HIV Diagnostic Testing

This is a PDF version of the following document:
Section 1:Screening and Diagnosis
Topic 3:HIV Diagnostic Testing

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Background

HIV Testing and the Care Continuum

HIV diagnostic testing is the crucial first step in the HIV care continuum.[1] Establishing a diagnosis of HIV has important implications for both HIV treatment and prevention. Accumulating evidence shows that persons living with HIV who take antiretroviral therapy without interruption have better clinical outcomes and reduced HIV transmission.[2,3,4,5,6] Based on Centers for Disease Control and Prevention (CDC) estimates for 2016 in the United States, persons living with HIV who were unaware of their HIV diagnosis accounted for an estimated 37.6% of all new HIV transmissions during that year.[7] The highest HIV transmission rates occur among persons with acute HIV infection who are unaware of their diagnosis.[7,8,9,10,11]

Approach to HIV Testing in the United States

In 2014, the CDC and the Association of Public Health Laboratories (APHL) released an HIV diagnostic algorithm to allow for more accurate diagnosis of acute HIV-1, more diagnosis of HIV-2, fewer indeterminate results, and faster turnaround time for completion of the testing algorithm.[12] This HIV diagnostic algorithm, which was updated in 2018, is discussed in detail below in the section CDC HIV Testing Algorithm; the CDC recommended HIV testing approach consists of initial screening with an antigen-antibody test, with follow-up testing of reactive samples using an HIV-1/2 differentiation assay (which can also provide antibody confirmation).[12,13] Since none of the initial antibody or antigen-antibody screening tests are considered definitive for a diagnosis of HIV, confirmatory testing is always required. From a practical standpoint, the same patient blood sample can be used for the initial screening test and the HIV differentiation assay. When using point-of-care sampling, such as an oral swab or fingerstick blood sample, the confirmatory testing requires obtaining an additional sample.

Clinical Laboratory Improvement Amendments (CLIA) Criteria

With a range of HIV diagnostic tests now available, the testing process can occur in a wide range of clinical and nonclinical settings. Most HIV testing is performed in a laboratory setting and the time required to perform the tests varies significantly, but some laboratory tests can be performed in less than an hour. Several point-of-care, single-use rapid tests are now available that can be performed in clinical or nonclinical settings. In the United States, the Centers for Medicare and Medicaid Services (CMS) regulates all clinical laboratory testing through the Clinical Laboratory Improvement Amendments (CLIA). As part of this process, CLIA has established a three-level test complexity criteria and this applies to the different HIV testing procedures:

- **Waived:** These tests are considered simple to perform, low-risk, and can be performed with minimal training; specimens do not require centrifugation for testing.
- **Moderate Complexity**: Although these tests are considered simple to perform, the testing involves using plasma or serum specimens, and program participation in an external proficiency testing program.

- **High Complexity**: These tests involve multiple-step protocols and require trained laboratory personnel to perform, participation in an external proficiency testing program, and frequent checks on quality control.
Timing of Laboratory Markers following HIV Infection

Fiebig Staging System

Laboratory markers of HIV infection appear in a consistent sequence after the infection and delineate the period from initial exposure to established HIV infection. The Fiebig staging system, first published in 2003 defines 6 distinct stages of initial HIV infection, ranging from stage I (emergence of HIV RNA) to stage VI (full Western blot reactivity) (Figure 1).[14] Following HIV acquisition, HIV RNA is first detectable on standard laboratory tests approximately 10-11 days after infection.[14] The next marker to appear is p24 antigen, which typically reaches detectable levels 4 to 10 days after the emergence of HIV RNA. Next, IgM antibodies are detectable about 3 to 5 days later, and are gradually replaced by IgG antibodies, which appear 2 to 6 weeks after initial HIV RNA detection.[14]

Early HIV Test Reactivity and Terminology

The CDC and Association of Public Health Laboratories document outlines the sequence of when laboratory markers turn positive (Figure 2) and the document defines the following laboratory-based and clinically relevant terms related to the timing of laboratory markers of HIV infection becoming positive after acquisition of HIV.[12, 15, 16]

- **Eclipse Period**: The eclipse period is the initial interval after HIV infection when no existing diagnostic test is capable of detecting HIV (Figure 3). The HIV nucleic acid test (NAT) is the first test that can detect HIV following HIV acquisition.
- **Window Period**: The window period is most often defined as the time between acquisition of HIV and the time when the laboratory test will accurately detect HIV infection in almost all persons. The window period depends on the diagnostic test used. From a practical standpoint, the window period is used as the time period to rule out HIV infection after a potential exposure. The CDC recommends using 45 days as the window period for laboratory-based HIV-1/2 antigen-antibody assays, and 90 days after all HIV antibody tests and all HIV point-of-care tests.
- **Seroconversion Window Period**: The seroconversion window period specifically refers to the interval between HIV infection and the first detection of anti-HIV antibodies; the seroconversion window varies depending on the different HIV antibody assay used.
- **Acute HIV Infection**: The interval between detectable HIV RNA and the detection of anti-HIV antibodies. During acute HIV infection, the HIV RNA is always detectable and the HIV p24 antigen is usually positive.
- **Recent Infection**: This term describes the phase after acute HIV infection out to 6 months when anti-HIV antibodies are developing.
- **Early Infection**: The time period after infection out to 6 months that includes both acute and recent HIV infection.
- **Established HIV Infection**: This term refers to the time after early HIV infection in which anti-HIV IgG antibody responses have fully developed.
Tests Used for the Diagnosis of HIV

Updated Nomenclature for HIV Diagnostic Tests

In the United States during the early years of HIV testing, the FDA-approved HIV antibody tests were categorized as first-, second-, and third-generation tests, based on evolving techniques and significant improvement in assay sensitivity.[17,18] The first-generation assays detect immunoglobulin G (IgG) antibodies using crude viral lysates, the second-generation assays detect IgG antibodies using recombinant (synthetic) antigens or proteins, and the third-generation assays detect IgG and IgM with the antigen-antibody sandwich technique (Figure 4).[12,17,19] The first- and second-generation antibody assays are now more commonly referred to as IgG-sensitive tests and the third-generation assays as IgM/IgG-sensitive tests.[18] The availability of HIV-1/2 antigen-antibody immunoassays, which were previously referred to as fourth-generation assays, enabled earlier detection of HIV than prior HIV antibody testing methods.[18] More recently, experts have noted that with many of the newer HIV assays, a clear distinction between the HIV generations has blurred and use of the HIV test “generation” nomenclature is no longer recommended.[20] Instead, the current emphasis is on describing the type of test as either laboratory-based or point-of-care.[18,20] Further, use of the term rapid HIV test to describe point-of-care tests is no longer a distinguishing category since a number of newer instrumented, laboratory-based tests have the capacity to generate HIV test results in less than one hour. The following summarizes the major FDA-approved HIV diagnostic tests.[21]

HIV Antigen-Antibody Laboratory-Based Tests

The HIV antigen-antibody laboratory-based immunoassays detect HIV-1 p24 antigen and antibodies (IgM and IgG) to HIV-1 and HIV-2 (Figure 5).[12,18,22] These assays, formerly referred to as fourth-generation HIV tests, can detect HIV infection significantly earlier than laboratory-based antibody tests, point-of-care antigen-antibody tests, and point-of-care HIV antibody tests.[18,23] Data from a CDC study evaluated reactivity of HIV tests in specimens from HIV-1 seroconverters and found the HIV-1/2 antigen-antibody immunoassay was positive in 50% of persons at 17.8 days and in 99% at 44.3 days (Figure 6).[23] Accordingly, the CDC considers laboratory-based HIV-1/2 antigen-antibody immunoassays to have a window period of 45 days.[20] All reactive HIV-1/2 antigen-antibody tests require confirmatory testing. None of the HIV-1/2 immunoassays can detect HIV-2 core antigen (p26 antigen), but cross-reactivity to HIV-1 p24 antigen can occur in persons infected with HIV-2. Six laboratory-based HIV-1/2 antigen-antibody immunoassays are FDA-approved for use in the United States.

