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HIV Diagnostic Testing

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Module 1: Screening and Diagnosis
Lesson 3: HIV Diagnostic Testing

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Background

HIV Testing and the Care Continuum

Diagnostic testing is the first step in the HIV care continuum (Figure 1).[1] Establishing a diagnosis of HIV has important implications for both HIV treatment and prevention. Accumulating evidence shows that persons with HIV who take antiretroviral therapy and maintain suppressed plasma HIV RNA levels have good clinical outcomes and do not sexually transmit HIV to others.[2,3,4,5,6] Improving rates of HIV testing and awareness of HIV status is critical because a high proportion of HIV transmissions occur from persons unaware of their HIV diagnosis.[7] Persons with acute HIV who are unaware of their diagnosis have the highest HIV transmission rates.[7,8,9,10,11] The Centers for Disease Control and Prevention (CDC) estimated that in 2022, approximately 13% of people with HIV in the United States were unaware of their HIV diagnosis.[12] Universal testing is also important because individuals who test negative but have a risk of acquiring HIV can be offered HIV preexposure prophylaxis (PrEP).

Approach to HIV Testing in the United States

The CDC and the Association of Public Health Laboratories (APHL) recommend using an HIV-1/2 antigen-antibody immunoassay as the intal screening test, with follow-up testing of reactive samples using an HIV-1/HIV-2 differentiation immunoassay. The latter test can differentiate HIV-1 from HIV-2 and provide antibody confirmation.[13,14] Indeterminate or ambiguous results based on the initial HIV-1/2 antigen-antibody test and HIV-1/HIV-2 differentiation assay require further evaluation with an HIV nucleic acid test (NAT), such as an HIV-1 RNA PCR assay (or rarely an HIV-2 PCR).[14] This diagnostic algorithm is discussed in detail below in the section Recommended HIV Testing Algorithm. A wide range of HIV diagnostic tests are now available, and the testing process can occur in both clinical and nonclinical settings. Although most HIV testing is performed in a laboratory setting, several point-of-care, single-use, rapid tests are now available that can be performed in clinical or nonclinical settings. When using point-of-care testing, such as an oral swab or fingerstick blood sample, reactive tests require confirmatory testing with laboratory-based HIV testing.

Timing of Laboratory Markers following HIV Infection

Fiebig Staging System

Laboratory markers appear in a consistent sequence after acquisition of HIV. The Fiebig staging system defines 6 stages of early HIV infection that occur after the eclipse phase and these stages range from the emergence of HIV RNA (Stage I) to full Western blot reactivity (Stage VI) (Figure 2).[15]

Early HIV Test Reactivity and Terminology

The CDC/APHL document and the Adult and Adolescent ART Guidelines have defined the following laboratory-based and clinically relevant terms related to acute HIV.[14,16] Note that some of these terms are not standardized, and alternative terminology, such as primary HIV (instead of acute HIV), is often used (Figure 3).

- **Eclipse Phase**: The eclipse phase is the initial interval after HIV infection when no existing diagnostic test, including the HIV NAT, is capable of detecting HIV. During this phase, even the HIV RNA PCR assays, which are the first tests that can detect HIV following HIV acquisition, are negative because the HIV RNA levels have not yet reached levels detectable by standard laboratory assays. The duration of the eclipse phase is roughly 8–10 days and may vary based on the sensitivity of the HIV PCR assay used.
- **Window Period**: The HIV seroconversion window period refers to the interval between HIV acquisition and the first detection of anti-HIV antibodies; the length of the window period varies depending on the specific HIV antibody assay used.
- **Acute HIV**: The term acute HIV (also referred to as primary HIV) typically describes the interval between the detection of 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 often positive. People with acute HIV may have an abrupt onset of clinical symptoms.
- **Recent Infection**: The term recent infection usually describes the period after acute HIV when anti-HIV antibodies are developing up to 6 months after HIV acquisition.
- **Early Infection**: Early infection is generally used to describe both acute and recent HIV time periods, which extend out to 6 months after HIV acquisition.
- **Established HIV Infection**: The term established infection refers to the time after early HIV, during which anti-HIV IgG antibody responses have fully developed.

Timing of HIV Test Reactivity with Modern Assays

The CDC and Association of Public Health Laboratories (APHL) document, based on several CDC-related studies, have outlined the sequence of contemporary laboratory markers that turn positive following the acquisition of HIV (Figure 4).[17,18] Following HIV acquisition, HIV RNA becomes detectable on standard laboratory tests approximately 8–10 days after infection.[15] The next marker to appear is p24 antigen, which typically reaches detectable levels about 7 days after the emergence of HIV-1 RNA, with a positive p24 antigen test usually developing when the HIV-1 RNA exceeds 20,000-30,000 copies/mL.[14,16] Next, the EIA (IgM antibody test) turns positive approximately 5 days later, and the IgM antibodies are gradually replaced by IgG antibodies, which appear 2 to 6 weeks after initial HIV RNA detection.[15]

