Diagnostic assays for HIV-1 infection

By Hong-Ha M. Truong, PhD, MS, MPH, and Jeffrey D. Klausner, MD, MPH

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LEARNING OBJECTIVES

The objectives and test were prepared by Dianne M. Cearlock, PhD, CLS(NCA), MT(ASCP), professor and coordinator, Program in Clinical Laboratory Sciences, College of Health and Human Sciences, Northern Illinois University, DeKalb, IL.

Upon completion of this article the reader will be able to:

1. Discuss several characteristics of standard (current) HIV diagnostic testing for which improvement is desirable and describe how technologic advances are addressing those issues.
2. Describe the principles and materials (lysates, recombinant proteins, monoclonal antibodies, etc.) used in standard, alternative sample, and rapid diagnostic tests for HIV infection.
3. Explain the principles, advantages, and disadvantages of newer HIV diagnostic procedures, using nucleic acid amplification and combination antigen/antibody EIA techniques.

Worldwide, a total 40 million people were estimated to be living with HIV/AIDS through the end of 2003.[1] According to the World Health Organization and the Joint United Nations Programme on HIV/AIDS, over 5 million new infections occurred in 2003, which translates to roughly 14,000 new infections daily. Since the first case of HIV infection was diagnosed in the United States in 1981, there have been an estimated 886,575 AIDS diagnoses through 2002.[2,3] Currently, there are an estimated 800,000 to 900,000 persons living with HIV in the United States, with an estimated 40,000 new infections per year.[3]

The ability to diagnose HIV infection is a critical component in the efforts to stem the continued spread of the epidemic, because knowledge of individual HIV-serostatus can help prevent secondary transmission of infection. Diagnostic assays for HIV infection have evolved substantially since the introduction of antibody testing in 1985. This article summarizes a number of HIV test technologies cleared by the United States Food and Drug Administration (FDA) for diagnostic use in the United States and reviews several recently developed assays that represent further progress in diagnostic technology.

Standard HIV assays

HIV testing should be performed for patients with certain behavioral risk factors or clinical manifestations associated with HIV infection (Table 1).[4] Increasingly, HIV testing is being incorporated into routine medical care away from specialized counseling and testing centers. Serological responses develop soon after HIV infection. Following initial infection, there is a slow rise in HIV-specific antibody titers (Figure 1). The timing of antibody response varies, depending on both host and viral factors.

In the United States, HIV-1 is the causative agent for the majority of HIV infections. A second virus, HIV-2, is predominantly found in Africa, especially in parts of West Africa. HIV-2 infection occurs by the same modes of transmission as HIV-1, and both are associated with similar opportunistic infections and AIDS, although immunodeficiency develops more slowly and is milder with HIV-2. Since the prevalence in the United States is very low, routine HIV-2 testing is not recommended. Indications for HIV-2 testing include persons who have a sex partner known to be infected with HIV-2; persons from a country where HIV-2 is endemic; persons who have received a blood transfusion or shared needles with a person from a country where HIV-2 is endemic; and children of women who have risk factors for HIV-2 infection or are known to be infected with HIV-2.[5]

The standard HIV testing algorithm is comprised of two assays. Specimens, either serum or plasma, are first screened for HIV antibodies using an enzyme immunoassay (EIA). For specimens testing negative by EIA, no further testing is required. For specimens in which HIV antibodies are detected by EIA, a follow-up confirmatory test is conducted. The confirmatory testing is usually a Western Blot or immunofluorescence assay (IFA). In some resource-poor, high-prevalence settings, a second EIA is used as the confirmatory test.

Enzyme immunoassays. These assays use HIV-1 antigens coated onto the wells of microwell plates for the detection of antibodies specific for HIV-1. Specimens containing HIV antibodies form immune complexes through the interaction between antiHIV in the specimen and HIV antigens coated on the microwell. First-generation EIAs were based on viral lysate-based immunoglobulin G (IgG) tests, and the second generation incorporated recombinant protein and synthetic peptide antigens. Third-generation EIAs use antigen sandwich techniques to detect IgG and IgM, while the third-generation-plus assays enable the detection of HIV-1 group O.[6]

Currently, there are several EIA kits for HIV-1 testing that are FDA cleared. HIVAB HIV-1/HIV-2 (rDNA) EIA (Abbott Laboratories, Abbott Park, IL) uses recombinant HIV-1 *env* and *gag* and HIV-2 *env* proteins. The HIV-1 antigen for the Vironostika HIV-1 Microelisa System (bioMérieux Inc., Durham, NC) is derived from purified and inactivated HIV-1 virus propagated in T-lymphocyte culture. Genetic Systems rLAV EIA (Bio-Rad Laboratories, Redmond, WA) is manufactured from both a strain of HIV-1 designated LAV that is propagated in a CEM cell line and from an *E coli* recombinant protein containing antigenic regions of the HIV-1 envelope protein gp41.

