

## Perspective

# HIV Diagnostic Testing: Evolving Technology and Testing Strategies

*Detection of acute HIV infection is important to public health because this stage is one of high infectiousness and appears to account for a disproportionate amount of HIV transmission. Newer technologies in HIV testing, including third-generation enzyme immunoassays (EIAs) that detect anti-HIV IgM and IgG antibodies, fourth-generation combination EIAs that detect both anti-HIV antibodies and HIV p24 antigen, and nucleic acid–based testing for HIV RNA, have markedly reduced the interval between infection and detection of infection. Rapid diagnostic tests including assays for IgG and IgM anti-HIV antibodies have high sensitivity and specificity. The availability and wide use of these newer technologies have motivated review of recommended HIV testing algorithms. Individuals' knowledge of their HIV serostatus contributes to reducing transmission risk behaviors. Thus, widespread testing, facilitated by newer technology, allows more individuals to know their serostatus and is the first step in any successful effort to curb the incidence of HIV infection. This article summarizes a lecture by Demetre Daskalakis, MD, at the New York City IAS–USA continuing medical education program held in November 2009 and re-presented in December 2010.*

The US Public Health Service HIV testing algorithms have not changed substantially since 1989, despite the introduction and wide use of new technology.<sup>1</sup> It is still recommended that positive test results not be given to test recipients until screening test results are repeatedly positive on the same specimen and supplemental, more-specific tests such as the Western blot have been used to validate initial results.<sup>2</sup> There is considerable interest in revising testing guidelines to more accurately reflect new technology and associated challenges.

### New Technology: Focus on Acute Infection

The various measures of HIV infection have specific “detectable moments” during the natural history of infection (Figure 1).<sup>3,4</sup> For example, the early peaking of plasma virus level is matched in time by a peak in the HIV p24 antigen level, and the declines in

viral load and p24 antigen levels are coincident with increased levels of HIV anti-p24 antibody and then HIV anti-envelope antibody.

### Old and New Enzyme Immunoassays

In first- and second-generation (“indirect”) enzyme immunoassays (EIAs), plasma or serum is added to antigen-coated wells containing viral lysate (first-generation assays) or recombinant HIV proteins or synthetic peptides (second-generation assays). Anti-HIV IgG antibody in a sample binds to the antigens, and an enzyme linked to anti-human IgG antibody is added to the well and binds to the anti-HIV IgG. A color reagent is then added, and any color change indicates the presence of anti-HIV IgG in the sample.

In third-generation (“sandwich”) EIAs, the antigen-coated well contains recombinant proteins or synthetic peptides, and anti-HIV IgG and IgM antibodies in a sample bind to the antigen. In the enzyme-detection step, an enzyme linked to HIV antigen (rather than to anti-IgG antibody) is added to the well and binds to anti-HIV IgG and IgM. A change in color upon addition

of the color reagent indicates the presence of anti-HIV IgG and IgM in the sample. Because third-generation assays detect IgM as well as IgG, they allow antibody responses to be detected earlier than with the first- and second-generation anti-IgG–based systems.

In fourth-generation (“combination”) EIAs, the wells are coated with HIV antigen and p24 antibody (Figure 2). HIV antibodies in the sample bind the antigen, and the anti-p24 antibody captures free p24. The detection system uses both enzyme-linked HIV antigen and enzyme-linked p24 antibody. A color change after addition of the color reagent indicates the presence of either anti-HIV antibody or p24 antigen, and 2 different fluorescent labels can be used for independent detection of p24 antibody or HIV antigen. By detecting p24 antigen, fourth-generation assays permit even earlier detection of HIV infection than previously available assays, because they can detect viral antigen before an antibody response can be detected.

### Rapid Diagnostics

Available rapid diagnostic tests using samples of oral fluid, whole blood, plasma, or serum are lateral flow devices able to detect anti-HIV IgG and IgM but not HIV antigen. A sample added to a well moves along filter paper via capillary action through a zone containing colloidal gold conjugated to protein A or HIV antigen. Protein A nonspecifically binds antibody and HIV antigen binds anti-HIV antibody (Figure 3). The fluid reaches a test line coated with HIV antigen, and the colloidal gold produces a color change at the line if cross-binding with antigen occurs. The fluid then reaches an internal control line coated with anti-human IgG; binding at this line also causes a color change, indicating that the device has worked properly. Color