- **ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay**: This two-wash antigen-antibody sandwich immunoassay detects HIV-1 p24 antigen, antibodies to HIV-1 (group M), and antibodies to HIV-2.[24] This assay does not differentiate between p24 antigen and HIV antibodies. The ADVIA Centaur HIV Ag/Ab Combo Assay was evaluated in more than 7,000 samples and found to have a sensitivity of 98.4% and specificity of 99.7%.[25] This test is a chemiluminescent microparticle immunoassay (CMIA) and it requires less than 1 hour to perform.

- **ARCHITECT HIV Ag/Ab Combo**: This laboratory assay can detect HIV-1 p24 antigen, antibodies to HIV-1 (groups O and M), and antibodies to HIV-2.[26] This test does not distinguish antigen from antibody, nor does it distinguish HIV-1 from HIV-2. The Architect HIV Ag/Ab Combo assay has been evaluated in several studies and has demonstrated detection rates over 99% for established HIV infection and detection rates from 61% to 83% for acute HIV (with specificity above 98% in both established and acute HIV infection).[27,28] The ARCHITECT HIV Ag/Ab Combo is a CMIA and it takes less than 30 minutes to perform.

- **BioPlex 2200 HIV Ag-Ab Assay**: This laboratory assay detects HIV-1 p24 antigen, antibodies to HIV-1 (groups O and M), and antibodies to HIV-2.[29] This test can differentiate between HIV-1 p24 antigen, antibodies to HIV-1 antibodies, and antibodies to HIV-2 and thus...
can help identify persons recently infected with HIV-1. Investigators prospectively tested the BioPlex 2200 HIV Ag-Ab Assay on 1,505 routine serum samples and the assay had a sensitivity of 100% and specificity of 99.5%.[30] Additional testing on samples known to be positive for HIV-1 or HIV-2 and the differentiation capability of the assay for HIV-1, HIV-2, both HIV-1 and HIV-2, or early HIV infection was 100%, 90.7%, 100%, and 90.9%, respectively.[30] This test is a multiplex flow immunoassay and it takes 45 minutes to perform.

- **Elecsys HIV Combi PT**: This laboratory assay can detect HIV-1 p24 antigen, antibodies to HIV-1 (groups O and M), and antibodies to HIV-2.[31] A reactive test does not distinguish p24 antigen from antibodies to HIV and it does not distinguish HIV-1 from HIV-2. This test is an electrochemiluminescence immunoassay (ECLIA) and it takes 27 minutes to perform.

- **GS HIV Combo Ag/Ab EIA**: This laboratory assay can detect HIV-1 p24 antigen, antibodies to HIV-1 (groups O and M), and antibodies to HIV-2.[32] A reactive test does not distinguish p24 antigen from antibodies to HIV and it does not distinguish HIV-1 from HIV-2. In a performance evaluation, the GS HIV Combo Ag/Ab EIA was shown to be 100% sensitive in detecting established previously confirmed HIV infection and more than 85% sensitive in detecting acute HIV infection, with specificity above 99% in both groups.[33] This test uses an EIA microwell format and it takes at least 3 hours to perform.

- **VITROS HIV Combo Test**: This laboratory assay can detect HIV-1 p24 antigen, antibodies to HIV-1 (groups O and M), and antibodies to HIV-2; this assay is part of the VITROS 3600 Immunodiagnostic System.[34,35] A reactive test does not distinguish p24 antigen from antibodies to HIV and it does not distinguish HIV-1 from HIV-2. This assay is an immunometric 2-stage reaction and it takes 48 minutes to perform.

### HIV Antigen-Antibody Single-Use Point-of-Care Tests

In the United States, there is only one FDA-approved point-of-care HIV-1/2 antigen-antibody test for the diagnosis of HIV.

- **Alere Determine HIV-1/2 Ag/Ab Combo**: This assay is a point-of-care, single use, rapid test that can detect HIV-1 p24 antigen, antibodies to HIV-1 (group O), and antibodies to HIV-2.[36] The Alere Determine HIV-1/2 Ag/Ab Combo assay is the only FDA-approved point-of-care HIV-1/2 antigen-antibody test. This assay can differentiate HIV-1 p24 antigen from HIV antibody, but it does not differentiate HIV-1 and HIV-2 antibodies.[37] The sensitivity of this assay for acute or very recent HIV infection is less than with laboratory-based HIV-1/2 antigen-antibody assays.[38,39,40] With this assay, use of fingerstick whole blood specimens is not as sensitive as with plasma samples.[41] The Alere Determine HIV-1/2 Ag/Ab Combo is a lateral flow immunochromatographic assay that takes 20 minutes to perform.

### HIV Antibody Laboratory-Based Tests

The HIV enzyme immunoassay (EIA) antibody test for HIV diagnosis was first licensed in the United States in 1985.[17] Since 1985, for almost 20 years, HIV antibody tests were widely used as the initial laboratory diagnosis test in the HIV testing algorithm. Since 2014, however, the use of HIV antibody tests as an initial screening test has been replaced by HIV antigen-antibody assays.[12,13] A positive HIV antibody test always requires further confirmatory HIV testing with another HIV assay.[12] The laboratory-based HIV antibody tests are based on color change or fluorescence, which is analyzed relative to a standard cutoff.[17] Most of the current used laboratory-based HIV antibody tests are IgM/IgG sensitive assays that can detect HIV IgM antibodies and these tests first turn positive at approximately 23-25 days after infection.[18] The CDC considers the HIV antibody tests to have a window period of 90 days (even the IgM/IgG sensitive assays); in this context a window period of 90 days means a negative HIV EIA test at 90 days after a possible HIV exposure rules out HIV acquisition from that exposure.[20] The following list of FDA-approved laboratory HIV antibody tests includes three IgM/IgG-sensitive antibody assays and one IgG-sensitive assay.
- **ADVIA Centaur HIV 1/O/2 Enhanced**: This laboratory IgM/IgG-sensitive antibody assay can detect antibodies to HIV-1 (groups M and O) and/or antibodies to HIV-2.\[42,43\] A reactive test does not distinguish infection with HIV-1 from HIV-2. The test is a chemiluminescent microparticle immunoassay (CMIA) that takes less than 1 hour to perform.

- **Avioq HIV-1 Microelisa System**: This laboratory IgG-sensitive antibody assay only detects antibodies to HIV-1 (groups M and O).\[44\] A reactive test does not distinguish infection with HIV-1 from HIV-2. The test is an EIA that uses whole viral lysate antigens (HIV-1) plus native gp160; this test takes at least 3 hours to perform.