In 1990, a more divergent strain of HIV-1, known as group O, was isolated and characterized.[7] HIV-1 infections attributable to group O viruses in the United States are rare, with the majority of cases having been identified in Africa, with some cases in Europe.[8,10] The country with the highest prevalence of HIV-1 group O infections is Cameroon.[10,11] Antibodies specific for group O viruses are difficult to detect using assays that do not use whole viral lysate and/or contain group O-specific epitopes as antigens.[12,13] There are two EIA kits cleared by the FDA for the detection of type O viruses. Vironostika HIV-1 Plus O Microelisa System (bioMérieux Inc., Durham, NC) uses inactivated, purified HIV-1 viral lysate proteins, purified viral envelope proteins, and a synthetic peptide with amino acid sequence corresponding to the transmembrane immunodominant domain of the HIV-1 group O (ANT 70) isolate. Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio-Rad Laboratories, Redmond, WA) employs purified gp160 and p24 recombinant proteins derived from HIV-1, a peptide representing the immunodominant region of the HIV-2 transmembrane glycoprotein gp36, and a synthetic polypeptide mimicking an artificial specific epitope of HIV-1 group O.

In addition to antibody detection, the EIA technique can also detect viral components in blood. The HIV p24 antigen assay detects viral capsid p24 protein in sera, which usually appears earlier during acute infection before antibody formation.[14] Use of the p24 antigen assay for diagnosing HIV infection is discouraged, however, because the estimated time from detection of p24 antigen to detection of HIV antibody by standard EIA is on average six days, and not all recently infected persons have detectable levels of p24 antigen.[4] The Coulter HIV-1 p24 Antigen Assay (Beckman Coulter Corp., Miami, FL) uses a murine monoclonal antibody to bind HIV-1 p24 antigen coated onto microtiter strip wells. Purified HIV-1 p24 antigen reagent derived

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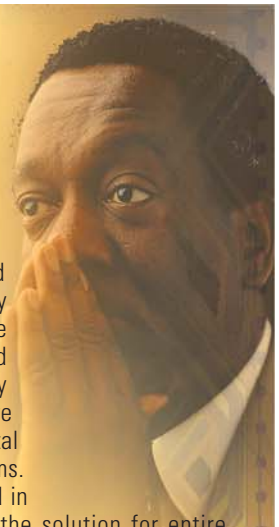
Bringing AIDS testing to developing countries

By Angela Vernon



In industrialized countries, antiretroviral treatment has turned AIDS from a death sentence into a chronic illness. Many countries in the developing world, however, do not have the funds to carry out effective care and treatment programs and lack the infrastructure required to support them. Today, nearly all HIV/AIDS treatment programs in developing countries are small-scale pilot programs, often carried out by nongovernmental organizations that are outside the mainstream health systems. These pilot programs are important, but they are often limited in their resources and geographic scope and cannot provide the solution for entire countries. Ultimately, groups must coordinate their efforts, and the mainstream health infrastructure has to be substantially upgraded to aggressively address the AIDS pandemic.

One of these groups, the William J. Clinton Presidential Foundation, has the battle against HIV/AIDS as a key focus. The Clinton Foundation HIV/AIDS Initiative aims to assist developing nations in implementing large-scale integrated care, treatment, and prevention programs to turn the tide of the AIDS epidemic. It partners with countries in Africa and the Caribbean to develop operational business plans to scale up HIV/AIDS care and treatment. The Clinton Foundation HIV/AIDS Initiative has been at work for the past year helping individual governments in these nations to develop scalable AIDS care, treatment, and prevention strategies. In the Caribbean, the Foundation is working with nine countries and three territories that together comprise over 90% of people living with HIV/AIDS in that area. The Foundation is also working with Mozambique, Rwanda, South Africa, and Tanzania, representing about one-third of all people living with HIV/AIDS on the African continent.

Building the capacity to run effective care and treatment programs in resource-poor countries is very difficult. It requires setting up protocols and organizing substantial treatment networks at the local and national levels. It requires regular testing and involves procuring and distributing drugs, ensuring affordable prices, adequate security for delivery of the drugs, and compliance by patients in taking the prescribed medications.

