Review Article
Laboratory Tests for HIV: Diagnosing, Monitoring and Managing AIDS - An Overview
Shibani Shetty, Sudeendra Prabhu, Kaveri Hallikeri, Rekha Krishnapillai

Abstract: The AIDS epidemic has already led to serious consequences for health care systems worldwide. The information attained by the laboratory tests is useful for prophylaxis, medical management, safety of blood, sentinel surveillance, to motivate behavioral modifications and to monitor trends of epidemic. This review highlights and updates various laboratory methods for diagnosing, monitoring and managing acquired immunodeficiency syndrome (AIDS) patients.

Key words: Immunologic tests; HIV infections; acquired immunodeficiency syndrome; HIV seropositive; HIV seroconversion; oral; body fluids.

Introduction
The Human Immunodeficiency Virus (HIV) was unknown until the early 1980's but since that time it has infected millions of persons worldwide. The result of HIV infection is relentless destruction of the immune system leading to onset of the Acquired Immunodeficiency Syndrome (AIDS). The AIDS epidemic has already resulted in the deaths of over half its victims. 1 The debate about the HIV antibody test had been long, complex and anguished. No single diagnostic test in the history of modern medicine has had such a momentous impact on the lives of the individuals who rely on it. Since the beginning of the AIDS crisis, people have had very dramatic responses to the test lapsing into severe chronic depression and anxiety. Given that the test holds such power, its flaws and shortcomings are extremely significant. Unfortunately, it is only now that this immensely important subject is being investigated. 2 The development of increasingly sophisticated virologic and immunologic techniques has further enhanced our ability to diagnose HIV-1 infection early and accurately. Despite the potential value of newer techniques, various modifications of the original solid phase serologic methods have remained the standard means by which most HIV-1 infections continue to be diagnosed in the United States and in many developed nations. 3 The ability to accurately determine viral and infected cell burden is essential in understanding the natural history of HIV infection, predicting disease progression and assessing the efficacy of various therapeutic drug regimens and vaccines 4. The present work is thus aimed at reviewing the laboratory methods used in the diagnosis of HIV infected patients.

Discussion
Once the HIV enters the human body via infected blood, semen, vaginal secretions etc, the virus immediately targets cells which display the viral receptors - CD4. These receptors are seen on lymphocytes (T helper cells and regulatory T cells) and other cells such as, monocytes, macrophages, and dendritic cells. The virus enters these cells, forms DNA, integrates into the host genome, multiplies and persists for many years. 5, 6 After a period of 3-12 weeks, the host mounts an immune response against the virus which is detected as antibody in the blood. This stage is called seroconversion. Current routine laboratory diagnosis of HIV is mainly based on the detection of these specific anti-HIV antibodies. The period following the entry of HIV into the body and the appearance of detectable levels of antibodies with the available tests is called the ‘window period’. During the window period, the patient is highly infectious but the antibody test is negative. This is also a worrying time for patients who have had an exposure and then want to be tested for HIV. 5, 7 Although many tests can be used to detect virus in general, specific tests has been described for detecting HIV-1 & 2 (Table 1). 3, 8

Serological Assays:
1. HIV -1 antibody screening assays:
   a) Enzyme Linked Immunoasorbant Assay [ELISA]: The first enzyme immunoassay test to detect antibodies against HIV was introduced in 1985. ELISA is widely applied in immunodiagnosis and enzyme linked antibody is the reagent...
Table 1: Diagnostic and monitoring tests for HIV employed to detect antibody-antigen reactions in the assay. ELISA commercial kits are most often used as screening tests. These commercially available ELISA utilizes whole virus extract from infected cells or recombinant proteins of HIV-1 that are affixed to the plastic surface at the bottom of microtitre wells. These are the binding assays that depend upon the antigen antibody reactions as the basis and enzyme reaction as a marker for the proof of reaction. A wide variety of assay principles that can be used in ELISA techniques are as follows (Table 2).

### Type 1
- Indirect or antiglobulin

### Type 2
- Competitive assay

### Type 3
- Class specific capture assay

### Type 4
- Double antigen sandwich or Immunometric assay

Table 2: Principles used in ELISA technique.

### Advantages: Sensitivity is > 99.5 %, useful in screening large number of blood samples as it is automated. Easy to perform, cost effective, and specific. Disadvantages: Shows high incidence of false positive reaction when used to screen people at low risk of infection.3, 9–15

### b) Home access HIV-1 test system/dried blood spot: In May 1996, the FDA approved two products for home sample collection for HIV testing. The kits are marketed directly to consumers, who perform a finger stick to obtain a dried blood specimen on filter paper. Dried blood spots is particularly useful under field conditions, where large numbers of samples can be relatively easily be collected, stored and transported for testing (Fig 1).