Tests Used for the Diagnosis of HIV

Updated Nomenclature for HIV Serologic Tests

In the United States, HIV serologic tests have historically been categorized as first-, second-, third-, fourth-, and fifth generation tests, based on evolving techniques and significant improvement in assay sensitivity.[19,20] With many of the new and improved HIV assays, a clear distinction between the HIV generations has blurred, and thus, use of the HIV test "generation" nomenclature is no longer routinely used.[21] Nevertheless, from a conceptual standpoint, it is still important to understand the framework of generations of HIV tests. The first- and second-generation antibody assays are now referred to as IgG-sensitive tests, third-generation assays as IgM/IgG-sensitive tests; fourth-generation tests are antigen-antibody immunoassays that provide a combined result based on detection of any component of the test (p24 antigen, HIV-1 antibodies, or HIV-2 antibodies); and fifth generation tests are antigen-antibody immunoassays that can differentiate between HIV-1 antibodies, HIV-2 antibodies, and the p24 antigen (Figure 5).[14,19,20,22] Further, the use of the term "rapid HIV test" to describe point-of-care tests is no longer recommended, since a number of instrumented, laboratory-based tests now have the capacity to generate HIV test results in less than one hour. Instead, the type of test is considered either laboratory-based or point-of-care.[20,21]

Summary of Test Methods used for Diagnosing HIV

The major tests used as screening tests for diagnosing HIV are (1) HIV antigen-antibody laboratory-based tests, (2) HIV antigen-antibody point-of-care tests, (3) HIV antibody laboratory-based tests, and (4) HIV antibody point-of-care tests.[23,24] There are also two FDA-approved HIV antibody tests for use and interpretation at home.[25] Further, HIV diagnostic testing includes HIV-1/2 differentiation assays and HIV nucleic acid tests (NATs). The NAT testing includes options for qualitative HIV RNA or proviral DNA PCR assays or a quantitative RNA PCR assay, which is commonly referred to as a viral load test. The following summarizes the major FDA-approved HIV screening and differentiation assays.[13,20,24] Detailed lists of specific assays for these tests are provided in the CDC document that summarizes FDA-approved HIV tests.[24]

HIV Antigen-Antibody Laboratory-Based Tests

The HIV antigen-antibody laboratory-based immunoassays are the preferred screening tests for HIV. These immunoassays detect HIV-1 p24 (capsid) antigen and antibodies (IgM and IgG) to HIV-1 and HIV-2 (Figure 6).[14,20,26] The HIV-1/2 antigen-antibody immunoassays detect HIV significantly earlier than laboratory-based antibody tests, point-of-care antigen-antibody tests, and point-of-care HIV antibody tests.[20,27] All reactive HIV-1/2 antigen-antibody tests require confirmatory testing. None of the HIV-1/2 antigen-antibody immunoassays can detect HIV-2 core antigen (p26 antigen), but cross-reactivity to HIV-1 p24 antigen can occur in persons with HIV-2.

- Laboratory-based HIV-1/2 antigen-antibody immunoassays detect p24 antigen, HIV-1 antibody (typically groups O and M), and/or HIV-2 antibody. Most of the approved and available assays give a positive result if any of these components are detected and they cannot distinguish which part was identified. Only a few of the assays can distinguish p24 Ag from HIV-1 antibody and/or HIV-2 antibody (such as by giving an overall positive result and then a sub-result that identifies specific test component reactivity.
- The antigen-antibody immunoassays use varying laboratory testing strategies (e.g., chemiluminescent microparticle immunoassays, electrochemiluminescence immunoassays), but are similar in that they can all be performed in a laboratory setting and can be completed in under one hour (and some can be completed within 20–30 minutes). The antigen-antibody combination immunoassays demonstrate excellent sensitivity (approaching 100%) and high specificity (typically 99.8-100%) for detecting HIV infection. These performance characteristics are better than point-of-care tests, which have more false-negative results (due to a longer window period) and a higher rate of false-positive results.

HIV Antigen-Antibody Point-of-Care Tests

The point-of-care HIV-1/2 antigen-antibody test is a, single-use, rapid test that can detect HIV-1 p24 antigen, antibodies to HIV-1, and antibodies to HIV-2.[28] This assay is a point-of-care, single-use, rapid test that can detect HIV-1 p24 antigen, antibodies to HIV-1, and antibodies to HIV-2.[28,29] This assay can differentiate HIV-1 p24 antigen from HIV antibody, but it does not differentiate HIV-1 and HIV-2 antibodies.[30] The sensitivity of this assay for acute or very recent HIV infection is less than with laboratory-based HIV-1/2 antigen-antibody assays.[31,32,33] With this assay, the use of fingerstick whole blood specimens is not as sensitive as with plasma samples.[34]

HIV Antibody Laboratory-Based Tests

The HIV enzyme immunoassay (EIA) antibody test for HIV diagnosis was first licensed in the United States in 1985.[19] For more than 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.[13,14] A reactive HIV antibody test always requires further confirmatory testing with another HIV assay.[14] Most current laboratory-based HIV antibody tests are IgM/IgG-sensitive assays (as opposed to IgG-only assays) and can detect HIV IgM antibodies at approximately 23 to 25 days after HIV acquisition.[20] The CDC considers these HIV antibody tests to have a window period of 90 days (even the IgM/IgG-sensitive assays); in this context, a negative HIV EIA test 90 days after a possible HIV exposure is considered to effectively rule out HIV acquisition from that exposure.[21]