In addition to working with governments and health ministries in developing countries, the Clinton Foundation has been actively working with selected manufacturers of instruments, tests, and drugs to develop a streamlined, cost-effective procurement process.

One of the companies working with the Clinton Foundation to supply HIV/AIDS-related products to some of these developing countries is Beckman Coulter, which — through this program — provides a diagnostic package specifically designed for use in resource-poor areas. In late March, the company was awarded the first tender in South Africa for its CD4 monitoring test and COULTER EPICS XL flow cytometers. The first shipment has already been delivered, and flow cytometers are currently being installed in South Africa.

"We were pleased when the Clinton Foundation approached us to participate in this HIV/AIDS prevention and treatment program. They have made tremendous progress in their effort to assist the developing world in combating the AIDS crisis," says Bonnie Anderson, vice president of Beckman Coulter's Translational Solutions Business Center. "This opportunity has expedited our plans to take the CD4 monitoring test we licensed from the National Health Laboratory Service in South Africa and deliver it to millions of individuals whose conditions might not otherwise be monitored."

Other companies working with the Clinton Foundation on its HIV/AIDS initiative are Bayer Diagnostics, BD, bioMérieux, and Roche.

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Table 1: Common indications for HIV testing
Clinical signs or symptoms suggesting HIV infection
■ Fever or illness of unknown origin ■ Mucocandidiasis (oral thrush) ■ Chronic, recurrent skin manifestations (psoriasis) ■ Unexplained lymphadenopathy with or without fatigue or weight loss
Diagnoses suggesting increased risk for HIV infection · Sexually transmitted disease · Hepatitis B or C infection
■ Recurrent pneumonia or bacteremia ■ Tuberculosis ■ Opportunistic infection (e.g., pneumocystis, cryptococcosis, cytomegalovirus) ■ Cervical or anal cancer, lymphoma, Kaposi's sarcoma
Self-reported HIV risk behaviors
■ Injection drug use ■ Men who have sex with men ■ Unprotected vaginal or anal intercourse with partner who might be HIV infected ■ Unprotected vaginal or anal intercourse with more than one sex partner
Pregnant women
Possible acute occupational exposure

Adapted in part from: Revised guidelines for HIV counseling, testing and referral. MMWR, 2001

from a human T-cell line infected with the HTLV-IIIB strain of HIV-1 is used as a quantitative calibration standard. Although Coulter discontinued production of this assay for clinical testing as of April 2004, already produced FDA cleared kits are still currently available. The assay will continue to be available for research purposes.

HIV antibodies are usually detectable within six to 12 weeks following infection using the earlier generations of assays. Third-generation assays can detect antibodies within three to four weeks, while p24 antigen assays can further shorten the diagnostic window to two weeks.[15] HIV antibody detection assays have high sensitivity and specificity (Table 2).

The instrumentation needed to perform EIA, such as a spectrophotometer plate reader for determining absorbance values, has utility for testing other conditions, including sexually transmitted diseases. Specifically designed for screening a high volume of specimens, EIAs are optimal for use in surveillance and centralized blood-transfusion services. Automation of EIA methods allow for increased efficiency and economy. Testing, however, needs to be performed in well-equipped laboratories by highly trained personnel.

Western Blot. Specific HIV-1 proteins are fractionated according to molecular weight by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecylsulfate. The resolved protein bands are electrotransferred to a nitrocellulose sheet. During the incubation period, HIV-1 antibodies present in the specimen bind to the major HIV-1 antigens (p17, p24, p31, gp41, p51, p66, gp120, gp160). The antigen-antibody complexes are visualized on the nitrocellulose strip as bands corresponding to the position of the HIV-1 proteins, if the HIV-specific antibodies are present in sufficient concentrations. A positive test result, as established by the interpretive criteria set by the CDC, is defined by the presence of any two of the following bands: p24, gp41, and gp120/160.[16] Distinguishing gp120 band from gp160 band is often very difficult; therefore, these two glycoproteins can be considered as one reactant for the purposes of interpreting Western Blot test results. Genetic Systems HIV-1 Western Blot Kit (Bio-Rad Laboratories, Redmond, WA) employs a purified and inactivated HIV-1 strain LAV grown in the CEM cell line. Cambridge Biotech HIV-1 Western Blot (Calypte Biomedical Corp., Rockville, MD) is manufactured from purified and inactivated HIV-1 propagated in an H9/HTLV-IIIB T-lymphocyte cell line.

