HIV testing: an update

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HIV testing has significantly improved since the first diagnostic test in 1985. Increased understanding of HIV transmission and the clinical course of the infection, together with the availability of new techniques, have changed the strategies used for HIV testing. Furthermore, now that HIV testing is recommended in the routine medical evaluation of patients, an increase in screening and the identification of HIV-infected patients is expected. To adequately understand HIV testing, a review of the virus, the natural course of the infection, and the test characteristics is helpful.

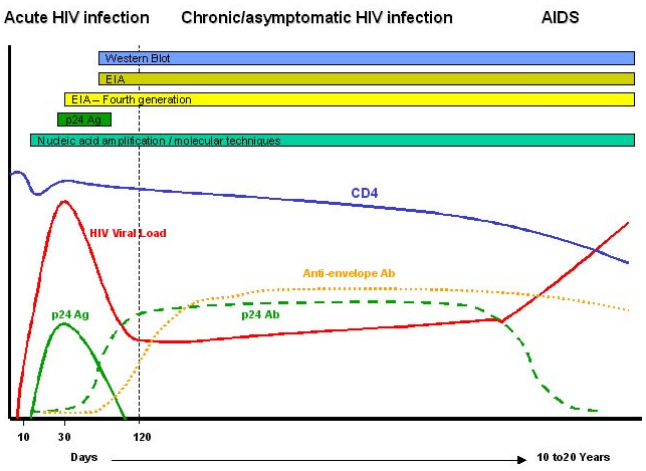
Virology and natural history

HIV is a retrovirus that consists of an envelope (outer membrane) and a viral core. The viral envelope is taken from the membrane of a human cell during viral budding and carries Env, a complex viral protein. The Env protein consists of a cap (made from glycoprotein (gp) 120), and a stem (made of gp41). Within the envelope, the viral capsid is made of thousands of copies of another viral protein, p24. These three proteins are highly immunogenic and are the antigens used in many diagnostic tests. The capsid surrounds two single strands of HIV RNA, each of which has a copy of the virus' nine genes. Three of these — *gag*, *pol*, and *env* — encode structural proteins. Three regulatory genes (*tat*, *rev*, and *nef*), and three auxiliary genes (*vif*, *vpr*, and *vpu*) contain information necessary for the production of proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. The core of HIV also includes the HIV nucleocapsid protein (p7) and three enzymes: reverse transcriptase, integrase, and protease. Some of the new HIV tests (e.g. molecular RNA or DNA technologies) target some of these genes and enzymes.

Host and viral markers of infection change depending on the time of the infection and will influence the selection of a test and its performance (see Figure 1). Soon after HIV infection, the virus starts an active replication, enabling the detection of viral RNA at very early stages (first marker of infection). Within the first few weeks after the acute infection, the capsid protein p24 (gag) appears. Its presence in the serum is transient and its disappearance coincides with the development of specific antibodies (see Figure 1). A transient immunoglobulin M antibody response against core (gag) and/or envelope (env) proteins is usually the first to appear and is followed by a long-lasting IgG response.[1] Following a similar sequence to their respective inducing antigens, IgG antibodies against the gag (p24) and env (gp160, 120, 41) appear first, followed by antibodies against HIV viral enzymes.[2] Most patients remain asymptomatic during the acute infection, but some others might develop a constellation of symptoms that include fever, rash, malaise and myalgias, diarrhea, and/or headache, among the most common.[3] At this time, the humoral response is still at early stages and most patients will have negative antibody tests (regardless of the technology used).

The acute infection is followed by a period of clinical latency that last several months to years. During this time, viral replication remains active and causes a progressive decline in T-helper lymphocyte (CD4+ T cell) counts. Both viral and host factors will determine the rate of decline in the CD4 T cell count, which ultimately results in loss of immune function and increased susceptibility to infection.

Figure 1: HIV testing technologies, HIV infection and host immune response



HIV testing technologies

The HIV testing assays are categorized in three main classes: tests that detect HIV antibodies, tests that detect antigens (in particular p24), and tests that detect or quantify viral nucleic acids.

Enzyme immunoassays (EIA) detect HIV-1 specific antibodies by using HIV-1 antigens coated onto the wells of microwell plates. EIA was the first technology developed for HIV diagnosis in 1985. Since then, serologic tests have been modified to include antigens for enhanced detection of viral variants (see Table 1) and to enable the diagnosis of HIV infection at earlier stages. In general, this technology has very high sensitivity and specificity, is inexpensive, simple, suitable for testing sizeable numbers of samples, and easily adapted to automate platforms. These characteristics make it the ideal test for the diagnosis of patients with chronic infection. A partial list of test kits can be found in Table 1.