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

Single-use, point-of-care (rapid) HIV test kits have self-contained testing reagents and materials and typically can yield a test result within 40 minutes.[19,35,36] There are multiple point-of-care (rapid) tests that the CDC identifies as suitable for use in clinical and nonclinical settings.[35] Most of these tests detect antibodies to HIV-1 and HIV-2. These antibody test results are either reactive or nonreactive. Hence, none of the currently used point-of-care antibody tests can differentiate HIV-1 from HIV-2. Single-use, point-of-care rapid antibody tests are less sensitive than laboratory-based antigen-antibody tests for the detection of early HIV.[37,38,39,40] Among the point-of-care tests, oral fluid samples have shown the most problems with false-negative and false-positive test results. All positive point-of-care HIV test results are considered to be a presumptive positive test and require further supplemental testing for confirmation of HIV.[14] Single-use, point-of-care testing is primarily used for testing (1) in emergency room encounters where follow-up might be problematic, (2) women in labor who had no HIV testing performed during their pregnancy, (3) in an occupational exposure to HIV when immediate results may be needed, and (4) in other clinical settings where a low likelihood of follow-up for HIV test results is anticipated.[19,41,42]

HIV-1/2 Supplemental (Differentiation) Assays

Several tests can distinguish HIV-1 from HIV-2 and these tests are referred to as supplemental or differentiation assays.[43,44] he result may indicate reactivity for HIV-1, reactivity for HIV-2, or reactivity for both in cases of HIV-1/HIV-2 coinfection. Differentiating HIV-1 and HIV-2 is important to avoid misclassification of HIV infection.[13,14] These tests, which are antibody immunoassays, are also used to confirm an initial reactive HIV-1/2 antigen antibody test.

HIV-1 Western Blot Laboratory Tests

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 8).[45] The HIV Western blot typically becomes positive after about 5 to 6 weeks following HIV acquisition; as more protein bands become detectable, the Western blot typically evolves from a pattern of negative, then

indeterminate, then positive.[45]

HIV Self Testing

When HIV testing is performed by the person undergoing testing, it is referred to as self testing or in-home testing. Self-testing is done in its entirety at home (or in another private location). With the specimen is collected at home (or in another private location) and mailed in for testing. Commercially available home tests provide individuals with an option for anonymous HIV testing. Self-testing may be preferable for some people who are reluctant to undergo HIV testing in medical settings.[46,47] Studies have shown that self-testing is feasible and acceptable for persons undergoing testing,[47,48,49,50,51] though several concerns persist, including (1) the cost of the test, (2) low sensitivity for detecting recent HIV acquisition, (3) lack of appropriate counseling and confirmatory testing for a positive test result, and (4) insufficient resources for linkage to care for persons with a positive test or linkage to HIV PrEP services for people with a negative test result.

HIV Nucleic Acid Diagnostic Laboratory Tests

In the United States, HIV nucleic acid tests, including qualitative HIV RNA and quantitative HIV RNA assays, are used as part of the HIV diagnostic algorithm.[13] Given the very low limit of detection of most quantitative HIV RNA assays, many clinicians now use quantitative HIV RNA tests (also known as viral load tests), rather than the FDA-approved qualitative HIV RNA assays, for diagnostic purposes. The quantitative tests are more widely available than the qualitative tests, as they are routinely used in the clinical management of persons with established HIV.[19,52] The HIV NAT tests are not typically used as an initial HIV screening test due to high cost, technical complexity, and failure to detect HIV RNA in elite controllers (the approximately 0.5% of individuals with HIV who maintain undetectable HIV RNA levels without antiretroviral therapy).[53] The diagnostic HIV NATs are primarily used for diagnostic purposes in the following situations.[13,54,55]

- In the CDC/APHL HIV diagnostic algorithm to evaluate discordant results when a specimen has a reactive HIV-1/2 antigen-antibody immunoassay, but a nonreactive or indeterminate HIV-1/HIV-2 differentiation assay. In this setting, a positive HIV NAT may indicate possible acute infection.
- When a high suspicion of acute HIV exists and the initial HIV-1/2 antigen-antibody immunoassay is negative. In this scenario, a negative HIV-1/2; antigen-antibody immunoassay and a positive HIV NAT confirm the diagnosis of acute (primary) HIV.
- As part of screening for HIV infection, in addition to an antigen-antibody assay, for persons planning to start or already receiving HIV PrEP.

Recommended HIV Testing Algorithm

The CDC and Association of Public Health Laboratories (APHL) HIV testing algorithm, which was last updated in 2018, recommends using an HIV-1/2 antigen-antibody immunoassay as the initial HIV test, with positive test results followed by an HIV-1/2 differentiation immunoassay (Figure 9).[13,14,56] This HIV testing algorithm will detect HIV in most people with recent HIV-1 infection and virtually all people with chronic (HIV-1 and/or HIV-2) infection.[13,14] Although the use of this algorithm enhances earlier detection of acute HIV-1 infection than with HIV antibody testing alone, it does not accurately detect HIV immediately following HIV acquisition (during the eclipse phase).[57] The rationale for including HIV-2 testing in the algorithm is that HIV-2 infection, though uncommon in the United States, significantly differs from HIV-1 with respect to natural history and response to antiretroviral treatment regimens. Indeterminate or ambiguous results based on the initial HIV-1/2 antigen-antibody test and HIV-1/HIV-2 differentiation assay require further evaluation with an HIV nucleic acid test (NAT). From a practical standpoint, the same patient blood sample can be used to perform the initial screening test and the HIV differentiation assay.