Immunofluorescence assays. Designed to identify the presence of HIV-1 specific antibodies, the assay uses immortalized human T-cells expressing surface viral antigens. HIV antibodies present in the specimen bind to the HIV-1 antigens fixed on the surface of an IFA glass slide. The Ag-Ab complex is detected using antihuman immunoglobulin conjugated to fluorescein isothiocyanate (FITC), which binds to human antibodies and fluoresces when exposed to ultraviolet light. The presence of HIV-1 antibodies will generate a characteristic pattern of fluorescence. Interpretation of the results is evaluated by comparing the degree and pattern of fluorescence between uninfected and infected cell wells for each sample. Fluorognost HIV-1 IFA (Sanochemia Pharmazeutika AG, Vienna, Austria) utilizes HIV-1 antigens from an HTLV-IIIB isolate. Although there may be some degree of cross-reactivity with HIV-2 antibodies that recognize and bind to the viral antigens on the IFA slides, the Fluorognost HIV-1 IFA is not intended to identify HIV-2 antibodies or to distinguish HIV-2 from HIV-1.

Alternative specimen assays

The collection of noninvasive specimens like oral fluid and urine provides a good alternative for persons who avoid HIV testing because of their dislike for blood draws. There is the added safety advantage of eliminating the risk of accidental needlestick exposure to potential bloodborne pathogens, such as HIV and Hepatitis B and C. There

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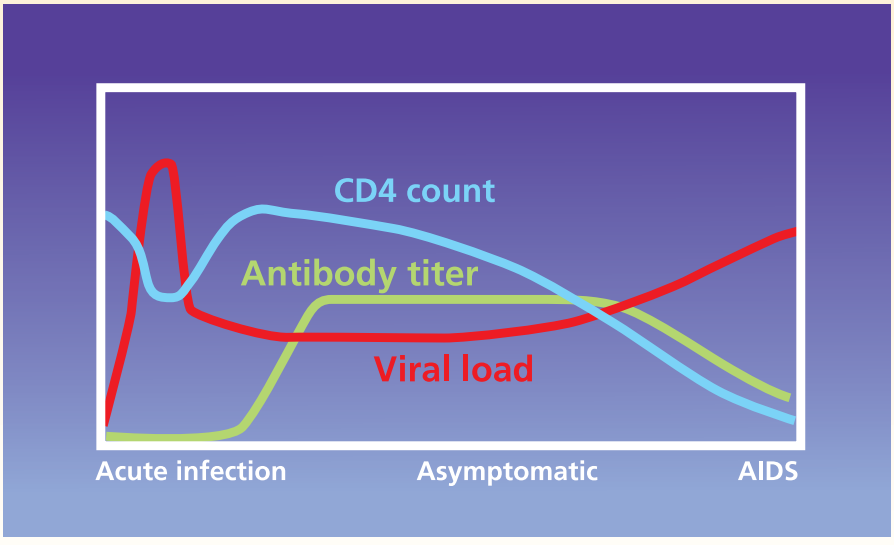

Figure1: Viral load, CD4 count, and HIV antibody titer with progression of HIV disease.

Table 2: Performance of selected HIV-1 diagnostic assays

	Sensitivity (%; range)	Specificity (%; range)
Enzyme Immunoassays HIVAB HIV-1/HIV-2 (rDNA) EIA	100	99.9
Vironostika HIV-1 Microelisa System	100	100
Genetic Systems rLAV EIA	100	99.9
Vironostika HIV-1 Plus O Microelisa System	100	99.6 to 100
Genetic Systems HIV-1/HIV-2 Plus O EIA	100	99.9
Coulter HIV-1 p24 Antigen Assay	95.5 to 100	99.9
Western Blot		
Genetic Systems HIV-1 Western Blot	100	87.2 to 89.3
Cambridge Biotech HIV-1 Western Blot Kit	*	*
Immunofluorescence assays		
Fluorognost HIV-1 IFA	99.7 to 100	99.0
Oral fluid tests		
Vironostika HIV-1 Microelisa System	98.6 to 99.1	97.7 to 99.6
OraSure HIV-1 Western Blot	97.5 to 99.5	60.8 to 63.1
Urine-based tests		
Calypte HIV-1 Urine EIA	97.9 to 99.0	99.1
Cambridge Biotech HIV-1 Urine Western Blot	99.1 to 100	94.5 to 100
Rapid tests		
OraQuick	99.6	100
Reveal Rapid HIV-1 Antibody Test	99.8	99.1
Uni-Gold Recombigen HIV Test	100	99.7 to 99.8

* As the first Western Blot FDA cleared in 1987 and considered the "gold standard" for HIV-1 blood confirmatory testing, there has not been historical performance evaluations for sensitivity and specificity (as cited by Calypte Biomedical Corp.).

is, however, reduced sensitivity and specificity of testing with oral fluids and urine compared to blood specimens (Table 2).