Figure 1: Dried blood specimen on filter paper and mailing procedure.

**Advantages:** It is rapid, inexpensive, simple, does not require use of organic solvents or extraction procedures. Collection of sample is simplified as it requires only a small amount of blood; sample processing time is reduced, and also gives high sensitivity and specificity.

**c) Rapid tests:** Rapid HIV tests can play an important role in HIV prevention activities and expand access to testing in both clinical and nonclinical settings. Rapid HIV formats include:

- **A.** *Agglutination tests* use different types of particles to produce clumping or settling patterns of the particles when a specimen is positive.
  
  i. **Autologous red cell agglutination** method detects HIV antibodies with a hybrid antigen antibody reagent which agglutinates the particles in red blood cells.
  
  ii. **Latex particle agglutination** detects HIV antibodies by the agglutination of minute latex particles when mixed with the patient’s blood.

- **B.** *Flow through cassettes* or *membrane immuno concentration devices*, capture and detect HIV antibody in a specimen flowing through a porous membrane. A visible dot or line forms on the membrane when HIV antibodies are present.

- **C.** *Solid phase tests* include dipstick “comb” assay. This assay uses a solid plastic matrix to which an HIV antigen is fixed. When HIV antibodies are present, a
spot or dot will be visible when processed with a signal reagent.

**D. “Immunochromatographic strip (ICS)” tests utilize a one-step method in which the patient’s blood specimen is combined with a signal reagent and migrates through a special membrane. A positive reaction is seen as development of a line or the membrane.**

Four rapid HIV antibody tests are now available which are approved by US Food and drug administration (FDA). They are: 1. Oraquick rapid HIV-1/2 antibody tests (Fig 2) 2. Reveal rapid HIV-1 antibody tests 3. Uni-Gold recombinigen HIV test 4. Multispot HIV-1/HIV-2 rapid tests.\(^{19,20}\)

**Figure 2: Oraquick Advance Kit**

1. Oraquick is mainly used in: Labour and delivery, ambulatory clinical sites, emergency departments, hospital inpatient services, occupational exposures and, in military battlefield operations

**d) Rapid latex agglutination assay:** Latex agglutination is a direct assay for antibodies. It is a procedure that can be performed within a matter of few minutes and that requires minimum of reagent and technical skills. This is a modification of standard latex agglutination that is based on the use of recombinant proteins derived from a highly conserved region of HIV-1 genome that are chemically linked to polystyrene beads. **Advantages:** Shows high sensitivity and high specificity. It is simple, rapid, requires minimum of reagents and technical skills.\(^{3,21,22}\)

**e) Dot blot assay:** Dot immunobinding assays is a rapid screening test that was developed as an alternative to conventional ELISA and western blot testing because of its simplicity, rapidity, cost effectiveness, sensitivity and specificity. In this type of assay, a lysate of viral antigens is prepared from HIV-1, harvested from cell culture and is dotted onto a grid of absorbent nitrocellulose paper. Appropriate dilutions of test sera (with panel of positive and negative controls) are spotted onto the areas containing viral antigens and allowed to react. The dots are developed by the addition of an appropriate substrate for the bound enzyme, resulting in a colorimetric reaction. HIV TRI DOT is most commonly used commercially available rapid visual test for the qualitative detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. **Advantages:** It is easy to perform, and also sensitivity and specificity is equivalent to ELISA.\(^{23,24}\)

2) HIV-1 antibody confirmatory antibody assays:

Confirmatory tests must be highly specific to ensure that individuals who test reactive in screening assays are correctly identified as being HIV infected (Fig 3).

**Figure 3: Algorithm for the use of ELISA & western blot in the diagnosis of HIV-1 or HIV-2 infection.**\(^{15}\)

**a) Western blot:** A more specific assay for the presence of HIV antibodies in serum, and is the commonly used confirmatory test. A conventional HIV western blot is an immunoblot preparation consisting of a crude lysate of HIV-1 /HIV-2 obtained from tissue culture, partially purified by differential centrifugation after cell lysis, separated by molecular weight into individual viral proteins through gel electrophoresis, and then electrophoretically transferred onto nitrocellulose paper. In accordance with their electrophoretic migratory pattern larger proteins are at the top of the strip and smallest proteins at the bottom. A western blot for HIV-1 contains HIV-1 envelope proteins (gp 160, gp120, gp 41), gag core proteins (p55, p24, p17), polymerase chain proteins (p66, p51,p31). HIV-2 western blots are similar but differ slightly in terms of molecular size of the three gene products. If antibodies to any of these proteins are present in serum, they bind to the immobilized HIV protein on the strip.