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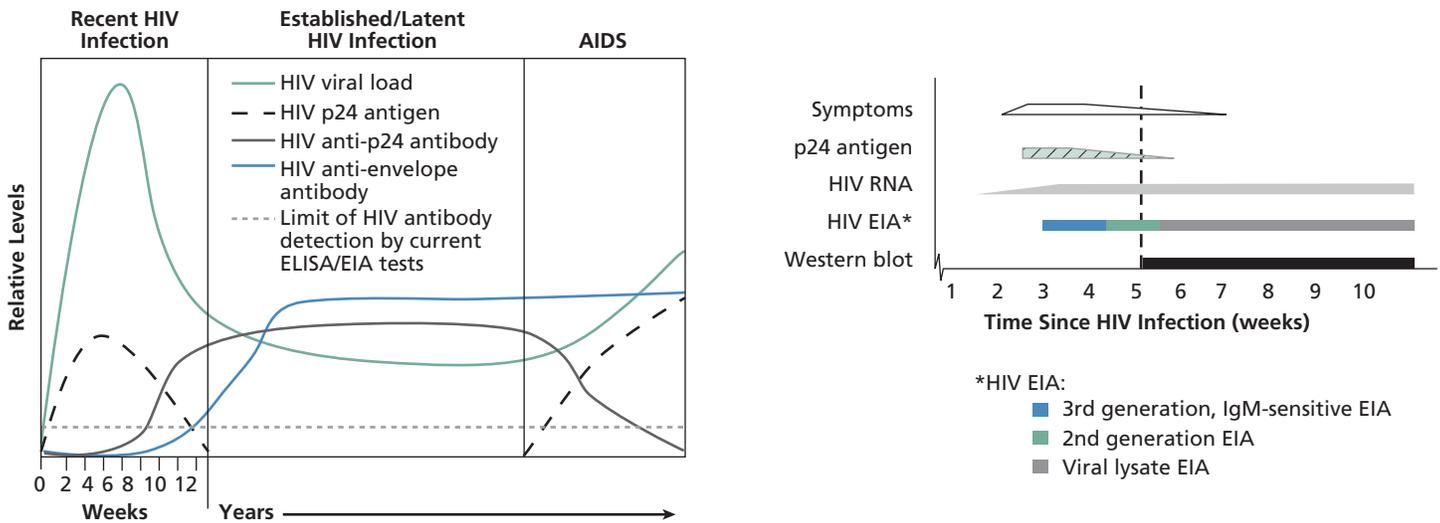


Figure 1. **Left**, Time of appearance and relative levels of HIV viral load, p24 antigen, and anti-HIV antibodies during the natural history of untreated HIV infection. Adapted from Daskalakis et al.<sup>3</sup> **Right**, Time during acute infection at which positive results of diagnostic tests can be obtained. Vertical dashed line indicates the earliest time at which Western blot testing could fully confirm a positive result. EIA indicates enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay. Adapted from Fiebig et al<sup>4</sup>; original illustration courtesy of Bernard M. Branson, MD.

change at both lines indicates a positive result.

The sensitivities and specificities of available lateral flow devices range from 99.3% to 100% and 99.7% to 99.9%, respectively, with narrow 95% confidence intervals, per the product information of these rapid diagnostics. Tests recently approved by the US Food and Drug Administration (FDA) include rapid automated serologic tests with enhanced sensitivity and specificity. In addition, an improved nucleic acid-based test has been approved that can be used both to detect acute HIV infection and to confirm positive serology results, although its use in the latter capacity remains outside of current guidelines.

### Comparison of New Technologies with Western Blot Testing

Newer diagnostic techniques permit earlier detection of HIV infection during acute infection than does Western blot testing (Figure 1). After infection, symptoms may appear within 2 weeks. HIV p24 levels (measurable with fourth-generation EIAs) typically peak after the onset of symptoms, at about 2.5 weeks to 3 weeks after infection; plasma HIV RNA levels (measurable by nucleic acid amplification tests [NAATs]) begin to increase at about 1.5

weeks to 2 weeks, peaking at around 3 weeks to 6 weeks after infection. With the subsequent occurrence of antibody response, third-generation EIAs can detect antibody as early as 3 weeks to 4 weeks after infection, and second-generation tests can return positive results at around 4.5 weeks to 5 weeks after infection. By comparison, Western blot testing first begins to show positive results at around 5 weeks. Direct comparison of some of the newer techniques with Western blot testing has shown that positive results are obtained days to weeks before the Western blot test yields positive or even indeterminate results.

Indeterminate Western blot test results are frequently associated with detection of anti-p24 antibody in the setting of both false-positive and negative screening EIAs. Indeed, anti-p24 antibody is the most commonly detected antibody in the setting of false-positive EIAs and indeterminate-result Western blots.<sup>5</sup> The fact that rapid HIV testing techniques do not include a p24 assay may allow such tests to avoid a proportion of false-positive results.