Oral fluid testing. Saliva is a complex mixture of parotid, submandibular, sublingual, and minor salivary gland secretions mixed with mucin, bacteria, leukocytes, sloughed epithelial cells, and gingival crevicular fluid. Gingival crevicular fluid, or mucosal transudate, is derived from the passive transport of serum components through the oral mucosa into the mouth. Use of oral fluids for HIV-1 testing has been problematic due to specimen instability and assay insensitivity.[17]

The OraSure HIV-1 Specimen Collection Device (Epitope Inc., Beaverton, OR) enhances the flow of mucosal transudate across the mucosal surfaces onto an absorptive cotton pad and includes preservatives effective for protection of antibodies against degradation by proteases found in oral fluid. The specimen is centrifuged to elute the oral fluid from the collection pad, and the eluate is diluted and added to microelisa wells. The Oral Fluid Vironostika HIV-1 Microelisa System (bioMérieux Inc., Durham, NC) uses antigen derived from HIV-1 virus propagated in T-lymphocyte culture from an HTLV-III isolate. The purified and inactivated HIV-1 antigen is coated onto the microelisa wells. OraSure HIV-1 Western Blot (OraSure Technologies Inc., Bethlehem, PA) employs a whole-cell viral lysate propagated in an H-9/HTLV-IIIB T-lymphocyte cell line that is purified and inactivated.

Urine-based testing. The Calypte HIV-1 Urine EIA (Calypte Biomedical Corp., Alameda, CA) uti-

lizes a recombinant HIV-1 envelope protein to detect the presence of antibodies to HIV-1. Antibodies present in the specimen will bind to the recombinant gp160 envelope protein absorbed onto the wells of the microwell plate. Cambridge Biotech HIV-1 Urine Western Blot (Calypte Biomedical Corp., Rockville, MD) uses a partially purified and inactivated virus propagated from an H9/HTLV-IIIB T-lymphocyte cell line.

Rapid testing

With the standard testing protocol, laboratory results are typically reported to the testing site within 48 hours to two weeks. Therefore, the patient is required to return to the clinic for a second visit in order to receive test results. This waiting period can be a source of anxiety for many patients, and some may never return for their test results. Rapid testing offers the convenience of point-of-care testing, providing patients with test results and immediate counseling during the initial visit. The rapid assay enables testing of pregnant women who are unaware of their HIV status at time of delivery and permits initiation of antiretroviral therapy for the mothers during labor and for their infants postpartum, thus greatly reducing the risk of vertical transmission.[18]

In the United States, an estimated 600,000 to 1,000,000 needlestick injuries occur each year among healthcare workers, with more than 380,000 occurring in U.S. hospitals.[19] The Centers for Disease Control and Prevention (CDC) received voluntary reports of 57 documented cases of HIV seroconversion associated with occupational exposure and an additional 138 possible cases through the end of 2001.[20] Rapid testing allows for timely treatment initiation (and potential discontinuation) after accidental exposures to body fluids from infected individuals. The rapid immunoassays are performed manually and visually read, so results can be obtained in 20 minutes or less. These tests are technically simple and can be performed by personnel with minimal training. CDC protocols recommend that reactive rapid HIV tests must be confirmed with a Western Blot or IFA, even with a subsequent nonreactive EIA. Recommendations also include follow-up testing for persons with negative or indeterminate confirmatory test results with blood specimens collected four weeks after the initial reactive rapid test result.[21]

The immunoreactive test system captures HIV-1 antibodies present in serum or plasma that are then visualized by the addition of a colormetric detection reagent. Reveal HIV-1 (MedMira Laboratories Inc., Halifax, Nova Scotia, Canada) is a single-use test cartridge containing an immunoreactive test membrane comprised of synthetic peptides corresponding to conserved regions of HIV structural proteins coated onto a membrane matrix, and is for use with serum or plasma specimens. Uni-Gold Recombigen HIV Test (Trinity Biotech, Wicklow, Ireland) employs genetically engineered recombinant proteins representing immunodominant regions of the HIV-1 envelope proteins. OraQuick Rapid HIV-1 Antibody Test (OraSure Technologies Inc., Bethlehem, PA) utilizes a lateral flow immunonassay procedure, whereby a plastic housing device holds an assay test strip comprised of a matrix for the immunochromatography of the specimen. In addition to finger-stick whole blood or venipuncture whole blood and plasma as specimens, in March 2004, this assay became the first rapid HIV test to be FDA cleared for use with oral fluid.