Initial Test in Algorithm

The recommended initial test in the CDC/APHL HIV testing algorithm should be a laboratory-based HIV-1/2 antigen-antibody immunoassay; these tests can detect antibodies to HIV-1, antibodies to HIV-2, and HIV-1 p24 antigen.[13,14] A reactive HIV-1/2 antigen-antibody immunoassay requires confirmation and differentiation of HIV-1 from HIV-2 infection.[13,14] A person with a nonreactive HIV-1/2 antigen-antibody immunoassay is considered to not have HIV infection, unless a recent exposure to HIV has occurred. If no recent exposure to HIV has occurred, further HIV testing is not required for evaluation of current HIV status.[13,14] A CDC study evaluated the reactivity of HIV tests in specimens from individuals with HIV-1 seroconversion and showed that an HIV-1/2 antigen-antibody immunoassay was positive in 75% at 24 days and in 99% at 44.3 days (Figure 10).[27] Accordingly, a nonreactive laboratory-based HIV-1/2 antigen-antibody immunoassay virtually excludes HIV infection if 45 days or more have passed since the last possible exposure to HIV.[21] In situations where it is not feasible to perform a laboratory-based initial HIV-1/2 antigen-antibody immunoassay, a point-of-care HIV-1/2 Ag/Ab test can be used as the initial test in the HIV diagnostic laboratory algorithm.[58]

Differentiation Assay

If the initial screening HIV-1/2 antigen-antibody immunoassay is reactive, a second (supplemental) HIV test is needed to confirm the initial test and to differentiate whether the infection is caused by HIV-1, HIV-2, or both.[13,24,56,59] For this purpose, the CDC algorithm recommends using an HIV-1/HIV-2 antibody supplemental (differentiation) assay. Samples that are reactive with the HIV-1/2 antigen-antibody immunoassay and the HIV supplemental assay are considered positive for HIV and should be classified as HIV-1, HIV-2, or HIV-1 and HIV-2.[13] 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 (qualitative or quantitative RNA assay) to evaluate the possibility of acute HIV (false-negative differentiation assay due to the window period) 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.

HIV Nucleic Acid Testing

If the initial HIV-1/2 antigen-antibody immunoassay is reactive, but the HIV-1/HIV-2 differentiation assay is nonreactive, further testing with an HIV-1 NAT should be performed.[13] This is generally accomplished by drawing a sample for a qualitative or quantitative HIV-1 RNA assay, unless the individual has a substantial risk for HIV-2 infection or known exposure to HIV-2, in which case an HIV-2 RNA assay should be added. If both the HIV-1/2 antigen-antibody immunoassay and the HIV-1/HIV-2 differentiation assay are reactive, then quantitative HIV RNA testing (viral load) is indicated—HIV-1 quantitative or HIV-2 quantitative, depending on whether HIV-1 or HIV-2 is identified on the differentiation assay.

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 45 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 person has HIV-1 infection.
- If the HIV-1/2 antigen-antibody immunoassay is reactive and the HIV-1/HIV-2 differentiation assay result shows nonreactive HIV-1 and reactive HIV-2, 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 result shows reactive HIV-1 and reactive HIV-2, then conclude the patient has HIV-1 and HIV-2 coinfection.
- 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 RNA is indicated. If the HIV-1 RNA is positive, the patient has acute HIV-1. If the HIV-1 RNA 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. Alternatively, in a person with risk factors for acquiring HIV-2, these test results could theoretically indicate acute HIV-2. Follow-up testing with HIV-2 NAT should be considered if an individual has epidemiologic risk factors for exposure to HIV-2.

Performance of Diagnostic Tests

The principles that define a good screening test are not unique to HIV infection and apply to medical screening in general. An ideal screening test will accurately identify individuals with the clinical condition of interest, but without mistakenly diagnosing individuals who do not have the condition. In addition, the use of screening tests is most effective when limited to conditions for which there exists available, effective treatment that can directly target the disease and improve prognosis and outcomes.[60]

False-Negative HIV Tests

A false-negative HIV test result refers to a negative HIV test result in a person who actually has HIV (Figure 11). A false-negative HIV antigen-antibody test result most often occurs when performing HIV testing in a person with acute HIV (the p24 antigen usually becomes reactive by day 17) or when a person received potent antiretroviral therapy very early after HIV acquisition.[61,62,63] Additional causes of false-negative results include failure to generate anti-HIV antibodies due to defects in immunity, acquisition of HIV while receiving HIV PrEP, ypogammaglobulinemia, recent receipt of potent immunosuppressant medications, or laboratory error.[64,65,66,67,68] In adults with chronic HIV, the loss of HIV antibodies (seroreversion) is exceedingly rare.[69] A false-negative HIV NAT can occur in the first 8–10 days after HIV acquisition during the eclipse phase and in the rare persons with chronic HIV who inherently have very strong immunologic control of HIV (elite controllers) 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 (Figure 12). 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 (or other vaccines), autoimmune disorders, receipt of an investigational HIV-1 vaccine, receipt of gamma globulin, prior blood transfusions, human T-lymphotropic virus (HTLV) infection, recent viral infection (including COVID-19), collagen vascular diseases, and laboratory errors.[70,71] Recently, several reports have described false-positive HIV NATs in persons who received chimeric antigen receptor (CAR) T-cell therapy, due to the lentivirus used as the vector in manufacturing these individualized therapies; in these cases, the lentivirus vector used had incorporated a plasmid that contained part or all of the HIV *gag* sequence.[72] When trying to determine whether a person's HIV screening test result is accurate, the pretest probability—the likelihood before the test was performed that the patient has HIV—can help with interpretation. 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.[73]

Special Diagnostic Situations

Diagnosis of Acute HIV-1

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 13).[16,74,75] Use of HIV-1/2 antigen-antibody immunoassays will detect HIV about 17 days after HIV acquisition, which is significantly sooner than with HIV laboratory-based HIV antibody tests, all point-of-care HIV tests, and in-home HIV tests.[18,57,76,77] 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 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 (a mononucleosis-like syndrome).[78,79,80] 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. Ideally, if acute HIV is suspected, a quantitative RNA assay (as opposed to a qualitative RNA assay) should be ordered since HIV RNA levels are typically very high at this stage.