The potential value of rapid testing in this respect is illustrated by findings in a study in which women in labor for whom no HIV test results were available were screened.<sup>6</sup> Among 7680

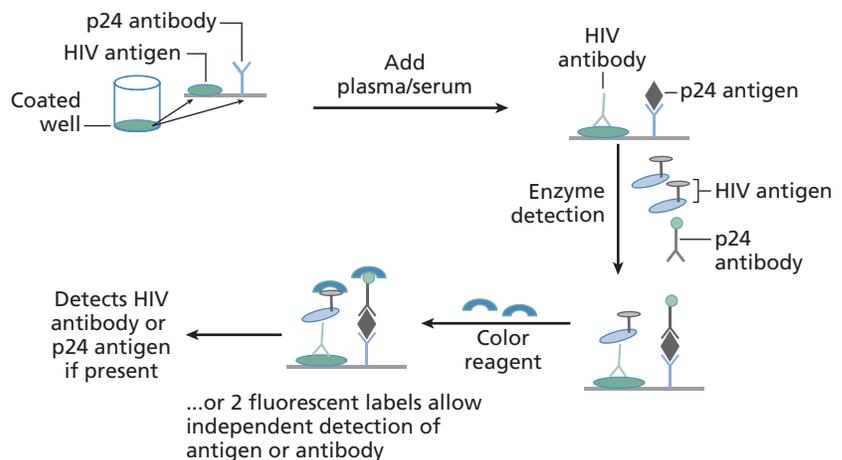


Figure 2. Schematic of fourth-generation combination enzyme immunoassay. Adapted from original figure courtesy of Bernard M. Branson, MD.

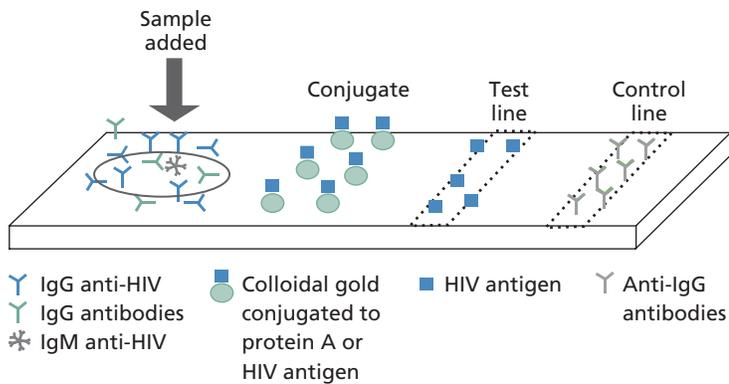


Figure 3. Schematic of rapid diagnostic, lateral flow devices. Adapted from original figure courtesy of Bernard M. Branson, MD.

women screened, 54 (0.7%) new HIV infections were identified. Oral rapid testing yielded 6 false-positive results and no false-negative results. EIA testing yielded 15 false-positive results, of which 7 were positive for p24 only and 8 were negative on Western blot testing. Overall, specificities for the oral rapid test and the EIA were 99.92% and 99.80%, respectively, and positive predictive values were 90% and 76%, respectively. Such findings indicate that p24-excluding rapid tests may be less prone to false-positive results in the labor and delivery setting, potentially avoiding unnecessary exposure of mothers and infants to antiretroviral drugs.

Given the intricacies and interactions of rapid HIV testing, standard EIA testing, and Western blot testing, workgroups from the Centers for Disease Control and Prevention (CDC) currently are investigating new HIV testing algorithms for both point-of-care and laboratory settings. Possibilities being considered are the use of combinations of rapid tests and the use of NAAT-based confirmatory testing. It is also possible that the Western blot test may be phased out of use.

### HIV Antigen Detection: Technology and Strategies

Detection of acute HIV infection is essential to public health, as the acute phase is a period of enhanced infectiousness. Data from Montreal indicate that 50% of HIV transmission is attributable to recently infected persons,

and other data indicate that recent and late-stage infections are associated with enhanced transmission regardless of viral load.<sup>7,8</sup> Standard HIV testing, with the exception of the fourth-generation EIAs, misses much of the acute stage of infection. Diagnosis of acute infection is hampered by the fact that many patients may not have major symptoms during acute infection and that symptoms, even when present, frequently are missed in history and examination. It is estimated that some symptoms are present in 92% of cases of acute infection, but that the diagnosis on the basis of symptoms is missed 80% of the time.<sup>9</sup>

Options for testing for acute HIV infection include enhancing the screening test with pooled results from HIV NAAT; however, this tends to be available only through specific programs. An alternative is to request an individual NAAT test to assess viral load after a negative rapid test result or while awaiting results of a standard EIA. In pooled screening, 100 patient samples are arrayed in 10 pools of 10 samples each. For any pool that includes positive test results, samples from each of the patients contributing to that pool are individually retested using a NAAT. A positive viral load test result is indicative of acute infection in an individual with a negative rapid test result and positive EIA result, and the viral load test should likely be repeated to confirm that HIV viremia has been detected in the setting of no detected seroconversion.

