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Chapter

Streamlining Laboratory Tests for HIV Detection

Ramakrishna Prakash and Mysore Krishnamurthy Yashaswini

Abstract

HIV is a retrovirus that primarily infects CD4 presenting cells of the human immune system, such as macrophages and dendritic cells. People die of AIDS because the disease remains undetected for long periods of time. HIV diagnostic testing has come a long way since it was introduced in the early 1980s. Early diagnosis is key to successful treatment of HIV. Assay selection is based on initial screening results and clinical information provided by the physician, both of which are essential for the laboratory’s ability to make accurate diagnoses. Detecting HIV with high specificity and sensitivity in the early stages of infection requires simple, accurate and economical methods. In this chapter we have described the indications & criteria’s for HIV testing, HIV diagnosis by utilizing variety of immunological and molecular methods, like ELISA, rapid diagnostics, Western blotting, indirect immunoassays, and nucleic acid-based tests. Diagnostic laboratories must use testing algorithms to ensure the accuracy of results and the optimal use of lab resources. Participation in laboratory quality assurance programs are also essential to ensure that diagnostic laboratories provide accurate, timely and clinically relevant test results. HIV testing is the first step in maintaining a healthy life and preventing HIV transmission.

Keywords: generations, HIV antibody test, HIV diagnosis, HIV testing algorithm, quality assurance, Western Blot

1. Introduction

Early detection and diagnosis is key to ending the HIV/AIDs pandemic by 2030. The need for timely and quality programs to enhance rapid, timely, accurate and relevant tests as the initial step in reducing HIV and maintaining the quality of life is a fundamental process. This chapter describes the known tests for HIV Infection. The author bases his discussion on significant systematic review of literature of HIV diagnostic tests. The aim is to explain and provide rationale for the use of tests available. The chapter also describes, and discusses the various indications, criteria for HIV testing, detection and identifies phases and stages of laboratory detection. A variety of immunological and molecular methods are outlined and discussed. These include the simplest from point of care such as ELISA diagnostic tests, rapid tests and brands. It also extends the discussion to more advanced tests including indirect immunoassays, nucleic based assays and the Western blot confirmatory tests. The laboratory algorithms are discussed to promote quality assurance in practice. The
paper discusses the difficulties encountered during the early window period of infection and suggests appropriate detection tools. The staging and the dynamics of HIV viremia post infection and its implications for detection is discussed. The classifications of tests from first generation to forth generation is described and recommendations made on their appropriate usage for early and sustained quality in detection of infection. The challenges of detection during the acute and the window period post infection is discussed and suggestions made. Establishing several testing stages is discussed to support quality HIV detection and ideal screening and confirmatory tests for each stage are recommended. The review has a potential benefit to improve HIV [1, 2].

2. Indications for HIV testing

HIV testing should be considered in the following situations. The healthcare team should be aware of the screening recommendations [3].

- All the patients in the age group 13 and above.
- Patients with risky sexual behavior
- Occupational exposure of patients or healthcare workers
- Before providing pre-exposure prophylaxis
- Signs and symptoms suggestive of HIV
- Patients sharing needles for substance abuse
- Pregnant women

3. Criteria’s for HIV testing

There are ample of tests which can detect HIV starting from the point of care testing to confirmatory test. The test algorithm to be followed has been released by the Centre for Disease Control (CDC) and Association of Public Health Laboratories (APHL) as well as the national organizations in every country [4, 5].

3.1 Clinical laboratory improvement amendments (CLIA)

Centers for Medicare and Medicaid Services (CMS) has regulated for all the clinical laboratory testing to be done through the CLIA. As per this amendments, a three-level test complexity criteria has been established for HIV testing procedures. The criteria are as follows [4, 5]:

- **Waived:** These are the tests which are simple to perform with low-risk, it can be performed by any person with minimal training and the specimens do not require any centrifugation for testing.
• **Moderate Complexity**: These are the tests which are simple to perform but requires the use of plasma or serum samples as well as participation in an external quality assessment or proficiency testing program.

• **High Complexity**: These are the tests which need multiple steps to be performed as well as well-trained laboratory technician to perform the test, it also needs the participation in an external quality assessment or proficiency testing program and internal quality control regularly.