An enzyme and substrate are added to generate a colorimetric reaction and
produce a colored band on the strip representing antigen antibody complex. The spectrum of bands present is used as the interpretive criterion for the test (Table 3). If no colored bands present, the western blot is interpreted as negative (Fig 4).

![Western blot interpretation](image)

**Table 3: Interpretive criteria for a positive HIV-1 western blot test**

<table>
<thead>
<tr>
<th>ORGANIZATION</th>
<th>CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ASTPHLD/CDC [Association of State and Territorial Public Health Laboratory Directors / Centres For Disease Control and prevention]</td>
<td>Any two: p24-gp41, gp120/160</td>
</tr>
<tr>
<td>2) DuPont</td>
<td>p24+p31+gp41 Or gp120/160</td>
</tr>
<tr>
<td>3) ARC [American Red Cross]</td>
<td>Three or more bands, one from each gene product Group: gag, pol, env</td>
</tr>
<tr>
<td>4) CRSS [Consortium for retroviruses Serological standardization]</td>
<td>Two or more bands: p24 or p31 and gp41 or gp120/160</td>
</tr>
<tr>
<td>5) WHO [World Health Organization]</td>
<td>Two env bands with or without gag or pol</td>
</tr>
</tbody>
</table>

**Advantages:** It shows sensitivity of about 96%, is highly specific and defines the antibody profile to specific viral gene products. **Disadvantages:** It is an inappropriate initial screening test for HIV, expensive, labour intensive and needs expertise to interpret. It may give unequivocal / Indeterminate result and is time consuming (so not used as screening tests).

b) **Indirect immunofluorescence assay:**
Immunofluorescence assay (IF) is widely used for the rapid diagnosis of virus infections by the detection of virus antigen in clinical specimens, as well as the detection of virus specific IgG or IgA or IgM antibody. The technique makes use of a fluorescein labelled antibody to stain specimens containing specific virus antigens, so that the stained cells fluoresces under UV illumination. There are mainly two types of IF assay such as direct and indirect IF assays.

**Advantages:** Usually turns positive earlier in the course of infection than a conventional ELISA or western blot. **Disadvantages:** Unlike western blot, it does not permit precise delineation of specific patterns of antibody reactivity.

c) **Radio immunoprecipitation assay [RIPA]:** An alternative test that is sometimes used as a confirmatory assay over the conventional western blot. Here the infected lymphocyte cells such as H9-T cells are grown in presence of amino acids radiolabelled with 35S–methionine and 35S-cystine to permit incorporation of radiolabel into HIV-1 proteins. Some laboratories use surface labelling of HIV-1 with 125I after purification of virus from a host cell line.

**Advantages:** Detects early seroconversion and resolution of higher molecular weight envelope proteins is better than western blot. **Disadvantages:** Though it detects early seroconversion, it becomes less sensitive soon after infection.

d) **Line immunoassay:** In this assay, recombinant or synthetic peptide antigens are applied on a nitrocellulose strip rather than electrophorosed as in the western blot.

**Alternative Antibody Testing Technologies**
Noninvasive methods provide alternatives to diagnostic blood tests and have high patient acceptance, increased safety and reduced costs. Currently saliva, gingival crevicular fluid, oral mucosal transudates and urine are considered to be the alternatives to blood.

**a. Oral fluids:**
Whole saliva, glandular duct saliva or mucosal transudates are the specimens that can be collected for tests to detect antibody to HIV in oral secretions. The fluid most frequently employed for salivary diagnostic purposes is expectorated whole saliva, and oral mucosal transudates.

Several devices are commercially available for the collection of oral mucosal transudate specimens for the detection of HIV antibodies (Fig 5). These include:

- A. Salivette™
- B. Orapette™
- C. Omni-SAI™
- D. OraSure™

**Screening for HIV Antibody in Oral Fluids:** A number of different screening assays have been employed for the detection of HIV antibodies in oral fluids.
These include both conventional enzyme immunoassays (EIA) and rapid tests designed for use with serum or plasma samples, as well as an IgG antibody capture radioimmunoassay (GACRIA) and enzyme-linked immunosorbent assay (GACELISA) optimized for the detection of HIV antibody in specimens that contain low concentrations of immunoglobulin.