Investigations of pooled RNA screening have shown the ability to detect infections missed by antibody testing. In a study in North Carolina (2003), 0.02% of 109,250 individuals (0.5% antibody-positive) were found to have HIV antibody-negative and HIV RNA-positive test results with pooled screening.<sup>10</sup>

In Florida (2007), 0.02% of 45,288 individuals (1.2% antibody-positive) yielded antibody-negative and HIV RNA-positive results.<sup>11</sup> In Los Angeles (2007), 0.05% and 0.09% of 30,289 individuals had antibody-negative and HIV RNA-positive results in studies using 3 different screening tests (1.2% were antibody-positive on both tests).<sup>11</sup>

Other investigations have used pooled RNA screening in high-risk settings. A study in San Francisco City Clinic in 2004 found a 0.3% frequency of antibody-negative and HIV RNA-positive individuals among 3789 tested (3.2% antibody-positive), and another in 2007 found a 1.1% rate among 1092 tested (7.5% antibody-positive).<sup>12</sup> A study in Los Angeles (2004) found that 0.05% of 2523 individuals (0.9% antibody-positive) had antibody-negative and HIV RNA-positive pooled-screening results.<sup>13</sup> In Atlanta (2004), a frequency of 0.2% in 2202 individuals was found (2.9% antibody-positive).<sup>14</sup> In Seattle, 0.2% of 3525 individuals (2.3% antibody-positive) had antibody-negative and HIV RNA-positive results.<sup>15</sup>

The use of fourth-generation EIAs has also increased the ability to detect acute HIV infection. For example, an Australian study found that a third-generation versus a fourth-generation EIA identified 66% versus 92% of 53 cases of acute infection in 2005, 67% versus 97.7% of 43 cases in 2006, and 56.5% versus 90% of 30 cases in 2007, respectively (Bernard M. Branson, MD; written communication, September 2009). For all 3 years combined, the third-generation assay identified 63.2% of acute infections, and the fourth-generation assay identified 93.2%.

### HIV Testing Expansion

According to 1 model, the estimated 25% of individuals unaware of their HIV infection are responsible for 54% of new infections.<sup>16</sup> Many HIV health care practitioners believe that individuals' knowledge of their HIV serostatus is an effective preventive intervention, and this belief is supported by available data. A meta-analysis of 11 studies showed a 68% reduction in unprotected anal or vaginal sex in HIV-infected

patients aware of their serostatus versus those who were unaware.<sup>16</sup> Quantitative analysis of a cohort of 28 persons showed statistically significant behavior changes at 2 months after receipt of a diagnosis of acute or recent HIV infection, including reductions in total number of partners and the proportion of unprotected sexual acts occurring with uninfected partners (serosorting). The subjects reported that these changes occurred because they were motivated to prevent transmission, although it was also found that there was no increase in condom use.<sup>17</sup>

The test-and-treat model of HIV intervention indicates that there would be a dramatic reduction in HIV incidence with widespread testing and immediate institution of antiretroviral therapy for individuals with positive test results (thereby lowering the “community” viral load).<sup>18</sup> Testing is the first step in any effort to substantially curb the HIV epidemic. In recognition of this fact, the 2006 revised CDC guidelines for testing recommended HIV screening for patients in all health care settings once the patients are notified that testing will be performed unless they decline (opt-out screening).<sup>2</sup> It is also recommended that persons at high risk of infection be screened at least annually. Separate written consent for testing is not required because general consent for medical care is considered sufficient to encompass consent to HIV testing. Not all locales comply with these recommendations, however.

### Testing Initiatives in New York City

A number of efforts to increase HIV testing are under way in New York City (NYC). For example, the NYC Health and Hospital Corporation (HHC), the largest public health delivery system in the United States, has committed to increasing routine HIV testing in its facilities. In fiscal year 2005, 62,023 tests were performed, representing 6.3% of the 984,265 eligible HHC clients. In fiscal year 2008, a total of 160,900 tests were performed, representing 15.4% of the eligible population (n = 1,040,432)

(Judith A. Aberg, MD; written communication, October 2009).