- **Genetic Systems (GS) HIV-1/HIV-2 Plus O EIA**: This laboratory IgM/IgG-sensitive antibody assay can detect antibodies to HIV-1 (groups M and O) and/or antibodies to HIV-2.\[45\] A reactive test does not distinguish infection with HIV-1 from HIV-2. This test uses an EIA micro-well format and requires at least 3 hours to perform.

- **VITROS Anti-HIV 1+2 Assay**: This laboratory IgM/IgG-sensitive antibody assay can detect antibodies to HIV-1 (groups M and O) and/or antibodies to HIV-2.\[46\] A reactive test does not distinguish infection with HIV-1 from HIV-2. This test is a CMIA and it takes less than 1 hour to perform.

**HIV Antibody Single-Use Point-of-Care Tests**

Single use, point-of-care HIV test kits have self-contained testing reagents and materials and typically can yield a test result within 40 minutes.\[17,47,48\] There are 7 FDA-approved, rapid, point-of-care tests HIV antibody tests that the CDC identifies as suitable for use in clinical and nonclinical settings.\[47\] Six of these tests detect both HIV-1 and HIV-2 and one detects HIV-1 only. None of the currently used point-of-care antibody tests can differentiate HIV-1 from HIV-2 infection. Multiple reports have shown problems with false-negative and false-positive test results with the oral fluid point-of-care test rapid test. Single-use, point-of-care rapid antibody tests are less sensitive than the laboratory-based antigen-antibody tests for the detection of early HIV infection.\[49,50,51,52\] All positive point-of-care HIV test results are considered presumptive positives and require further supplemental testing.\[12\] The main situations where single-use, point-of-care testing is performed include (1) emergency room encounters where it is unlikely that individual tested will return for the results, (2) at hospitals for women in labor who had no HIV testing performed during their pregnancy, (3) occupational blood exposure for HIV when immediate results may be needed to determine whether to offer postexposure prophylaxis to a health care worker, and (4) in other clinical settings where a low likelihood of follow-up for HIV test results is anticipated.\[17,53,54\]

- **Chembio DPP HIV 1/2 Assay**: This IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.\[55\] This assay can be performed on samples of oral fluid, fingerstick whole blood, venous whole blood, serum, or plasma. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This immunochromatographic test utilizes the Dual Path Platform (DPP) and requires 10-15 minutes to perform with blood samples and 25-40 minutes for oral fluid.

- **Chembio HIV 1/2 STAT-PAK Assay**: This IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.\[56\] Tests can be performed using fingerstick whole blood, venous whole blood, serum, or plasma samples. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This immunochromatographic lateral-flow test requires 15 minutes to perform.

- **Chembio SURE CHECK HIV 1/2 Assay**: This IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.\[57\] This test is approved for use with fingerstick whole blood, venous whole blood, serum, or plasma samples. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This immunochromatographic lateral-flow test requires 15 minutes to perform.

- **INSTI HIV-1/HIV-2 Antibody Test**: This IgM/IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.\[58\] This test is approved for use with fingerstick whole blood, venipuncture whole blood, and plasma samples. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This flow-through test...
immunoassay requires only 2 minutes to perform.

- **OraQuick ADVANCE Rapid HIV-1/2 Antibody Test:** This IgM/IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.[59] This assay can be performed on samples of oral fluid, fingerstick whole blood, venous whole blood, or plasma. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This lateral flow immunoassay requires 20-40 minutes to perform.

- **Reveal G4 Rapid HIV-1 Antibody Test (Reveal G4):** This IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1.[60] The following samples are FDA-approved for testing: fingerstick whole blood, venipuncture whole blood, serum, and plasma. This vertical flow immunoassay requires less than 3 minutes to perform.

- **Uni-Gold Recombigen HIV-1/2:** This IgM/IgG-sensitive assay is a single use, point-of-care, rapid test that can detect antibodies to HIV-1 and/or HIV-2.[61] This test is indicated for use with fingerstick whole blood, venous whole blood, serum, or plasma samples. A reactive test does not differentiate HIV-1 antibodies from HIV-2 antibodies. This lateral-flow immunoassay requires 15-20 minutes to perform.

**HIV-1/2 Differentiation Assays**

Although several tests can distinguish HIV-1 from HIV-2, only two—the Geenius HIV-1/2 Supplemental Assay and the Multispot HIV-1/HIV-2 Test—have been approved by the FDA for use as HIV-1/HIV-2 differentiation assays. Currently, there is only one FDA-approved HIV-1/HIV-2 differentiation assay available, since the manufacturers of the Multispot withdrew this product from the market in July 2016 and replaced it with the instrumented, laboratory-based Geenius HIV-1/2 Supplemental Assay.[62] Differentiating HIV-1 and HIV-2 is important to avoid misclassification of HIV infection; studies have shown that the HIV-1 Western blot was erroneously interpreted as positive for HIV-1 in 46% to 85% of specimens from persons infected with HIV-2.[12]

- **Geenius HIV 1/2 Supplemental Assay:** The Geenius HIV 1/2 Supplemental Assay is a single-use immunochromatographic test that functions both as an HIV confirmatory test and an HIV-1 and HIV-2 differentiation assay (Figure 7).[63,64] This assay 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 contains 7 test lines, including the 6 HIV peptides and one control. A positive HIV-1 result requires at least 2 envelope peptides (gp160 and gp41) or 1 envelope peptide plus either the p24 or the polymerase peptide p31; a positive HIV-2 diagnosis requires reactivity to both HIV-2 envelope peptides gp36 and gp140.[63] Investigators have shown the Geenius HIV 1/2 Supplemental Assay is a reliable HIV confirmatory assay and this test is recommended by the CDC as the differentiation assay to use in the HIV testing algorithm.[62,65,66,67,68]

- **Multispot HIV-1/HIV-2 Rapid Test:** The Multispot HIV-1/HIV-2 Rapid test is now of historical significance only, since manufacturing for this HIV-1/HIV-2 differentiation assay was discontinued in July 2016.[69] Prior to July 2016, the Multispot HIV-1/HIV-2 Rapid Test had been widely used as the recommended HIV-1/HIV-2 differentiation assay.[12,70,71] The test requires fresh or frozen human serum or plasma and it can be completed in about 15 minutes. (Figure 8).

**HIV-1 Western Blot Laboratory Tests**

The HIV-1 Western blot separates HIV-1 proteins by gel electrophoresis, after which they are transferred to a paper-like membrane for analysis of a patient’s serum sample (Figure 9).[72] The HIV-1 Western blot has been largely replaced by more sensitive and specific HIV diagnostic tests. When used, the HIV-1 Western blot can detect human antibodies that react to HIV-1 proteins that originate from three HIV-1 gene regions: env (gp41, gp120/160), pol (p31, p51, p66), and gag (p15, p17, p24, p55) (Figure 10).[72] The HIV Western blot typically becomes positive after about 5 to 6 weeks following initial HIV infection; as more protein bands become detectable, the Western blot typically evolves from a pattern of negative to indeterminate to positive. In 1989, the CDC and the
Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) published criteria for interpretation of Western blot tests for HIV.\[72\] A positive Western blot indicates the presence of at least two of the following bands: p24, gp41, and gp120/160; a negative Western blot is defined by the absence of any bands; and 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 infection, pregnancy, or cross-reactivity with other antibodies, such as in persons who have recently received an influenza immunization or who have autoimmune disorder.