**Modifications for oral fluids:** The oral-fluid Vironostika HIV-1 Microelisa system has been licensed for use with the associated OraSure collection device. This EIA is identical to the serum-based Vironostika HIV-1 assay, except that the procedure has been modified by decreasing the sample dilution from 1 to 75 for serum to 1 to 2 for oral fluids. Rapid and simple "point-of-care" tests which use techniques involving membrane capture or particle agglutination have also been used for the detection of HIV antibody in oral fluids.30-34

**b. Urine analysis:** A high prevalence of antibodies to the glycoproteins gp120 and gp160 in urine samples among seropositive specimens led to the proposition of detection of antibodies specific for these glycoproteins. Several studies have utilized IgG antibody capture particle adherence tests and have concluded that performance of urine antibody screens is similar to that of serum tests.31,35-37

**c. Antibodies against HIV in vaginal mucosa:** A study has shown that "high-risk seronegative" subjects had IgA in their genital mucosa, that it likely was produced locally, and that it probably served as a barrier to systemic HIV-1 infection. Belec et al 199431 studied 150 paired serum and vaginal secretions obtained from HIV-1 seronegative women. The results showed detection of IgG antibody among 2.5 % of the vaginal secretions. Antibodies in such specimens were broadly reactive with HIV-1 core and env antigens.31

**Viral Identification Assays:**

1) **Polymerase Chain Reaction [PCR]:**
Polymerase chain reaction (PCR) has emerged as one of the most powerful tools for the amplification of genes and their RNA transcripts (Fig 6).

![Figure 6: Basic steps for a single cycle of PCR.43](image)

**Advantages:** Sensitivity and specificity is about 97% to 98%. **Disadvantages:** Highly subjective to false positivity by means of contamination.4,5,12,14,38

2) **Virus culture:** The qualitative culture enables one to isolate HIV and may be used to confirm infection in an individual with equivocal serologic studies, or in infants born to seropositive mothers. The quantitative assay enables one to determine the number of infected cells or titer of infectious virus in a given sample by evaluating serial dilutions of infected peripheral blood mononuclear cells (PBMC) or plasma.

Useful in several clinical settings such as:
- Characterization of isolates of virus mainly with regard to antiviral sensitivities
- Stratification of populations of patients
- Evaluation of the efficacy of experimental antiretroviral agents39,40

3) **p24 antigen capture assay:** This antigen can be detected relatively early after HIV-1 exposure in many patients and detection often precedes the process of seroconversion by several weeks. This rise in measurable p24 antigen correlates with the burst in viral replication, detectable by other methods such as plasma viremia which occurs shortly after primary infection.41,42

**Monitoring tests:**

**Lymphocyte analysis:** Lymphocyte subsets are usually quantitated in two related ways.
The first of these is as a percentage of the total T lymphocyte (CD3 bearing) population. This value is generated directly from the fluorescence activated cell sorter (FACS). The second measurement (more commonly used) of the CD4 subset is the total CD4 cell count, as an absolute number derived by multiplying the FACS’s percentage of CD4 cells by the total lymphocyte count. The CD4 T cell count is an important determinate of disease stage and prognosis in HIV infected individuals. Nonspecific markers such as CD4 cell count, is expressed either as an absolute value (normal adult range 600-1700 /mm³) or as a CD4:CD8 ratio (normal adult ratio 1.2:3.5). According to most guidelines followed, a CD4 T cell count < 350/µl is an indication of initiating antiretroviral therapy, and a decline in the CD4 T cell count of > 25% is an indication for considering change in therapy. Once the CD4 T cell count is < 200 /µl, patients should be placed on a regimen for pneumocystis carinii pneumonia prophylaxis and once count is < 50 /µl, primary prophylaxis for MAC (mycobacterium avium complex) infection is indicated. CD4 T cells (determined by flow cytometry) and total lymphocyte count (determined by the WBC count and differential percent) has been shown to correlate very well with the level of immunologic competence. Flow cytometry is an important method used to evaluate cell kinetics, in which large numbers of cells can be analyzed rapidly, providing distribution profile of several thousand cells at one time.\(^{33,44,45}\)

**Other surrogate markers:** 12, 46

The following are few surrogate markers which help in assessing the progress of the disease. They are as follows

- \(\beta_2\) – Microglobulin levels
- Neopterin levels
- Interleukin-2 receptor levels
- Titres of antibody to p24 antigen
- CD8 cell counts
- Serum IgA levels
- Total serum immunoglobulins
- Packed red cell volume
- Erythrocyte sedimentation rate
- Acid labile endogenous interferon levels
- Soluble CD16 levels
- Tumour necrosis factor levels
- gp120 antigen levels
- Anti gp120 antibody levels

**Viral load assay:** Currently various viral load assays are available. They target different regions of the HIV genome such as the gag and pol genes, and use of a variety of technologies, such as