New approaches to testing

Nucleic acid amplification. During acute HIV infection, virus can be detected in serum and plasma prior to the development of antibody responses. To detect these early antibody-negative/RNA-positive infections, a number of laboratories have begun implementing nucleic acid amplification testing as part of routine HIV diagnostic testing al-

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gorithms. Three major nucleic acid amplification methodologies include reverse transcriptase-polymerase chain reaction (Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems, Pleasanton, CA), nuclei acid sequence-based amplification (NucliSens HIV-1 QT Test, Organon Teknika, Bostel, The Netherlands), and signal amplification branched-chain DNA (Quantiplex HIV-1 RNA, Bayer Diagnostics, Emeryville, CA). One screening strategy entails combining aliquots of antibody-negative specimens to create a specimen pool to screen for HIV RNA. Individual specimens are tested for HIV RNA only if the first pool screens positive. Pooling strategies, especially in clinical HIV testing populations with few cases of acute, antibody-negative infections, increase cost efficiency and predictive value.[22] HIV RNA testing based on pooling of antibody-negative specimens can efficiently identify acute HIV infection in clinical settings.[23,24] Diagnosis of acute HIV infections allows for early initiation of medical care, referral to research studies for early infection, and, more importantly, for implementation of intervention strategies (e.g., partner notification) aimed at preventing secondary transmission.

Combination antigen and antibody EIA. To reduce the window between the time of HIV infection and laboratory diagnosis, new assays have been developed that combine p24 antigen EIA with traditional antibody EIA for simultaneous detection of HIV p24 antigen and antibodies using a single test. The fourth-generation EIA reduces the diagnostic window on average by four days, compared to the third-generation EIA.[19] Murex HIV Ag/Ab combination (Abbott Laboratories, Abbott Park, IL) uses a solid phase made up of HIV-1 antigen (p31 and gp41), HIV-2 antigen (p36) recombinant protein, HIV-1 group O peptide (pg41), and anti-p24 monoclonal antibodies. VIDAS HIV DUO Ultra (bioMérieux, Marcy l'Etoile, France) is comprised of a solid phase receptacle (SPR) with an upper surface coated with three different anti-p24 monoclonal antibodies for p24 antigen detection. The lower surface of the SPR is coated with HIV-1 gp160 and peptides representing the immunodominant regions of gp41 for HIV-1 group O and gp36 of HIV-2 for IgG, IgM, and IgA antibody detection. In evaluation studies, the assay demonstrated 100% sensitivity and a range of 98.1% to 100% specificity.[5,20] Combined antigen and antibody EIA are internationally available, but have not yet been cleared by the FDA for diagnostic use in the United States. Though these assays show higher sensitivity for acute HIV infection detection compared to conventional antibody tests, the detection limit is still higher than that of antigen assays, and highly sensitive antigen assays can detect acute HIV infection on average one to two days earlier.[27,28]

Conclusion

Selection of a particular test technology in a clinical or nontraditional setting should be based on several factors, including the accuracy of the assay, patient preferences and acceptability, ease of sample collection, availability of trained personnel, laboratory facilities required to perform the assay, and FDA clearance of the assay.[4] Well-established assays such as the EIA, Western Blot, and IFA enable detection of HIV antibodies, while the p24 antigen assay allows for early virus detection. The capability of using oral fluid and urine specimens that can be collected in a noninvasive manner and the development of rapid tests and point-of-care testing providing quick turnaround of results have removed some long-standing barriers to HIV testing.

Despite all these advances in HIV diagnostic-testing technologies, as many as one-third of the nearly 900,000 HIV-infected persons in the United States may be unaware of their HIV status.[29] Moreover, of the 2.5 million people who seek testing at publicly funded counseling and testing sites annually, over 30% never receive their HIV test results. Antiretroviral therapy has been shown to be effective in extending life and reducing morbidity for persons living with HIV/AIDS. With the advances in treatment, accurate and early detection of HIV infection is instrumental for the timely initiation of antiretroviral therapy and subsequent reduction in illness and death.

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