**In-Home HIV Testing**

In-home HIV testing typically refers to either performing the test in its entirety at home (OraQuick In-Home HIV Test) or collecting the test specimen at home and mailing it in for testing (Home Access HIV-1 Test System). Both of these commercially available tests provide individuals with an option for anonymous HIV testing. In-home testing or in-home specimen collection may be preferable for some persons who are reluctant to undergo HIV testing in medical settings, and it can be used by new sex partners for mutual testing prior to engaging in a sexual relationship.\[73,74\] Studies have shown that in-home testing is feasible and acceptable for persons undergoing testing,\[74,75,76,77,78\] though several concerns persist, including cost of the test, lack of appropriate counseling for a positive test result, access to confirmatory testing for a positive result, and low sensitivity for detecting recent HIV acquisition.

- **OraQuick In-Home HIV Test:** The OraQuick In-Home HIV Test is the only FDA-approved test for performing at home.\[79\] The test involves collecting an oral sample with a test device at home, placing the test device in a test kit vial that contains a developer solution, and then waiting 20 minutes to read the test result (the test must be read within 40 minutes). The client must read and interpret the test result; the OraQuick In Home HIV Test web site has printed and video "How-to" instructions. The in-home test costs approximately $40 and contains a full set of easy-to-follow instructions; in addition, the OraQuick web site has an online video on how to perform the test, information on understanding the test results, and phone numbers for a confidential Support Center that can answer customer questions in English and Spanish 24 hours a day and 7 days a week. As with all other rapid tests, a positive home HIV test result is considered a preliminary positive HIV test result and confirmatory HIV testing is required.

- **Home Access HIV-1 Test System:** The Home Access HIV-1 Test System is an anonymous HIV testing system that utilizes home collection of a serum specimen.\[80\] This Home access test involves multiple steps, including (1) calling a toll free number to register the Home Access test number anonymously and to receive pre-test counseling, (2) using a retractable safety lancet (provided in the kit) to self-collect a fingerstick blood sample at home, (3) shipping the sample in a prepaid shipping envelope to a manufacturer-designated accredited laboratory, and (4) calling a toll free number the next day and using the registration number to anonymously obtain test results and post-test counseling.

**HIV Nucleic Acid Diagnostic Laboratory Tests**

Qualitative HIV RNA nucleic acid testing (NAT) is used in three situations: (1) in the CDC/APHL HIV diagnostic algorithm to evaluate for possible acute infection when a specimen has a reactive HIV-1/2 antigen-antibody immunoassay, but a nonreactive or indeterminate HIV-1/HIV-2 differentiation assay, (2) when a high suspicion of acute HIV exists and the initial HIV-1/2 antigen-antibody immunoassay result is negative, and (3) to confirm chronic HIV-1 infection. Quantitative HIV RNA NAT is used routinely in clinical practice for monitoring the viral loads of patients who have already been diagnosed with HIV, and many commercially available tests are capable of detecting viremia as low as 20 copies/mL. Given the very low limit of detection of most HIV quantitative assays, many clinicians use quantitative HIV RNA tests ("viral load" tests) rather than qualitative tests for diagnostic purposes, since the quantitative tests are more widely available.\[17,81\] Despite the ability of HIV RNA tests to identify very early HIV infection, it is not used as a routine HIV screening
test due to cost and technical complexity.[17] In addition, approximately 0.5% of individuals who have chronic HIV infection and are not receiving antiretroviral therapy will have undetectable HIV RNA levels; these individuals are often referred to as elite controllers and would be misclassified as negative if HIV RNA were used as the sole screening test.[82] In the United States, there is currently only one HIV nucleic acid test that is FDA-approved for HIV diagnostic purposes.

- **APTIMA HIV-1 RNA Qualitative Assay**: This laboratory-based, instrumented nucleic acid test is the only FDA-approved NAT for the diagnosis of HIV-1 infection, including acute HIV-1 infection.[83] This assay can detect all major HIV-1 groups and subtypes and has a manufacturer reported detection rate of 98.5% for 30 copies/mL, 82.6% for 10 copies/mL, and 42.5% for 3 copies/mL.[84,85] The APTIMA HIV-1 RNA Qualitative Assay requires at least 3 hours to perform.
Laboratory HIV Testing Algorithm as Recommended by CDC/APHL

The CDC and APHL HIV testing algorithm, which was initially published in 2014 and then updated in 2018, allows for more accurate diagnosis of acute HIV-1, more accurate diagnosis of HIV-2, fewer indeterminate results (due to a shorter window period), and faster turnaround time than previous approaches (Figure 11). [12,13,62] Because no single test is capable of detecting HIV immediately following infection, some patients with very early HIV infection will escape detection with this test algorithm.[86]

Initial Testing

The recommended initial HIV test should be a laboratory-based HIV-1/2 antigen-antibody immunoassay that can detect antibodies to HIV-1, antibodies to HIV-2, and HIV-1 p24 antigen.[12,13] A positive HIV-1/2 antigen-antibody immunoassay requires confirmation and differentiation of HIV-1 from HIV-2 infection. A person with a negative initial HIV-1/2 antigen-antibody immunoassay is considered not infected with HIV infection, as long as a very recent (within 4 weeks) exposure to HIV has not occurred. If no recent exposure to HIV has occurred, further HIV testing is not required for evaluation of current HIV status. In situations where it is not feasible to perform an instrumented, laboratory-based initial HIV-1/2 antigen-antibody immunoassay, the rapid, point-of-care Determine HIV-1/2 Ag/Ab Combo test can be used with serum or plasma samples as the initial test in the HIV diagnostic laboratory algorithm. The Determine HIV-1/2 Ag/Ab Combo is not as sensitive as the laboratory-based HIV-1/2 antigen-antibody immunoassays for detecting HIV during acute infection.[87]

Differentiation Assay

If the initial screening HIV-1/2 antigen-antibody immunoassay is reactive, a second HIV test is needed to confirm the initial test and to differentiate whether the infection is caused by HIV-1, HIV-2, or both. For this purpose, the CDC algorithm recommends using an HIV-1/HIV-2 antibody differentiation assay. In the United States, only the Multispot HIV-1/HIV-2 Rapid Test and Geenius HIV 1/2 Supplemental Assay are FDA-approved for differentiating HIV-1 from HIV-2 infection.[62,65,88] Note that the Multispot is no longer manufactured. Samples that are reactive with the HIV-1/2 antigen-antibody immunoassay and the HIV differentiation assay are considered positive and should be classified as HIV-1, HIV-2, or HIV-1 and HIV-2.