### 3.2 Fiebig staging system

The Fiebig staging system (2003) defines six stages on initial HIV infection. Stage I is defined as the emergence of HIV RNA and Stage VI is defined as full Western blot reactivity. The markers which appear as per timeline after HIV infection are HIV RNA after 10 to 11 days, p24 antigen after 4 to 10 days after emergence of HIV RNA, IgM antibodies after 3 to 5 days later, IgG antibodies after 2 to 6 weeks after HIV RNA emergence [6].

### 4. Tests used for the diagnosis of HIV

HIV tests were classified as first, second, third and fourth generation tests based on the substrate used for testing. First generation HIV antibody tests were developed using separate HTLV III and lymphadenopathy virus (LAV) isolates proteins isolated from virus-infected tissue cultures as antigenic targets. Initially window period was up to 12 weeks or more post-infection. These assays detected only IgG antibody of HIV-1. Second generation HIV test were based on the recombinant antigens for HIV-1 p24. The window period was up to 4 to 6 weeks post-infection. Third generation HIV test can detect IgM antibody in addition to second generation tests. Fourth generation tests is a test which can detect both HIV-specific antigen p24 and HIV antibodies. This test reduced window period to approximately 2 weeks [7]. Fifth generation HIV test detects both HIV-specific antigen p24 and HIV antibodies with increased sensitivity in detection of p24 and it also identifies the individual HIV1 and HIV2 markers. The different generations of HIV tests are shown in Table 1.

<table>
<thead>
<tr>
<th>Assay progression</th>
<th>Indirect ELISA (HIV-1/2)</th>
<th>Sandwich ELISA HIV-1/2, IgG &amp; IgM</th>
<th>Sandwich ELISA HIV-1/2, IgG &amp; IgM + P24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generations</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Source of Antigen</td>
<td>Virus Infected Cell Lysate</td>
<td>Lysate &amp; Recombinant &amp; Synthetic peptides</td>
<td>Recombinant &amp; Synthetic peptides</td>
</tr>
<tr>
<td>Window period</td>
<td>8–10 weeks</td>
<td>4–6 weeks</td>
<td>2–3 weeks</td>
</tr>
</tbody>
</table>

*Source: Alexander [8].

Table 1. Different generations of HIV tests.*
With the invention of new HIV tests, the distinction between the different generations of ELISA test has been obscure. So the generation nomenclature is being modified as:

- IgG- sensitive tests for first and second generation antibody assays.
- IgM/IgG-sensitive tests for third generation assays
- Antigen-antibody immunoassays for fourth generation assays [5, 9–11].
- Laboratory-based assays and point-of-care assays are being used now instead of rapid HIV tests [10, 12].

4.1 Non-specific tests

The non-specific tests for HIV diagnosis are [13]:

- **Total and differential leucocyte count**: Lymphocyte count can decrease may be up to less than 400 per cubic mm with leucopenia.
- **T-lymphocyte subset assays**: Reversal of CD4:CD8 T-cell ratio up to around 0.5:1 from the normal ratio of 2:1.
- **Platelet count**: Thrombocytopenia will be seen in full blown HIV patients.
- **IgG and IgA levels**: Both levels will be raised in blood.
- **Skin tests for CMI**: Cell mediated immunity (CMI) will be diminished which can be evidenced by any skin allergy test.

4.2 Specific tests for HIV infection

These are the tests which are specifically done for testing of HIV.

4.2.1 Virus isolation

This is a time-consuming procedure which is not routinely done. The viruses are present in the lymphocytes in the peripheral blood and also seen in lymphocytes in bone marrow, plasma and other body fluids. The procedure used to cultivate HIV virus is called as Cocultivation. In this both infected and noninfected mononuclear cells will be co-cultivated. The culture may become positive for HIV p24 antigen and HIV reverse transcriptase by 7–14 days or by 28 days. This test will be useful when the viral load is high especially in the initial stage of the disease [14].