Diagnosing HIV In Persons Receiving HIV PrEP

The diagnostic accuracy and timing of early HIV infection in persons who acquire HIV while taking HIV PrEP with either tenofovir DF-emtricitabine, tenofovir alafenamide-emtricitabine, or long-acting injectable cabotegravir, may result in atypical laboratory patterns, such as delayed seroconversion, indeterminate results on HIV differentiation assays, low-level HIV RNA levels, or HIV RNA levels below the limit of the assay, even in persons with acute or early HIV.[81] Data from the Partners PrEP Study and the Bangkok Tenofovir Study showed persons receiving HIV PrEP who acquired HIV had marked delays in HIV seroconversion with point-of-care tests, especially when using oral fluid samples.[66,82] Other groups have reported false-negative or ambiguous HIV test results in persons taking HIV PrEP.[67,81] Problems with false-negative testing are greater in this setting when using point-of-care tests; thus, laboratory-based HIV testing is recommended when monitoring persons receiving HIV PrEP.[20] Of note, even laboratory-based, antigen-antibody tests may be affected by HIV PrEP exposure. In the HPTN 083 trial, which compared injectable cabotegravir to daily, oral tenofovir DF-emtricitabine, delays in diagnosis were documented in individuals with incident HIV acquisition in both arms, though delays were generally longer for individuals in the cabotegravir arm.[83] For this reason, the CDC HIV PrEP Guidelines, which were updated in 2021, now recommend routine HIV RNA monitoring for individuals receiving HIV PrEP, and an HIV RNA test prior to initiating HIV PrEP is recommended in several circumstances.[54] In situations where results are ambiguous or confusing, clinical consultation is recommended.

Diagnosis of HIV in Infants and Children Exposed to HIV

The 2014 and 2018 CDC HIV diagnostic algorithm does not address HIV diagnostic testing of infants and children exposed to HIV.[13,14] To diagnose HIV among infants younger than 18 months of age, the Pediatric ART Guidelines recommend using a virologic assay (HIV NAT) that directly detects HIV RNA or HIV DNA (either quantitative or qualitative tests can be used). This issue is addressed in detail in the section Diagnosis of HIV in Infants and Children in the lesson HIV in Infants and Children.

Diagnosis of HIV-2

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).[13,14,84] 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 RNA Quantitation) and the New York State Department of Health (HIV-2 Nucleic Acid Testing, which includes HIV-2 qualitative or quantitative options).[85] It is important to note, however, that a significant percentage of individuals with HIV-2 have undetectable HIV-2 RNA levels without antiretroviral treatment. Thus, in certain epidemiological settings (e.g., a person with risk factors 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.[86] This occurred because an HIV Western blot was used as the confirmatory test (instead of the currently used 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.[14,86,87]

Delivering Test Results

Follow-Up for Test Results

The use of multiple modalities for HIV testing and delivery of HIV test results has helped to optimize this process. In particular, delivery of HIV test results by telephone has been found to be both effective and acceptable, and increases the number of people who receive their test results.[88,89] In addition, the use of point-of-care, rapid tests, which provide a result at the same visit and augment the capacity to run tests quickly at mobile and community sites, provides an opportunity for real-time delivery of test results.[90] Implementation of rapid tests in outreach and community settings was found in this report to enhance testing of people at risk of acquiring HIV but who may not regularly have access to medical care. The availability of self-testing also increases the proportion of persons who undergo HIV testing and can immediately see their test result. That said, a positive self-test result always requires confirmatory testing and recent large studies found mixed results when assessing whether self-testing significantly reduces the number of individuals with undiagnosed HIV.[49,91,92]

Communicating Test Results

The CDC offers practical advice for medical providers who offer HIV testing in their clinical settings.[93] Medical providers should be prepared to deliver results to individuals undergoing HIV testing in a private area and in a direct, neutral tone. The health care professional delivering the test results should be knowledgeable about HIV, since persons undergoing testing may have questions about HIV, risk of transmission to partners, and disclosure of HIV status to partners. Any individual who receives a positive HIV test result should be linked to HIV care prior to leaving the testing setting and should have a scheduled appointment with an HIV medical provider as soon as possible. Availability of a case manager or social worker familiar with HIV and HIV-related resources can aid in the initial discussion with a person newly diagnosed with HIV. Being able to provide emotional support, medical information, and timely linkage to care is critical when delivering positive HIV results.[94] For persons who test negative for HIV, the medical provider should be prepared to provide HIV prevention counseling to help the individual remain HIV negative, including discussion of and referral for HIV PrEP, if 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 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 (1) more likely to detect acute HIV-1, (2) more accurately diagnoses HIV-2, (3) allows for faster turnaround time, and (4) leads to fewer indeterminate results.
- An ideal screening test is sensitive, specific, and limited to conditions for which there is effective treatment available that can directly target the disease and improve prognosis and outcomes.
- False-negative HIV screening test results can occur during acute HIV; false-positive HIV screening test results may occur due to laboratory errors, and rarely from cross-reactivity with other antibodies, such as during pregnancy, in persons who have an autoimmune condition, or following recent vaccine administration.
- 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 typically not detected in the first 10 days after induction during the eclipse phase.
- Single-use, point-of-care HIV tests and self-testing are additional options to help facilitate HIV screening and detection. A reactive result on a point-of-care or self-test should be considered a presumptive positive result and requires further testing.
- Challenges may occur with the diagnostic evaluation of acute HIV, when evaluating infants and children exposed to HIV, and in persons receiving HIV PrEP. Clinical consultation is recommended if a person has been exposed to HIV or there is suspicion or risk for HIV, yet HIV test results are ambiguous or indeterminate.