- Specimens that are reactive on the initial HIV-1/2 antigen-antibody immunoassay but either indeterminate or nonreactive on the differentiation assay require further testing with an HIV-1 NAT to evaluate the possibility of acute HIV infection versus a false-positive HIV-1/2 antigen-antibody test. In this situation, if the NAT is positive for HIV-1, the person is likely to have acute HIV-1 infection.
- With the recent implementation of the Geenius HIV-1/2 differentiation assay, several test results now occur that did not occur with the previously used Multispot HIV-1/HIV-2 test as the HIV differentiation assay.[62]
- In the case of a reactive (“preliminary positive”) result from a rapid test, the specimen should be submitted for testing according to the full 2014 algorithm, beginning with the combination antigen-antibody immunoassay.[12]

HIV Nucleic Acid Testing

If the initial HIV-1/2 antigen-antibody immunoassay is positive, but the HIV-1/HIV-2 differentiation assay is negative further testing with an HIV-1 nucleic acid test should be performed. If both the HIV-1/2 antigen-antibody immunoassay and the HIV-1/HIV-2 differentiation assay are positive, then quantitative HIV RNA testing (viral load) is indicated—HIV-1 quantitative, HIV-1 RNA, or quantitative...
Interpretation of Test Results

- If the HIV-1/2 antigen-antibody immunoassay is nonreactive, then the interpretation is no infection with HIV-1 or HIV-2, unless the individual undergoing testing has acquired HIV within the past 30 days. If acute HIV is suspected, then perform an HIV-1 RNA test.
- If the HIV-1/2 antigen-antibody immunoassay is reactive and the HIV-1/HIV-2 differentiation assay result is reactive for HIV-1 and nonreactive for HIV-2, then conclude the patient has HIV-1 infection.
- If the HIV-1/2 antigen-antibody immunoassay is reactive and the HIV-1/HIV-2 differentiation assay result shows HIV-1 nonreactive and HIV-2 reactive, then conclude the patient has HIV-2 infection.
- If the HIV-1/2 antigen-antibody immunoassay is reactive and the HIV-1/HIV-2 differentiation assay shows HIV-1 indeterminate (or negative) in conjunction with a nonreactive HIV-2, then several possibilities exist. In this scenario, follow-up testing with HIV-1 NAT is indicated. If the HIV-1 NAT is positive, the patient has acute HIV-1 infection. If the HIV-1 NAT is negative, the most probable scenario is that the initial reactive immunoassay result was a false-positive result and the individual undergoing testing does not likely have HIV-1 or HIV-2 infection. Alternatively, in a person with risk factors for acquiring HIV-2, these test results could theoretically indicate acute HIV-2 infection. Follow-up testing with HIV-2 NAT should be considered. Since HIV-2 RNA is not detected in at least half of individuals infected with HIV-2, making a definitive diagnosis may require testing of HIV-2 proviral DNA.

CDC Recommended HIV Testing Approach Prior to 2014

The HIV diagnostic algorithm recommended by the CDC prior to 2014 consisted of an initial EIA test (optimized for sensitivity) followed by a supplemental (confirmatory) Western blot (optimized for specificity).

This algorithm has several shortcomings. First, the initial EIA test does not detect acute HIV infection until approximately 25 days after acquisition. Second, the traditional algorithm relied on Western blot for confirmation of HIV infection, but the Western blot has a turnaround time of several days and can produce false negative or indeterminate results, particularly during early HIV infection, all of which complicate management decisions. Third, this traditional HIV diagnostic testing algorithm does not adequately identify persons infected with HIV-2.
Performance of Diagnostic Tests

Characteristics of an IDEAL Screening Test

The principles that define a good screening test are not unique to HIV infection but apply to medical screening in general. An ideal screening test will accurately identify individuals with the clinical condition of interest, without mistakenly diagnosing individuals who do not have the condition. In addition, use of screening tests is most effective when limited to conditions for which there is available, effective treatment that can directly target the disease and improve prognosis and outcomes.[89]

Sensitivity and Specificity

In relation to HIV testing, sensitivity refers to the proportion of true positives (persons who are infected with HIV) that are correctly identified by a screening test (Figure 12).[90] In general, very high sensitivity is desired for initial HIV screening tests since the ideal goal of the screening test is to not miss detecting anyone who has HIV infection. Thus, if the test is 100% sensitive and the person tests negative, you can be confident the individual tested does not have the infection. All HIV antibody tests approved for use in the United States have a sensitivity greater than 98% in diagnosing persons with chronic HIV infection.[15] Specificity is the proportion of true negative persons who are not infected with HIV that are correctly identified as HIV-negative by a screening test (Figure 13).[91] If a test is 100% specific and the person tests positive, you can be confident they have the disease and the test is not a false-positive result. In the United States, initial HIV antibody tests have greater than 99% specificity for chronic HIV infection, and the specificity increases to nearly 100% when the initial test is combined with a supplemental HIV test. [66]

Positive Predictive Value and Negative Predictive Value

In contrast to sensitivity and specificity, which refer to the diagnostic ability of a screening test, the predictive value of a test refers to the likelihood that the test will give the correct diagnosis.[90] Positive predictive value is the proportion of patients with a positive HIV result who are correctly diagnosed (i.e. who actually have HIV disease). Negative predictive value is the proportion of patients with negative HIV results who are correctly diagnosed (i.e. who are actually negative for HIV disease).[90] Because screening tests are neither 100% sensitive nor 100% specific, the predictive value of tests is also imperfect. It is possible for persons to receive an incorrect result from a diagnostic test: these results are termed false negative and false positive test results. It is important to understand that the prevalence of a disease in a community impacts the predictive value of a given test, and predictive values in one study or in one community do not apply to all other settings.[90]

False-Negative HIV Tests

A false-negative HIV test result refers to a negative HIV test result in a person who actually has HIV infection(Figure 14). A false-negative HIV antibody test result most often occurs when performing antibody testing in a person with acute HIV infection or from laboratory error. In addition, rare causes of false-negative results include (1) testing in persons who have defects in HIV-specific immunity and thus fail to generate certain antibodies,[92,93,94,95], (2) following receipt of potent antiretroviral therapy very early after HIV acquisition,[96,97] (3) in patients with hypogammaglobulinemia,[98] and (4) after administration of potent immunosuppressant medications.[99] In adults with chronic HIV infection, the loss of HIV antibody (seroreversion) is exceedingly rare.[100] A false-negative p24 antigen test can occur in the first several weeks after HIV acquisition (usually positive by day 17); in addition, many persons with untreated chronic HIV infection do not have persistently detectable p24 antigen levels, often due to p24 antigen complexing with p24 antibody. False-negative HIV RNA tests can occur in the first week or two after
HIV acquisition (typically positive by day 10) and in persons chronically infected with HIV who have inherently strong immunologic control of HIV and thus may have undetectable HIV RNA levels in the absence of antiretroviral therapy.

**False-Positive HIV Tests**

A false-positive HIV test result is defined as a positive HIV test result in a person who does not have HIV infection (Figure 15). A false-positive HIV test may occur due to polyclonal cross-reactivity, which is more common in the setting of pregnancy, recent inoculation with influenza vaccine, autoimmune disorders, receipt of an investigational HIV-1 vaccine, receipt of gamma globulin, prior blood transfusions, HTLV-1/2 infection, recent incident viral infection, collagen vascular diseases, and laboratory errors.\[101\] When trying to determine whether a patient's HIV screening test result is accurate, the pretest probability—the likelihood before the test was performed that the patient has HIV infection—can help with interpretation; for example, a person who injects drugs and shares needles has a higher pretest probability of having HIV than an asymptomatic pregnant woman who tested negative in her two previous pregnancies and has had no change in sexual partners. Further, the likelihood of an accurate HIV test result correlates directly with the prevalence of HIV in the testing community: the proportion of false-positive tests is higher in populations with low HIV prevalence (even if the screening test is highly sensitive and specific), whereas the proportion of false-negative tests is lower.\[90\]
Special Diagnostic Situations