4.2.2 Serologic tests

These tests include demonstration of antigens and antibodies in the serum. The tests have been classified as:

1. HIV antigen-antibody laboratory-based tests.
2. HIV antigen-antibody point-of-care tests.

3. HIV antibody laboratory-based tests.

4. HIV antibody point-of-care tests.

5. HIV 1 and 2 differentiation tests.

6. HIV-1 Western Blot test.

7. HIV Nucleic acid diagnostic tests.

8. In-home HIV tests.

4.2.2.1 HIV antigen-antibody laboratory-based tests

These immunoassay tests are the preferred screening tests which detect HIV-1 p24 (capsid) antigen and antibodies (IgM and IgG) to HIV-1 and HIV-2. (Figure 1A–C) These antigen-antibody tests detect HIV infection much earlier than the antibody-based tests. If found positive in these tests, then it may require a confirmatory test. Limitation of these tests are cross-reactivity to HIV-1 p24 antigen seen in HIV-2 infected persons. Examples are ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay, ARCHITECT HIV Ag/Ab Combo, BioPlex 2200 HIV Ag-Ab Assay, Elecsys HIV Combi PT, GS HIV Combo Ag/Ab EIA, & VITROS HIV Combo Test [10, 16].

4.2.2.2 HIV antigen-antibody point-of-care tests

This assay is a single use, rapid test which is a point-of-care test for the detection of HIV-1 p24 antigen, antibodies to HIV-1 (group 0), and antibodies to HIV-2. This test does not differentiate HIV-1 and HIV-2 antibodies. This test is less sensitive for acute or recent HIV infection when compared to laboratory-based HIV-1/2 antigen-antibody tests. Example: Abbott Determine HIV-1/2 Ag/Ab Combo [17–19].

4.2.2.3 HIV antibody laboratory based tests

Laboratory-based HIV antibody tests were the first to be used for screening HIV since 20 years which has been replace by HIV antigen-antibody tests. These tests can detect the IgM/IgG-sensitive assays for HIV IgM antibodies in 23–25 days after HIV infection. Window period is around 90 days. The positive result in this tests would require an confirmatory test. Examples are: ADVIA Centaur HIV 1/O/2 Enhanced, Avioq HIV-1 Microelisa System, Genetic Systems (GS) HIV-1/HIV-2 Plus O EIA, VITROS Anti-HIV 1 + 2 Assay [5, 6, 10, 12].

4.2.2.4 HIV antibody point-of-care tests

Single-use, point-of-care tests can yield result in 40 min. These tests can detect antibodies to HIV-1 or HIV-2 or both but they will not be able to differentiate between HIV-1 and HIV-2. These tests are primarily used for testing (1) emergency situations (2) pregnant women whose HIV status in not known (3) occupational, and (4) in patients for whom follow-up for HIV result will not be possible. Examples are: Chembio DPP HIV 1/2 Assay, Chembio HIV 1/2 STAT-PAK Assay, Chembio SURE
4.2.2.5 HIV 1 and 2 differentiation tests

These tests will be able to differentiate between HIV-1 and HIV-2. These tests utilize multiple recombinant or synthetic peptides to detect HIV-1 antibodies and HIV-2...
antibodies. These immunochromatographic tests will contain 7 lines which consists of 6 HIV peptides and one control. A minimum of 2 envelope peptides (gp160 and gp41) or 1 envelope peptide plus either the p24 or the polymerase peptide p31 for HIV-1 reactive or HIV-2 envelope peptides gp36 and gp140 should be present for HIV-2 reactive test. (Figure 2) Example: Geenius HIV 1/2 Supplemental Assay [15, 22].

The Geenius HIV 1/2 Supplemental Assay is a single-use immunochromatographic test that utilizes multiple recombinant or synthetic peptides to detect HIV-1 antibodies (p31, gp160, p24, and gp41) and HIV-2 antibodies (gp36 and gp140). The test cassette as shown here contains seven test lines, including the six HIV peptides and one control.

4.2.2.6 HIV-1 Western blot test

Western blot test is used as supplemental tests for those tests which are reactive by rapid tests. It can detect the human antibodies for three HIV-1 gene regions: env (gp41, gp120/160), pol (p31, p51, p66), and gag (p15, p17, p24, p55) (Figure 3A-D). This graphic shows the relationship of the HIV-1 genes and products with the corresponding band on the HIV-1 Western blot.

CDC and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) have published the criteria for interpretation of Western blot tests [23].

Positive: A positive Western blot indicates the presence of at least two of the following bands: p24, gp41, and gp120/160.