Tests can be grouped in four generations by the antigens used. The first generation of EIAs was based on viral lysate-based immunoglobulin G (IgG) test, and this was soon followed by a second generation of tests in which recombinant proteins and synthetic peptide antigens were incorporated for increased sensitivity and specificity. Looking to further increase the test performance, the third generation of EIAs incorporates the use of an "antigen sandwich." This technique detects both IgG and IgM, allowing the diagnosis of HIV infection at an earlier stage. Using the same approach, the third generation-plus incorporated specific HIV-1 group O antigens that enable its detection, broadening the spectrum of HIV strains that can be tested. The fourth generation uses the simultaneous detection of HIV-1 p24 antigen and antibodies to

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Table 1: HIV technologies

Name	Format	Sample	Target Molecule / Comments	Manufacturer	FDA
HIV antigen assay for serum and plasma					
Abbott HIVAG-1 monoclonal	EIA	Serum/ Plasma		Abbott Laboratories	Yes
Coulter HIV-1 p24 Ag assay	EIA	Serum/ Plasma	HIV-1 p24 antigen	Beckman Coulter	Yes
HIV Antibody Detection in Serum or Plasma					
a. First- and second-generation EIAs					
HIVAB HIV-1/HIV-2 (rDNA)	EIA	Serum/ Plasma	Recombinant HIV-1 *env* and *gag* and HIV-2 env proteins.	Abbott Laboratories	Yes
Vironostika HIV-1 Microelisa System	EIA	Serum/ Plasma Blood spot	Purified, inactivated HIV-1 virus propagated in T-lymphocyte culture	bioMerieux Inc.	Yes
b. Third-generation (Detect HIV-1 group M and group O) EIA					
Vironostika HIV-1 Plus O Microelisa System	EIA	Serum/ Plasma	Purified, inactivated HIV-1 viral lysate proteins, envelope proteins, and a HIV-1 group O (ANT 70) transmembrane protein.	bioMerieux Inc.	Yes
Genetic Systems HIV-1/HIV-2 PLUS O EIA	EIA	Serum/ Plasma Cadaveric Serum	Purified gp160 and p24 recombinant proteins from HIV-1, HIV-2 transmembrane glycoprotein gp36, and a synthetic epitope of HIV-1 group O.	Bio-Rad Laboratories.	Yes
c. Fourth-generation EIA					
VIDAS HIV DUO Ultra	Enzime linked	Serum/ Plasma	HIV-1 gp160, p24 antigen, and peptides representing regions of gp41 from HIV-1 group O and gp36 from HIV-2.	bioMérieux	No
Murex HIV Ag/Ab combination	EIA/p24	-	HIV-1 antigens p31 and gp41, HIV-2 p36 recombinant protein, HIV-1 group O gp 41, and anti-p24 monoclonal antibodies.	Abbott Laboratories	No
d. HIV Antibody Detection in serum or plasma: Western Blot					
Genetic Systems HIV-1 Western Blot Kit	WB	Serum/ plasma	Purifie and inactivated HIV-1 strain LAV grown in CEM cell line.	Bio-Rad Laboratories	Yes
Immunoblot Cambridge Biotech HIV-1 Western Blot	WB	Serum/ Plasma	Purified and inactivated HIV-1 propagated in an H9/HTLV-IIIB T-lymphocyte cell line.	Calypte Biomedical Corp.	Yes
e. Detection in oral specimen					
OraSure HIV-1 Specimen Collection Device	Collection Device	-	Device that enhances the flow of mucosal transudate into a cotton pad that has antibodies preservatives against dehydration and proteases.	Epitope Inc.	Yes
OraSure HIV-1 Western Blot	WB	Oral fluid	Uses whole-cell purified and inactivated viral lysate propagated in a T-lymphocyte cell line	OraSure Technologies Inc.	Yes
Oral Fluid Vironostika HIV-1 Microelisa System	EIA	Oral fluid	Purified and inactivated HIV-1 antigen coated onto microelisa wells.	bioMerieux Inc.	Yes
f. Detection in urine					
Calypte HIV-1 Urine EIA	EIA	Urine	Recombinant HIV envelope proteins (gp160) to detect the presence of specific antibodies.	Calypte Biomedical Corp.	No
Cambridge Biotech HIV-1 Urine Western Blot	WB	Urine	Partially purified and inactivated HIV-1 propagated in an H9/HTLV-IIIB T-lymphocyte cell line.	Calypte Biomedical Corp.	Yes
Home blood-collection for laboratory assays					
Home Access HIV-1 test System	Collection Device		Collection device for direct dried blood spot	Home Access Health Corp.	Yes
Rapid tests/Rapid immunoassay					
Reveal HIV-1		Serum/ Plasma	Single-use test cartridge. Uses synthetic HIV structural proteins.	MedMira Laboratories Inc.	Yes
Uni-Gold Recombigen HIV Test		Serum/ Plasma	Uses recombinant proteins representing regions of the HIV-1 envelope proteins.	Trinity Biotech	Yes
OraQuick Rapid HIV-1 Antibody Test		Serum/ Plasma	Lateral flow procedure	OraSure Technologies Inc.	Yes
HIV Viral Load Assays					
Amplicor HIV-1 Monitor Test	PCR	Plasma	Reverse transcriptase-polymerase chain reaction	Roche Diagnostic System	Yes
NucliSens HIV-1 QT Test	NASBA RNA amplific.	Plasma	Nucleic acid sequence-based amplification.	Organon Teknika, Bostel, The Netherlands	Yes
Quantiplex HIV-1 RNA	bDNA signal amplific.	Plasma	Signal amplification branched-chain DNA	Bayer Diagnostics, Emeryville, CA	No