Diagnosis of Acute HIV-1 Infection

The laboratory diagnosis of acute HIV-1 infection is most reliably made with a positive HIV RNA (or HIV-1 p24 antigen) with a concomitant negative HIV antibody assay; note that with very early acute HIV infection, the p24 antigen assay may be negative (Figure 16).\[102,103,104\] Use of HIV-1/2 antigen-antibody immunoassays will detect HIV at about 17 days after HIV infection, which is significantly sooner than with HIV laboratory-based HIV antibody tests, all point-of-care HIV tests, and all home HIV tests.\[16,86,105,106\] Even when using HIV-1/2 antigen-antibody immunoassays, the initial laboratory testing will fail to detect some individuals who have very early acute HIV infection. Thus, for individuals in whom initial HIV-1/2 antigen-antibody testing is nonreactive, but acute HIV infection is strongly suspected, HIV NAT (i.e. HIV RNA testing) should be performed. Increased awareness of acute retroviral syndrome by medical providers can help facilitate diagnosis in the early stages of infection. Among persons recently infected with HIV, it is estimated that at least half develop a nonspecific syndrome characterized by fever, myalgia, lymphadenopathy, pharyngitis, fatigue, headache, and rash.\[107,108,109\] Because HIV RNA levels are typically very high in persons with acute retroviral syndrome, an HIV NAT is uniformly positive at this stage of infection.

Diagnosing HIV In Persons Receiving Preexposure Prophylaxis

The diagnostic accuracy and timing of early HIV infection in patients who acquire HIV while taking preexposure prophylaxis with tenofovir DF-emtricitabine may result in atypical laboratory patterns, such as delayed seroconversion, indeterminate results on HIV differentiation assays, and low-level viremia in the setting of acute or early infection.\[110\] In particular, data from the Partners PrEP Study and the Bangkok Tenofovir Study showed persons receiving PrEP who acquired HIV had marked delays in HIV seroconversion with point-of-care tests, especially when using oral fluid samples.\[111,112\] Taken together, the findings from these studies have led to the recommendation to use laboratory-based HIV tests when monitoring persons receiving PrEP.\[18\]

Diagnosis of HIV Infection in HIV-Exposed Infants and Children

The 2014 and 2018 CDC HIV diagnostic algorithm does not address the diagnosis of infants and children exposed to HIV.\[12,13\] To diagnose HIV infection among infants younger than 18 months of age, the Pediatric ARV Guidelines recommend using a virologic assay (HIV nucleic acid testing, or NAT) that directly detects HIV RNA or HIV DNA (either quantitative or qualitative tests can be used). Virologic diagnostic testing should be considered at birth for infants considered high risk for acquiring HIV and all infants with perinatal HIV exposure should have virologic diagnostic testing following birth at 14-21 days, 1-2 months (preferably 2-4 weeks after cessation of antiretroviral therapy), and at 4-6 months).\[113\] In the United States, the Aptima HIV-1 RNA Qualitative Assay is the only FDA-approved qualitative test for HIV diagnosis; the previously used Amplicor HIV-DNA test is no longer commercially available.\[113\] Use of HIV p24 antigen testing is not recommended in this setting because of the lower sensitivity and specificity in the first months of life when compared with virologic tests such as HIV nucleic acid testing. Maternal and/or neonatal receipt of antiretroviral prophylaxis may decrease both HIV RNA and HIV DNA levels in the infant with HIV infection during the first 6 weeks of life and thus may compromise the sensitivity of HIV nucleic acid tests if performed during the postexposure prophylaxis period and likely for about 2 weeks after stopping prophylaxis.\[113,114,115\] Serologic tests are generally not useful in confirming a diagnosis of HIV in infants less than 18 months of age because maternal anti-HIV antibodies are passively transferred to the infant and persist for 12-18 months. In contrast, a negative HIV antibody test after month 12 can be used as an indicator to support the absence of HIV infection.

Diagnosis of HIV-2 Infection
The 2014 and 2018 HIV diagnostic algorithm improves the detection of HIV-2 by using an HIV-1/HIV-2 differentiation assay as the second step of the algorithm (following the initial HIV-1/2 antigen-antibody immunoassay); the Western blot is eliminated from the new testing sequence algorithm.\cite{12,13} Confirmation of HIV-2 infection can be challenging since HIV-1 RNA assays do not reliably detect or quantitate HIV-2. More recently, quantitative HIV-2 RNA assays have become available through the University of Washington Department of Laboratory Medicine (HIV-2 DNA/RNA Qualitative and HIV-2 RNA Quantitation) and the New York State Department of Health (HIV-2 Nucleic Acid Testing).\cite{116} It is important to note, however, that a significant percentage of individuals with HIV-2 infection can have undetectable HIV-2 RNA levels. Therefore, in certain epidemiological settings (e.g. a person with risk factor for acquiring HIV-2), a positive screening HIV-1/2 antigen-antibody test followed by a positive HIV-2 antibody on the differentiation assay should be considered HIV-2 positive even if plasma HIV-2 RNA is undetectable with an HIV-2 RNA assay. Prior to 2014, the diagnosis of HIV-2 was often missed or delayed due to improper classification as HIV-1.\cite{117} This occurred because an HIV Western blot was used as a second confirmatory test instead of an HIV-1/2 differentiation assay and HIV-2 infection may cause a negative, indeterminate, or positive HIV-1 Western blot due to cross-reacting antibodies.\cite{12,117,118} With HIV-2 infection, the HIV-1 Western blot pattern is often indeterminate, with the presence of gag bands (p55, p24, or p17) and pol bands (p66, p51, or p31), but absence of env bands (gp160, gp120, or gp41), because HIV-1 and HIV-2 share little similarity in the env gene (Figure 18).\cite{119}
Delivering Test Results

Follow-Up for Test Results

In the past decade, the percentage of people newly identified with HIV infection who received their test results has risen substantially. Data from CDC-funded HIV testing sites in 2013 showed 91-97% of persons who underwent HIV testing received their HIV test results.[120] Innovative methods of HIV testing and HIV test result delivery are helping increase these numbers. In particular, delivery of HIV test results by telephone has been found to be both effective and acceptable to patients, and in multiple studies was shown to increase the numbers of persons who received their test results.[121,122] From 2004-2006, the CDC examined the feasibility of HIV testing in outreach and community settings, including in bathhouses, needle exchange programs, public parks, bars, and shelters; in these settings, 1.1% of persons had a positive confirmatory test and 75% of those newly diagnosed received results of their confirmatory HIV testing.[123] Although not captured by the CDC-funded testing site data, the availability of home testing has likely also increased the proportion of persons who undergo HIV testing and receive their results; persons with positive results require confirmatory testing.[76]

Communicating Test Results

The CDC offers practical advice for providers who offer HIV testing in their practice settings.[124,125] Providers should be prepared to deliver results to patients in a private area and in a direct, neutral tone. The person delivering the test results should be knowledgeable about HIV, since patients may have questions about HIV infection, transmission to partners, and disclosure. Importantly, patients who receive a positive HIV test result should be linked to HIV care prior to leaving the testing setting, and have a scheduled appointment with an HIV provider. For patients who test negative for HIV, the provider should be prepared to provide HIV prevention counseling to help the patient remain HIV negative, including discussion of and referral for preexposure prophylaxis (PrEP) when indicated.
Summary Points