- Diagnostic testing for HIV should ideally link persons who test negative to appropriate preventive care and those who test positive to HIV treatment services.

Citations

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- Spivak AM, Brennan TP, O'Connell KA, et al. A case of seronegative HIV-1 infection. J Infect Dis. 2010;201:341-5.
[PubMed Abstract] -

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

Figures

Figure 1 HIV Care Continuum

Source: Adapted from HRSA. HIV Care Continuum

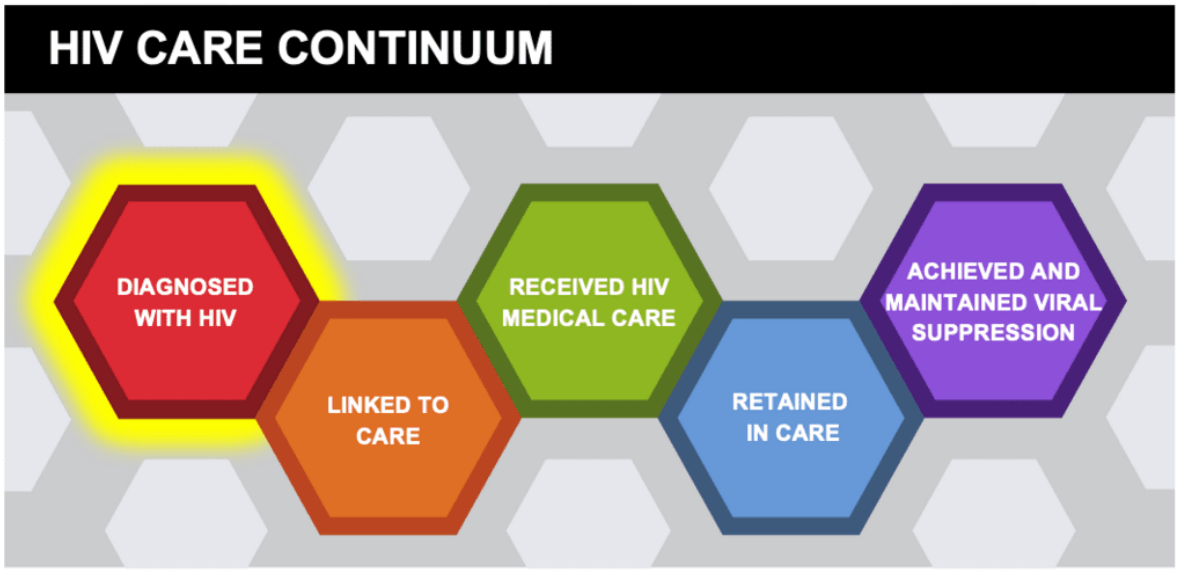

Figure 2 (Image Series) - Fiebig Classification for Early HIV-1 Infection (Image Series) - Figure 2 (Image Series) - Fiebig Classification for Early HIV-1 Infection
Image 2A: Duration of Stages

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. AIDS. 2003;17:1871-9.

Fiebig Laboratory Stages of Early HIV-1 Infection

Stage	Duration	HIV RNA	p24 Ag	*EIA	Western blot
Eclipse	11.0 days	(-)	(-)	(-)	(-)
I	5.0 days	(+)	(-)	(-)	(-)
II	5.3 days	(+)	(+)	(-)	(-)
III	3.2 days	(+)	(+)	(+)	(-)
IV	5.6 days	(+)	(+)	(+/-)	#Indeterminate pattern
V	69.5 days	(+)	(+/-)	(+)	Reactive, but absence of p31 (*pol*)
VI	Open-ended	(+)	(+/-)	(+)	Reactive, including p31 (*pol*)

* EIA = enzyme immunoassay (refers to IgM-sensitive 3rd generation assay)
# 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 2 (Image Series) - Fiebig Classification for Early HIV-1 Infection
Image 2B: Graphic of 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. AIDS. 2003;17:1871-9.