HIV-1 and HIV-2 to allow an even earlier diagnosis (reducing the diagnostic window by four days in average compared with third generation) and to include a broader range of strains.[4]

The WB assay is based on the recognition of the major HIV proteins (p24, gp41, gp120/160) by fractionating them according to weight by electrophoresis and then visualizing them by binding with specific antibodies over a nitrocellulose sheet. In contrast to EIAs that qualitatively detect antibodies without delineating the specific antigens to which the antibody reacts, WB shows the specific proteins to which the patient's antibodies are attached, making it a more specific test (which does not necessarily provide a higher test specificity). When two or more of the three major bands (p24, gp41, and/or gp120/160) are present, the test is called positive. The presence of any other combination of bands is called indeterminate.[5] Given its great negative predictive value, WB is probably the most widely accepted confirmatory assay and has been traditionally considered the "gold standard."

Immunofluorescence assay, or IFA, identifies HIV-1-specific antibodies by using immortalized human T-cells expressing surface viral antigens fixed on the surface of a glass. HIV antibodies from the specimen bind the HIV-1 antigens and the antigen-antibody complex is detected by using anti-human immunoglobulin conjugated to fluorescein isothiocyanate, or FITC, which is fluorescent when exposed to UV light. This technique can provide a definitive diagnosis in samples that test indeterminate with other confirmatory tests, but it requires a fluorescence microscope and well-trained operators.

Line immunoassay (LIA) incorporates separate HIV antigens on nitrocellulose strips, so each reaction can be visualized separately. The antigens used for this process are synthetic and recombinant, decreasing the background from non-specific host proteins, thereby decreasing the number of indeterminate results in non-infected patients and facilitating an easier interpretation. It offers better quality control and better reproducibility than WB. LIAs are also a popular confirmatory test.

Technically simple, a rapid HIV test can be performed manually by a person with minimal experience and visually read in 20 minutes. It allows testing in pregnant women that are unaware of their status before delivery, reducing the risk of vertical transmission by providing the appropriate interventions. Similarly, after needle-stick accidental exposure, rapid testing provides useful information for clinicians to select prophylactic treatment and potential early discontinuation. Rapid HIV diagnosis at point-of-care venues identifies high-risk infected patients, allowing the immediate establishment of support networks and medical care, reducing loss of follow up, time to establish care, and partner notification with great potential public-health implications.

Currently, most protocols still recommend confirming any positive rapid test result with either WB or EIA. Follow-up with WB or EIA four weeks after is also recommended for those with negative or indeterminate confirmatory tests.[6]

Oral fluid tests provide an alternative for people that do not want blood draws and avoid risk of needle-stick exposure for healthcare providers. IgG antibodies present in oral fluids can be detected by the technologies mentioned previously. Unfortunately, the use of oral fluids has been problematic due to specimen instability and assay insensitivity. Recently, the Food and Drug Administration (FDA) has cleared the OraQuick Advance HIV1/2 Antibody Test for use with oral fluid and plasma (previously approved only for whole-blood specimens). With 99.3% sensitivity and greater than 99.6 % specificity, this is the only rapid HIV test to be cleared in the United States by the FDA for use with oral fluid.[7] Newer strip technologies (Saliva-Strip HIV-1/2, Saliva Diagnostics Systems, Vancouver, WA) are being developed and seem to be highly concordant with blood-testing methods in developing countries. Although the FDA has not cleared the other tests, results from developing countries suggest that these technologies will provide a rapid, simple, non-invasive point-of-care test.