Negative: A negative Western blot is defined by the absence of any bands.

Indeterminate: An indeterminate Western blot results from the presence of any bands, but not meeting positive criteria. Possible causes of an indeterminate Western blot include early HIV infection, HIV-2, pregnancy, or cross-reactivity with other antibodies, such as in persons who have recently received an influenza immunization or who have autoimmune disorder.
4.2.2.7 HIV nucleic acid diagnostic tests

HIV RNA nucleic acid test (NAT) is used in case of 1. Reactive HIV-1/2 antigen-antibody immunoassay but a nonreactive or indeterminate HIV-1/HIV-2 differentia-
tion test, 2. HIV-1/2 antigen-antibody immunoassay is negative but there is high
suspicion of acute HIV, and 3. Confirmative test for chronic HIV-1 infection. The
limitation of these tests are cost, time taken to perform the test is 3 hours and the
expert is required to perform the test. Example: APTIMA HIV-1 RNA Qualitative
Assay [9, 24–26].

4.2.2.8 In-home HIV tests

This test can be performed at home by the client itself within 40 minutes by simply
collecting an oral sample and performing the test as per kit literature. A confirmatory
test will be required if this test is reactive. Example OraQuick In-Home HIV test [27].

5. HIV laboratory testing algorithms

There are several algorithms published for HIV laboratory testing among which
CDC, NACO (National AIDS Control Organization) and APHL are some of them
(Figure 4) [28].

This graphic shows the HIV testing algorithm as recommended in 2014 and 2018
by the Centers for Disease Control and Prevention (CDC) and Association of Public
Health Laboratories (APHL). Source: Centers for Disease Control and Prevention and
Association of Public Health Laboratories [4, 5].
6. Interpretation of HIV test results

- If any HIV-1/2 antigen-antibody immunoassay test is NONREACTIVE, then the test result should be interpreted as not infected with HIV-1 or HIV-2. If acute HIV is suspected, then there will be a need to perform HIV-1 RNA test.

- If any HIV-1/2 antigen-antibody immunoassay test is REACTIVE, then the test should be checked with HIV-1/HIV-2 differentiation assay result to check for whether the person is reactive to HIV-1 or HIV-2.

- If any HIV-1/2 antigen-antibody immunoassay is reactive and HIV-1/ HIV-2 differentiation test is indeterminate for HIV-1 and nonreactive for HIV-2, then it is indeterminate result. HIV-1 NAT should be done in this case.

7. Staging of HIV and tests recommended

Days following HIV acquisition which of the HIV diagnostic tests can show positivity for infection are shown in Figure 5 [14, 29].

This graphic shows the HIV testing algorithm as recommended in 2014 and 2018 by the Centers for Disease Control and Prevention (CDC) and Association of Public Health Laboratories (APHL).

The stages of HIV infection and the tests that are recommended are [5, 30].

- Eclipse Phase: This is the first phase of HIV infection during which no diagnostic test will be able to detect HIV infection. HIV nucleic acid test (NAT) is the test which can detect HIV infection at the earliest.

- Window Period: The time between HIV infection and the accurate detection of HIV infection by any laboratory test. This period can vary depending the type of test done to detect HIV infection. CDC has recommended around 45 days
window period for the HIV 1/2 antigen–antibody tests and 90 days for all HIV antibody tests and all HIV point-of-care tests.

- Seroconversion Window Period: The time interval between HIV infection and the detection of anti-HIV antibodies by any laboratory test. This period also can vary depending on the type of HIV test used.

- Acute HIV infection: The time interval between the detection of HIV RNA and anti-HIV antibodies.

- Recent Infection: The time interval from the HIV infection to 6 months of infection when anti-HIV antibodies are rising.

- Early Infection: The time interval which includes both acute HIV infection and recent HIV infection.

- Established HIV Infection: The full-blown HIV infection when the anti-HIV IgG antibody response is fully detectable.

8. Performance of diagnostic tests

8.1 An ideal screening tests

An ideal screening test should be able to accurately identify individuals with the HIV infection and rule out infection in individuals without HIV infection.

The characteristics that define a screening test are [31]

- The disease should be a health problem.
The disease should be treatable.

The disease should be diagnosable.

The disease should have a test for diagnosis.