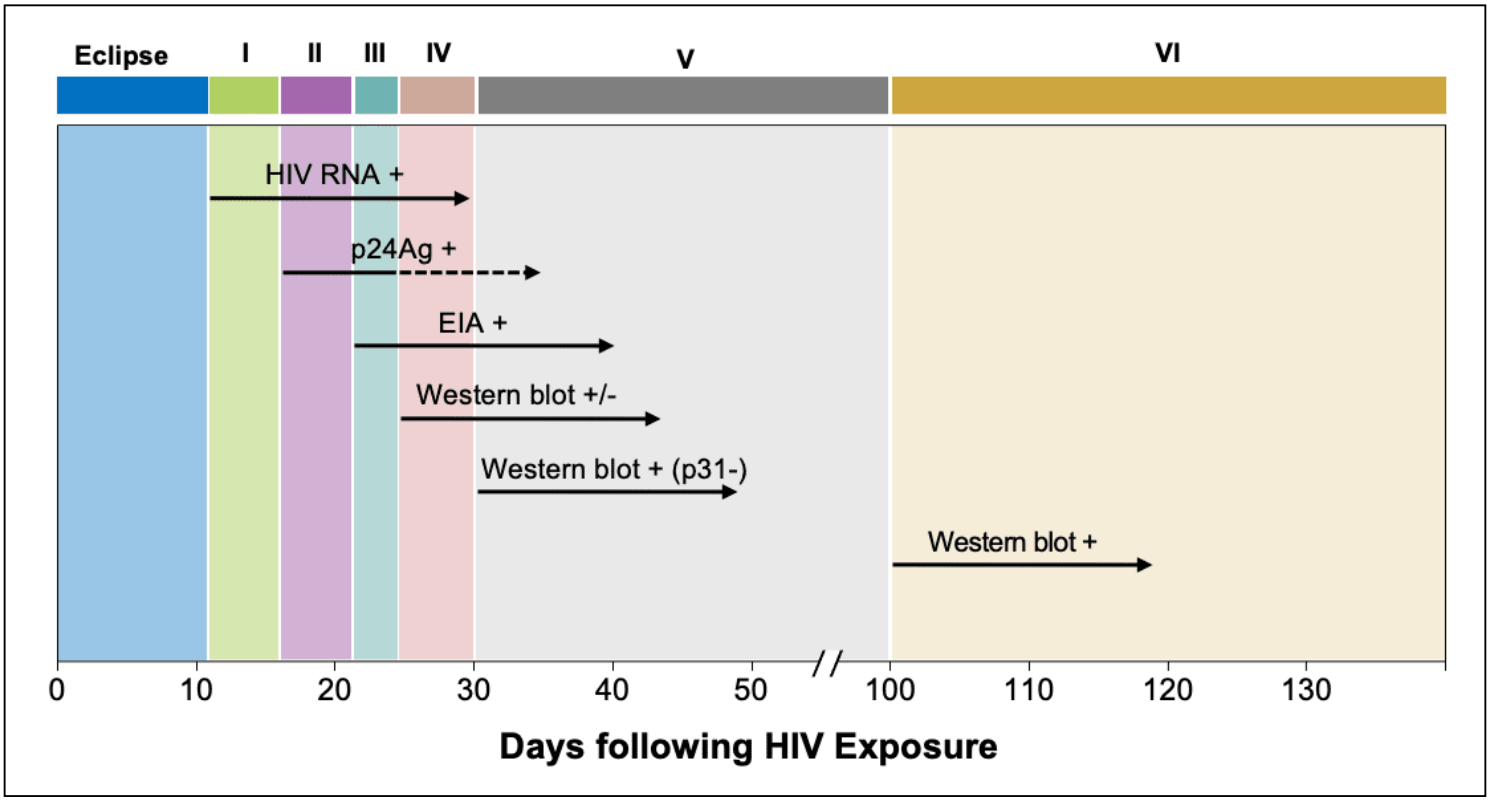

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition (Image Series) - Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition Image 3A: HIV Eclipse Phase