The aim of the Bronx-Wide HIV Testing Initiative (the Bronx Knows: What’s Your Status? at [www.nyc.gov/bronxhivtesting](http://www.nyc.gov/bronxhivtesting)) is to increase testing with the goals of having all Bronx residents aged 18 years to 64 years aware of their HIV serostatus and ensuring that all infected persons have access to good-quality care and prevention services. Testing is being performed at community health clinics, hospitals, community-based organizations, NYC Department of Health clinics, and jails in partnership with the Department of Health and Mental Hygiene (DOHMH). Testing increased by 28% in the first year of the program.<sup>19</sup>

Project BRIEF is an emergency department-based initiative started at the HHC Jacobi Hospital Center that combines informatics, including multimedia counseling, and a client-centered “white glove” connection to care. Upon receipt of a positive result indicating HIV infection, patients receive an escort to the HIV clinic for an automatic connection to care. On a recent assessment of performance of this program, 33,487 patients had been screened, and 0.45% of patients were found to be HIV-seropositive; 85% of HIV-infected persons were connected to care and 89% of eligible patients were receiving antiretroviral therapy (Jason Leider, MD, Yvette Calderone, MD; written communication, September 2009).

The NYC DOHMH recently initiated the use of pooled NAATs at their sexually transmitted disease (STD) clinics, and an analysis indicated detection of acute infection in 0.17% of patients tested. Many of these infections were in men who have sex with men (MSM), raising the possibility of more-targeted use of pooled NAAT in the MSM population.<sup>20</sup>

A recent analysis was made of outcomes from the Bellevue/New York University Men’s Sexual Health Project ([www.hivinfosource.org/testingproject](http://www.hivinfosource.org/testingproject)), which operates from satellite diagnostic areas of Bellevue Hospital Center and is based at commercial sex venues, events, and parties (eg, bathhouses,

sex clubs). The analysis showed that of more than 3000 testing visits conducted, 3.2% of individuals had newly diagnosed HIV infection and 0.5% had acute infection detected using pooled viral load testing (a rate approximately 3 times higher than that reported in DOHMH STD clinics). The program has achieved a 96% connection-to-care rate. High rates of syphilis, chlamydial infection, and gonorrhea were also detected, indicating the need to integrate HIV testing with testing for other STDs.

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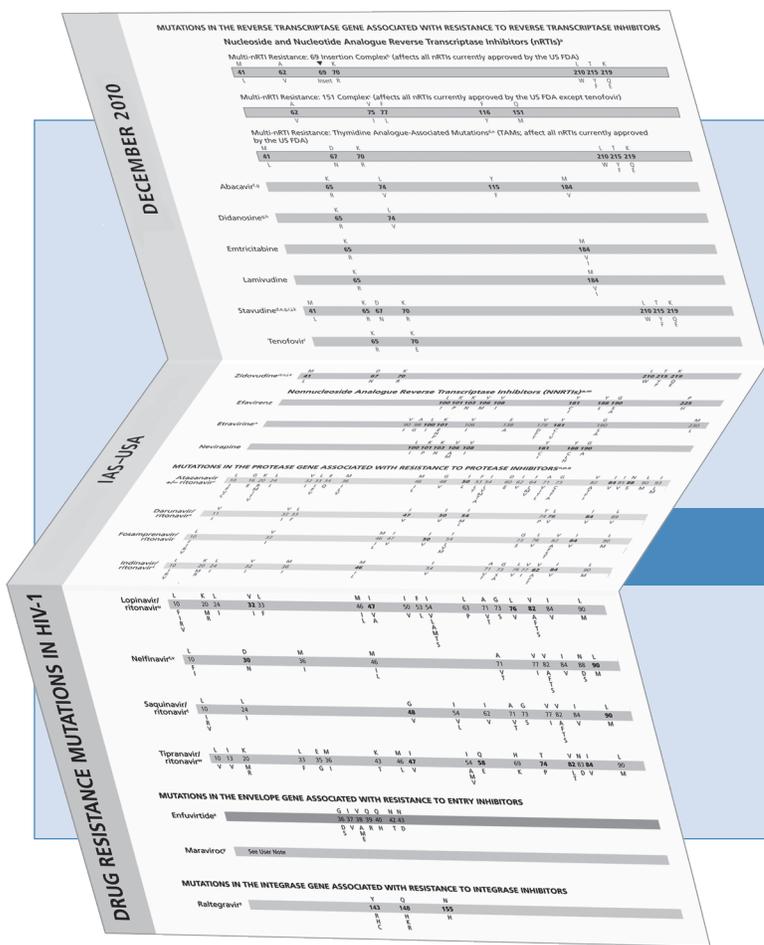
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