- Laboratory markers of HIV infection (HIV RNA, p24 antigen, anti-HIV IgM antibody, anti-HIV IgG antibody) appear in a consistent sequence and are the basis for all of the HIV diagnostic tests.
- In 2014, the Centers for Disease Control and Prevention (CDC) and the American Public Health Laboratories (APHL) jointly published new HIV diagnostic testing guidelines.
- The CDC HIV testing algorithm recommends initial testing with an HIV-1/2 antigen-antibody immunoassay, followed (for samples that are reactive on the antigen-antibody test) by an HIV-1/2 differentiation assay. Testing for HIV RNA should be done in cases where the initial test is reactive, but the differentiation assay is either nonreactive or indeterminate.
- Compared to previous screening algorithms, the current algorithm is more likely to detect acute HIV-1 infection, more accurately diagnoses HIV-2 infection, allows for faster turnaround time, and leads to fewer indeterminate results.
- An ideal screening test is sensitive, specific, and limited to conditions for which there is available, effective treatment that can directly target the disease and improve prognosis and outcomes.
- False-negative HIV screening test results can occur during acute HIV infection and false-positive HIV screening test results may occur due to lab errors, and rarely, cross-reactivity with other antibodies.
- Testing for HIV RNA may identify very early HIV infection (HIV RNA tests may be positive up to a week sooner than the antigen-antibody tests), but HIV RNA is not generally used for screening due to the cost and technical complexity of the test.
- Single-use, point-of-care HIV tests and home HIV tests are additional options to help facilitate HIV screening and detection. A reactive result on a point-of-care or home test should be considered as a presumptive positive and requires further testing.
- Diagnosis of acute HIV infection, infants and children exposed to HIV infection, and persons receiving PrEP may present diagnostic challenges and may require consultation.


21. Centers for Disease Control and Prevention. Advantages and disadvantages of FDA-approved HIV immunoassays used for screening by generation and platform. [CDC] -


24. U.S. Food and Drug Administration. ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay. [U.S. FDA] -


26. U.S. Food and Drug Administration. Abbott ARCHITECT HIV Ag/Ab Combo. [U.S. FDA] -


32. U.S. Food and Drug Administration. GS HIV Combo Ag/Ab EIA. [U.S. FDA]


36. U.S. Food and Drug Administration. Alere Determine HIV-1/2 Ag/Ab Combo. [U.S. FDA]


43. U.S. Food and Drug Administration. ADVIA Centaur HIV 1/O/2 Enhanced ReadyPack Reagents. [U.S. FDA] -

44. U.S. Food and Drug Administration. Avioq HIV-1 Microelisa System. [U.S. FDA] -


46. U.S. Food and Drug Administration. VITROS Immunodiagnostics Products Anti-HIV 1+2 Calibrator VITROS Immunodiagnostics Products Anti-HIV 1+2 Reagent Pack. [U.S. FDA] -


64. U.S. Food and Drug Administration. Geenius HIV 1/2 Supplemental Assay. [U.S. FDA]


79. U.S. Food and Drug Administration. OraQuick In-Home HIV Test. [U.S. FDA] -

80. U.S. Food and Drug Administration. Home Access HIV-1 Test System. [U.S. FDA] -


83. U.S. Food and Drug Administration. APTIMA HIV-1 RNA Qualitative Assay [U.S. FDA] -


87. Centers for Disease Control and Prevention (CDC). Technical Update: Use of the Determine HIV 1/2 Ag/Ab Combo Test with Serum or Plasma in the Laboratory Algorithm for HIV Diagnosis. October 4, 2017. [CDC] -


125. Centers for Disease Control and Prevention. Act against AIDS—testing and linking minority patients to care: Delivering test results. [CDC] -

References


Figures

Figure 1 (Image Series) - Fiebig Classification for Early HIV-1 Infection (Image Series) -
Figure 1 (Image Series) - Fiebig Classification for Early HIV-1 Infection

Image 1A: Characteristics of Fiebig Stages

Source: Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody
seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration</th>
<th>HIV RNA</th>
<th>p24 Ag</th>
<th>*EIA</th>
<th>Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclipse</td>
<td>11 days</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>1</td>
<td>5.0 days</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>5.3 days</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>3.2 days</td>
<td>(+)</td>
<td>(+)</td>
<td>IgM Positive</td>
<td>Absence of HIV-specific bands</td>
</tr>
<tr>
<td>4</td>
<td>5.6 days</td>
<td>(+)</td>
<td>(+)</td>
<td>IgM Positive</td>
<td>#Indeterminate pattern</td>
</tr>
<tr>
<td>5</td>
<td>88.6 days</td>
<td>(+)</td>
<td>(+/-)</td>
<td>Positive</td>
<td>Reactive, but absence of p31 (pol)</td>
</tr>
<tr>
<td>6</td>
<td>Open-ended</td>
<td>(+)</td>
<td>(+/-)</td>
<td>Positive</td>
<td>Reactive, including p31 (pol)</td>
</tr>
</tbody>
</table>

* EIA = enzyme immunoassay
# Indeterminate Western blot: presence of HIV-1 specific bands that fail to meet criteria established by
US FDA for positive HIV (reactivity to two of the following three bands: p24, gp41, gp120/160)
Figure 1 (Image Series) - Fiebig Classification for Early HIV-1 Infection
Image 1B: Graphic Timeline of Fiebig Stages

Figure 2 Timing of Positivity for HIV Diagnostic Tests

This graphic shows estimates for the mean number of days for HIV diagnostic tests to become positive after acquisition of HIV. Abbreviation: POC = point-of-care

**Figure 3 HIV Eclipse Period**

The HIV eclipse period is the time after acquisition of HIV when no existing diagnostic test is capable of detecting HIV. The HIV nucleic acid test (NAT) is the first test that can detect HIV following HIV acquisition.

Illustration: David H. Spach, MD
Figure 4 First, Second, and Third-Generation HIV Antibody Tests

This illustration shows that first-generation HIV assays detect immunoglobulin G (IgG) antibodies using crude viral lysates, second-generation assays detect IgG antibodies using recombinant (synthetic) antigens or proteins, and third-generation assays detect IgG and IgM with the antigen-antibody sandwich technique. Note that the first and second-generation assays detect IgG antibodies and the third-generation assays detect IgM and IgG antibodies.

Illustration: David H. Spach, MD
The HIV-1/2 antigen-antibody immunoassay contain components that will detect HIV-1 p24 antigen, antibodies to HIV-1, and antibodies to HIV-2. The HIV-1 and HIV-2 recombinant proteins vary from assay to assay.

Illustration by David H. Spach, MD
Figure 5 (Image Series) - Principles for Laboratory-Based HIV-1/2 Antigen-Antibody Immunoassays
Image 5B: Patient Sample Reacting with Components in HIV-1/2 Antigen-Antibody Immunoassay

In this example, the patient sample contains HIV-1 p24 antigen and anti-HIV antibodies that bind to the HIV-1 p24 capture antibody and the HIV recombinant proteins.

Illustration: David H. Spach, MD
The HIV-1/2 antigen-antibody immunoassay will turn positive with the presence of one or more of the following: HIV-1 p24 antigen, antibodies to HIV-1, or antibodies to HIV-2. With most of the laboratory-based assays, the positive reaction is nonspecific and thus does not differentiate HIV-1 p24 antigen, antibodies to HIV-1, or antibodies to HIV-2. In addition, most of the assays will not determine whether more than one of these components are present in a positive reaction.