Similar to oral fluids, urine contains IgG antibodies. Several tests are now available, and they seem to have performances similar to serum samples.[8]

The detection of p24 is a simple and cost-effective technique to demonstrate viral components in blood. Initially implemented in 1995 in the United States to supplement antibody screening of donated

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blood components, this test detects viral capsid (core) p24 protein in blood usually earlier than antibody after initial infection, and offers the same performance advantages as the EIA for antibody detection.

The presence of antigen is highly specific for infection, but a significant limitation of these assays is their analytic sensitivity, because low concentrations of antigen are difficult to detect and antigenemia occurs only transiently during different stages of infection (e.g., most tests will only detect 25% of infected-seronegative patients).

Although new molecular technologies have replaced the use of EIA detection of p24 for the diagnosis of acute HIV infection,[9] the addition of p24 antigen to the latest generation of EIAs has markedly improved their performance.

Implementation of HIV molecular tests has changed the way that HIV medicine is practiced. This technology targets the detection of the virus itself, increasing the sensitivity for the diagnosis of early infection and allowing quantification of the viral load and genotyping to determine mutations associated with antiretroviral resistance. Currently, reverse-transcription polymerase chain reaction (RT-PCR), nucleic-acid sequence-based amplification, or NASBA, and branched DNA, or bDNA, are the nucleic-acid amplification technologies available. The use of viral load determination is now widely used to predict clinical outcome, progression of disease, and determination of need for therapy.

Traditionally, the algorithm for HIV testing is comprised of two steps. Specimens are first screened for HIV specific antibodies using EIA. Specimens testing negative by EIA require no further work-up. For specimens testing positive, a confirmatory test (traditionally a Western Blot) is performed.

During chronic HIV infection, the diagnosis is relatively straightforward since current technologies have very high sensitivity and specificity at this stage. A positive screen followed by a positive confirmatory test is diagnostic of HIV infection and a negative screening test rules out infection. During the acute HIV infection and the earlier stages of infection, however, most of the initial screening technologies can be falsely negative. Although the new EIAs are significantly decreasing this diagnostic window, to overcome this problem the use of molecular techniques is becoming progressively more common. As mentioned above, nucleic-acid amplification can detect HIV infection at its earliest stage, but it is an expensive and labor-intensive test. Since the cost of this technique can be prohibitive from a public-health perspective, the use of pooled samples has been a common practice. In this approach, the samples testing negative by EIAs are pooled in groups and tested using molecular techniques. If one of those groups test positive, the samples are tested individually looking for the infected one.[10]

The setting in which the HIV testing will be applied should also affect the selection of the ideal testing algorithm. Targeted testing is offered to patients that are felt to be at risk or that have any symptoms suggestive of HIV infection (see Table 2). In certain situations, however, in which missing a case can have serious clinical and public-health consequences (like in blood banks or very high-risk populations), all the members of a defined population are screened with a highly sensitive test. The positive results are then confirmed. Most importantly, the negative ones are further tested for acute infection by using nucleic-acid amplification technologies. This approach might not be the most cost-effective in populations with low HIV prevalence. Furthermore, with the development of new testing technologies, the selection of the initial screening test and the confirmatory one will also be influenced by particular characteristics of the test and the population. Rapid HIV testing is now being used to diagnose patients at physician offices, emergency rooms, and clinics, facilitating the early identification and treatment of new cases. Common indications for HIV testing are listed in Table 2.

Table 2: Common indications for HIV testing

Clinical signs or symptoms suggesting HIV infection
- Fever of unknown origin - Oral candidiasis - Chronic and/or recurrent skin problems (prurigo nodularis, psoriasis, etc.) - Unexplained lymphomegaly with or without fatigue or weight loss
Diagnoses suggesting increased risk for HIV infection
- Diagnosis of sexually transmitted diseases - Diagnosis of hepatitis B or C - Recurrent pneumonia - Tuberculosis - Opportunistic infections - Cervical or anal cancer - Lymphoma, Kaposi's sarcoma
Self-reported risk behaviors
- Injection drug users - Men who have sex with men - Unprotected vaginal or anal sex with a partner that might be infected with HIV - Unprotected vaginal or anal sex with more than one partner
Pregnant women
Occupational exposure

HIV testing has dramatically improved during the last two decades. Different HIV-testing technologies will perform differently in different settings. Selection of the most appropriate test should incorporate considerations regarding accuracy of the test, patient preferences, ease of sample collection, availability of trained personnel, availability of laboratory facilities, and prevalence of disease in the population that is being tested, among many other factors. In most cases, however, an EIA can be used as a screening test, followed by one of the many confirmatory tests. Nucleic-acid amplification should be used when the detection of acute HIV infection is necessary. □

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