- Reverse transcriptase polymerase chain reaction (RT-PCR) analysis with colorimetric detection (Roche),
- DNA hybridization and branched DNA signal amplification (Bayer),
- Nucleic acid sequence based assay,
- Colorimetric detection (Organon/bioMerieux).\(^{47,48}\)

**HIV-2 tests:** The antigens of HIV-2 are similar to those of HIV-1, but the molecular weights may vary slightly. The gag proteins of HIV-2 have designations of p56, p26, and p16. The designations of pol proteins are p68 and p34 and the envelope glycoproteins gp36 (or gp41), gp140, and gp105. As with HIV-1 screening tests, a variety of test formats are available to detect antibodies to HIV-2, including ELISA beads, ELISA microtitre, and rapid/simple assays. Commercially available HIV-1/2 “combination tests,” which incorporate antigens from both viruses, can be used to screen sera in an attempt to identify either infection.

HIV-2 confirmatory tests include the Western blot and the RIPA. In addition, EIA tests and some rapid tests that use chemically synthesized peptides corresponding to a unique immunogenic region within the respective transmembrane glycoproteins exhibit good correlation with the Western blot and the RIPA for identifying and differentiating HIV-1 and HIV-2 antibodies. Furthermore, these tests are valuable for differentiating samples that produce reactions to both viruses (dual reactors). For HIV-2 confirmation the WHO requires reactivity to at least 2 HIV-2 envelope antigens, whereas other organizations require reactivity to p26 (gag) and to gp34 or gp105 (Env). To conclude, if a specimen is tested by both HIV-1 and HIV-2 Western blot, the blot exhibiting the strongest reactivity to envelope antigens usually indicates which infection is present.\(^{49}\)

**Laboratory diagnosis of HIV in infants:**

Laboratory diagnosis of HIV infection in infants is complicated by the fact that all infants born to infected women are seropositive due to passively acquired maternal antibodies, irrespective of their infection status. Sensitive diagnostic tests designed to detect small amounts of HIV antibodies, give positive results in uninfected infants for 12 to 15 months, till they
seroconvert. Therefore, a definitive diagnosis using traditional ELISA and Western Blot can be made only after 18 months of age when uninfected infants will lose all maternal antibodies and infected infants will develop their own HIV specific antibodies.

Several approaches have been used for early diagnosis of HIV infection in newborns. They are as follows:  
- Viral culture
- HIV-1 p24 antigen detection
- HIV-1 DNA detection
- HIV-1 RNA detection
- Enzyme linked immunospot assay (ELISPOT) and In vitro antibody production (IVAP)
- HIV specific IgA
- IgG capture EIA

**Conclusion**

Various confirmatory tests such as western blot test, indirect immunofluorescence assay, radio immunoprecipitation assay and assays using recombinant antigens are available. Among these supplemental tests, the western blot is the most informative and it is the current “gold standard” for confirmation of HIV serological assays. CD4 cell enumeration and HIV-1 antigen capture assay are useful in predicting the course of HIV-1 infection and in monitoring anti retroviral therapies. Non-invasive tests using saliva, GCF, mucosal transudates and urine has been tried for the diagnostic purposes as an alternative to serological tests. But it requires further research for the improvement and standardization of these alternative techniques. Clinicians should be aware of the different tests to diagnose HIV infection and their optimal use. Diagnostic testing protocols for HIV-1 should include tests that complement each other. When choosing an HIV test, the sensitivity, specificity, and cost of the test, the prevalence of HIV infection in the community, the return rates for test results, HIV risk of the patient being tested and the patient’s personal preferences should all be considered. To conclude, we advocate continued development of new technologies that will increase the objectivity, specificity and sensitivity of tests used for the diagnosis of HIV infections.

**Authors Affiliations:** 1. Dr. Shibani Shetty, Senior Lecturer, MR Ambedkar Dental College and Hospital, Bangalore, 2. Dr. Sudeendra Prabhu, Reader, 3. Dr. Kaveri Hallikeri, Professor, S.D.M. College of Dental sciences and Hospital, Dharwad, Karnataka, 4. Dr. Rekha Krishnapillai, Associate Professor, Dental Faculty, Garyounis University, Benghazi, Libya.

**Acknowledgement:** All Staff in the Department of Oral Pathology, S.D.M. College of Dental sciences and Hospital, Dharwad, Karnataka.

**References**


Corresponding Author:
Dr. Sudeendra Prabhu
Reader,
Department of Oral & Maxillofacial Pathology,
S.D.M. College of Dental Sciences & Hospital,
Dharwad - 580009 Karnataka, INDIA.
Mobil No: 08886970917
E-mail: drsudi78@yahoo.co.in

Source of Support: Nil, Conflict of Interest: None Declared