The test should be acceptable.

The test should cost-effective.

8.2 Sensitivity and specificity

Sensitivity and specificity refer to the diagnostic ability of a given test. Sensitivity refers to the percentage of individuals who are correctly identified as having disease if they are infected with HIV. A very high sensitivity is desirable for the initial screening test so that if we get a non-reactive result we can be 100% sure that the person is not having HIV infection [32]. Specificity refers to the percentage of individuals who are correctly identified as not having disease if the person does not have HIV infection. A very high specificity is desirable for the confirmation test as a reactive result means the person is suffering from HIV infection [33].

8.3 Positive predictive value and negative predictive value

The predictive value of a test refers to the accuracy of the test. Positive predictive value refers to the proportion of patients who are correctly diagnosed as reactive. Negative predictive value refers to the proportion of patients who are correctly diagnosed as non-reactive [32].

8.4 False negative and false positive HIV test

False negative HIV test result refers to the non-reactive report in a person who is possessing HIV infection.

A false negative HIV antigen–antibody test result can be seen in [34–46]:

- Common causes
  - acute HIV infection,
  - from error in laboratory reporting,
  - person on antiretroviral therapy,

- Rare causes
  - Immunosuppression.
  - Hypogammaglobulinemia.
  - Immunosuppressant medications.
  - Chronic HIV.
A false negative p24 antigen test can be seen in the window period and in chronic HIV. A false negative HIV RNA tests can be seen in first one to two weeks after HIV infection and chronic HIV.

False positive HIV test result refers to the reactive report in a person who is not possessing HIV infection.

A false positive HIV test result can be seen in [47, 48]

- Polyclonal cross-reactivity
- Recent Influenza vaccination
- Autoimmune disorders
- Trial HIV-1 vaccination
- Gammaglobulin therapy
- Prior blood transfusions
- HTLV-1/2 infection
- Recent viral infection
- Collagen vascular diseases
- Laboratory error in reporting

A false positive HIV NATs can be seen in persons receiving chimeric antigen receptor (CAR) T-cell therapy.

9. Special diagnostic situation

9.1 Diagnosis of Acute HIV-1

HIV RNA is the most reliable test for diagnosis of acute HIV-1 infection as this test can detect HIV in about 17 days after HIV infection which is much earlier when compared to all other methods of testing [49, 50].

9.2 Diagnosing HIV in persons receiving preexposure prophylaxis

Diagnosis of HIV infection in persons receiving preexposure prophylaxis is difficult due to delayed seroconversion, indeterminate results in HIV differentiation tests, and low viraemia [51].

9.3 Diagnosing HIV in HIV exposed infants and children

Antibody tests or antigen-antibody immunoassays will not be useful in diagnosis of HIV in infants or children as they may have maternal HIV antibodies. Nucleic acid tests like HIV RNA, HIV DNA polymerase chain reaction or RNA qualitative or
quantitative tests will be better option for HIV diagnosis in infants. Qualitative HIV proviral DNA PCR assays detects cell-associated virus as they are less affected by the antiretroviral drugs [52].

9.4 Diagnosis of HIV-2

Diagnosis of HIV-2 should be done using a HIV-1/2 antigen-antibody immunoassay followed by HIV-1/HIV-2 differentiation test. Confirmation of HIV-2 can be done by HIV-2 DNA/RNA Qualitative and Quantitative assays. Western blot can give a negative, indeterminate or positive HIV-1 result in HIV-2 infected individuals. Western blot will be indeterminate with the presence of gag and pol bands but the env bands will be absent in HIV-2 infection [53–57].

10. Laboratory quality assurance

The laboratory should participate in quality assurance program to ensure the quality of reports. The quality control should be monitored in preanalytical, analytical and post-analytical stages with Internal QC (quality control), external QC as well as test kit controls [58].

11. Conclusions

Testing an individual having HIV infection is important using the appropriate test at the appropriate time. Differentiation of HIV-1 and HIV-2 can be done using the differentiation assays. Diagnosis of HIV-2 and infection in infants and children requires Nucleic acid tests. Quality assurance needs to be maintained in all the labs which do HIV testing as the entire process has to be done in an appropriate manner to get the perfect results.

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Conflict of interest

“The authors declare no conflict of interest.”
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