Illustration: David H. Spach, MD
**Figure 6 Timing of HIV-1/2 Antigen-Antibody Immunoassay Reactivity Following HIV Acquisition**

This graphic shows the time course for test HIV-1/2 antigen-antibody immunoassay positivity in 25%, 50%, 75% and 99% of persons following HIV acquisition. As shown, 99% of persons have a positive HIV-1/2 antigen-antibody test at 44.3 days after HIV acquisition. These data are from 222 longitudinally collected plasma specimens from HIV-1 seroconverters in the United States.

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.


<table>
<thead>
<tr>
<th>HIV-2 Bands</th>
<th>HIV-1 Bands</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp36</td>
<td>p31</td>
<td></td>
</tr>
<tr>
<td>gp140</td>
<td>gp160</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>gp24</td>
<td>gp41</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7 Geenius HIV 1/2 Supplemental Assay
Figure 8 (Image Series) - Multispot HIV-1/HIV-2 Rapid Test

Figure 8A: Components of the Multispot HIV-1/HIV-2 Rapid Test

Source: Centers for Disease Control and Prevention.
Figure 8 (Image Series) - Multispot HIV-1/HIV-2 Rapid Test

Image 8B: Interpretation of the Multispot HIV-1/HIV-2 Rapid Test

- **Nonreactive**

- **HIV-1 Reactive**

- **HIV-2 Reactive**

- **HIV Reactive (undifferentiated)**
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot

Image 9A: Components Used in the HIV-1 Western blot

Illustration: David H. Spach, MD

HIV Western blot Strip

- Color Reagent
- Enzyme Detector
- Antihuman IgG Antibodies
- Human HIV Antibodies (from patient serum)
- HIV Antigens (on Western blot)
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9B: Separation of HIV-1 Antigens with Gel Electrophoresis

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9C: Transfer of HIV-1 Antigens to Nitrocellulose Membrane

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9D: HIV-1 Antigens on Nitrocellulose Membrane

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9E: Generation of HIV-1 Western blot Test Strips

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9F: Analysis of Patient Serum Sample on HIV-1 Western blot Test Strips

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9G: HIV-1 Antibodies Bound to HIV-1 Antigens on Western blot Test Strip

Illustration: David H. Spach, MD

Human Serum  HIV Western blot Strip

Antibodies to gp120 and p24

gp160
gp120
p66
p55
p51

Antibodies to gp120

gp160
gp120
p66
p55
p51

Antibodies to p24

p31

p24

p17

p15
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9H: Addition of Secondary Anti-human Antibody Linked to Enzyme Signal

Illustration: David H. Spach, MD
Figure 9 (Image Series) - Process for Performing HIV-1 Western blot
Image 9I: Explanation of Components on Western blot

Illustration: David H. Spach, MD
Figure 10 HIV-1 Western blot

This graphic shows the relationship of the HIV-1 genes and products with the corresponding band on the HIV-1 Western blot.

<table>
<thead>
<tr>
<th>HIV-1 Gene and Product</th>
<th>Band on Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>env</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor Protein</td>
<td>gp160</td>
</tr>
<tr>
<td>External Glycoprotein</td>
<td>gp120</td>
</tr>
<tr>
<td>Transmembrane Protein</td>
<td>gp41</td>
</tr>
<tr>
<td><strong>pol</strong></td>
<td></td>
</tr>
<tr>
<td>Reverse Transcriptase</td>
<td>p66</td>
</tr>
<tr>
<td>Reverse Transcriptase</td>
<td>p51</td>
</tr>
<tr>
<td>Endonuclease</td>
<td>p31</td>
</tr>
<tr>
<td><strong>gag</strong></td>
<td></td>
</tr>
<tr>
<td>Gag Precursor</td>
<td>p55</td>
</tr>
<tr>
<td>Core</td>
<td>p24</td>
</tr>
<tr>
<td>Matrix</td>
<td>p17</td>
</tr>
<tr>
<td>Nucleocapsid Precursor</td>
<td>p15</td>
</tr>
</tbody>
</table>
Figure 11 CDC and APHL Recommended Laboratory Testing for the Diagnosis of HIV Infection

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).

Figure 12 (Image Series) - Sensitivity of HIV Diagnostic Test (Image Series) - Figure 12 (Image Series) - Sensitivity of HIV Diagnostic Test
Image 12A: Example of Sensitivity of HIV Diagnostic Test

Persons Infected with HIV: n = 50

HIV Antibody Testing: 49/50 Positive

Sensitivity: 49/50 = 98%
Figure 12 (Image Series) - Sensitivity of HIV Diagnostic Test
Image 12B: Sensitivity: Mathematical Expression

\[
\text{Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}
\]
Figure 13 (Image Series) - Specificity of HIV Diagnostic Test (Image Series) - Figure 13 (Image Series) - Specificity of HIV Diagnostic Test

Image 13A: Example of Specificity of HIV Diagnostic Test

Persons NOT Infected with HIV: n = 50

HIV Antibody Testing: 48/50 Positive

Specificity: 48/50 = 96%
Figure 13 (Image Series) - Specificity of HIV Diagnostic Test
Image 13B: Specificity: Mathematical Expression

Specificity = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}
Figure 14 (Image Series) - False-Negative HIV Diagnostic Test (Image Series) - Figure 14 (Image Series) - False-Negative HIV Diagnostic Test
Image 14A: Test Results for HIV-infected persons
Figure 14 (Image Series) - False-Negative HIV Diagnostic Test
Image 14B: False-Negative Identified
Figure 15 (Image Series) - False-Positive HIV Diagnostic Test (Image Series) - Figure 15 (Image Series) - False-Positive HIV Diagnostic Test
Image 15A: Test Results for HIV negative persons
Figure 15 (Image Series) - False-Positive HIV Diagnostic Test
Image 15B: False-Positive Identified
Figure 16 Laboratory Markers with Acute HIV Infection

Days following HIV Acquisition
Figure 17 Diagnostic Test Performance in Acute HIV Infection

With acute HIV infection, the typical pattern is a positive HIV RNA, positive HIV p24 antigen, and negative anti-HIV antibodies. Note that with very early acute HIV infection, the p24 antigen test may be negative.

Illustration: David H. Spach, MD
### Figure 18 HIV-1 and HIV-2 Gene Products, Proteins, and Glycoproteins

<table>
<thead>
<tr>
<th>Gene and Product</th>
<th>HIV-1</th>
<th>HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>env</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precursor Protein</td>
<td>gp160</td>
<td>gp140</td>
</tr>
<tr>
<td>External Glycoprotein</td>
<td>gp120</td>
<td>gp105/125</td>
</tr>
<tr>
<td>Transmembrane Protein</td>
<td>gp41</td>
<td>gp36/41</td>
</tr>
<tr>
<td><strong>pol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse Transcriptase</td>
<td>p66</td>
<td>p68</td>
</tr>
<tr>
<td>Reverse Transcriptase</td>
<td>p51</td>
<td>p53</td>
</tr>
<tr>
<td>Endonuclease</td>
<td>p31</td>
<td>p31/34</td>
</tr>
<tr>
<td><strong>gag</strong></td>
<td></td>
<td></td>
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<tr>
<td>Gag Precursor</td>
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<tr>
<td>Core</td>
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