Illustration: David H. Spach, MD

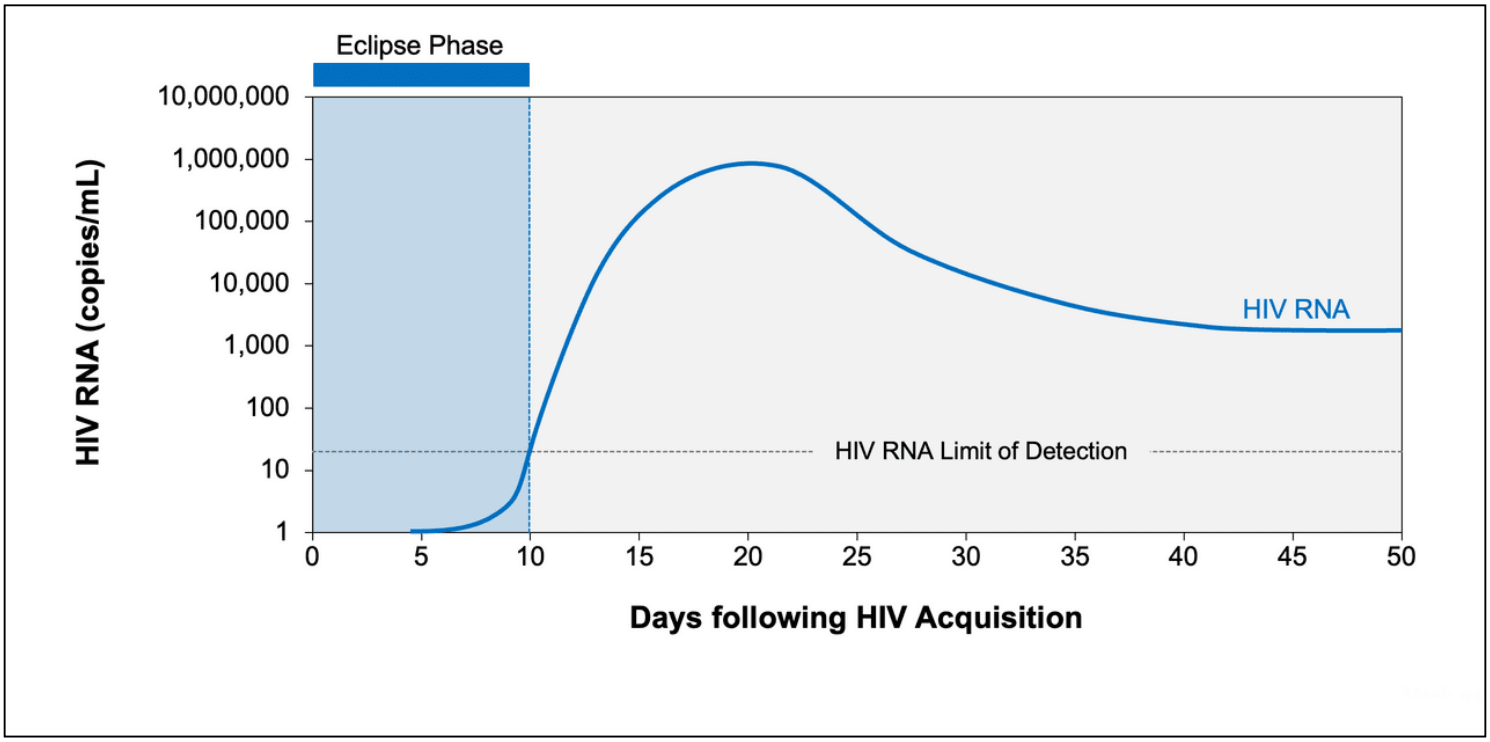

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition
Image 3B: HIV Seroconversion Window

Illustration: David H. Spach, MD

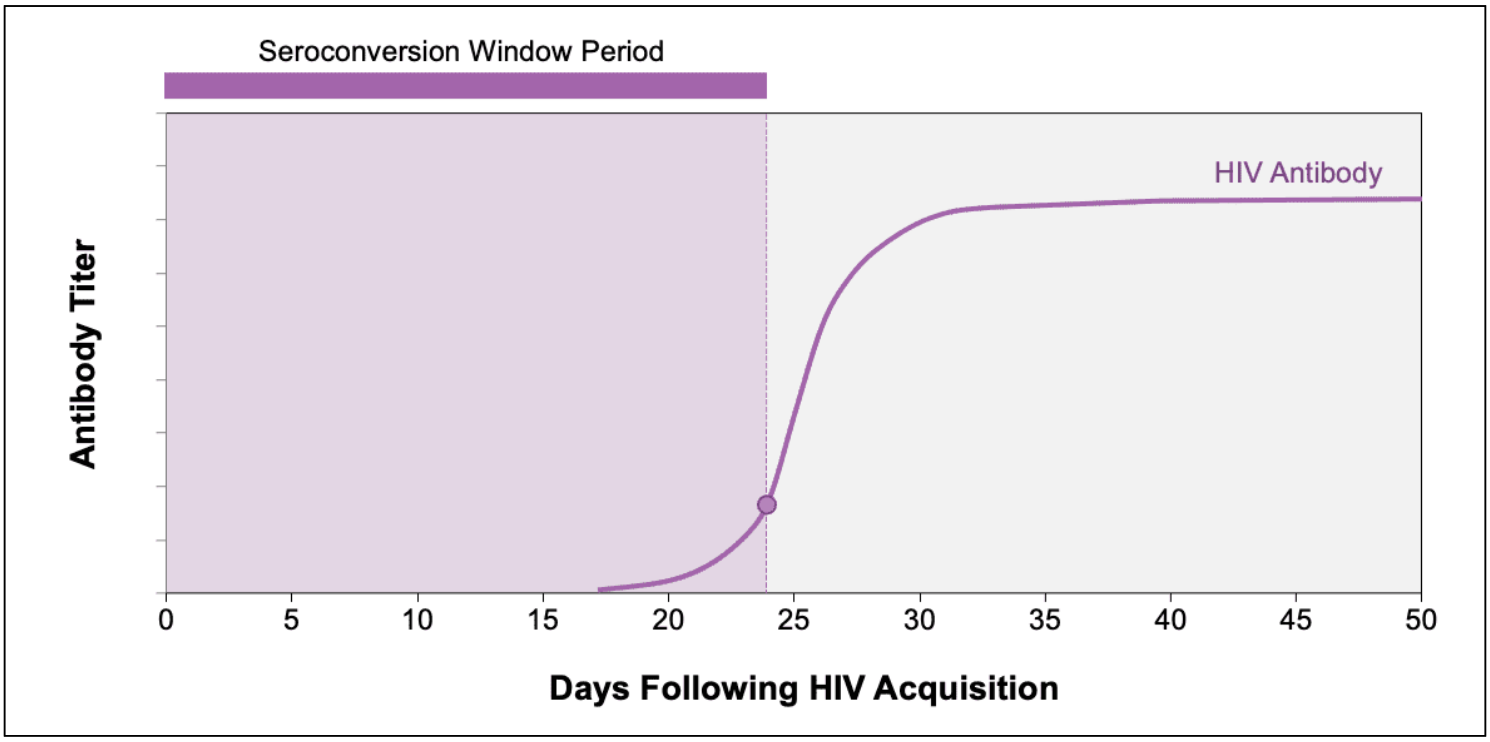

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition
Image 3C: Acute HIV

Illustration: David H. Spach, MD

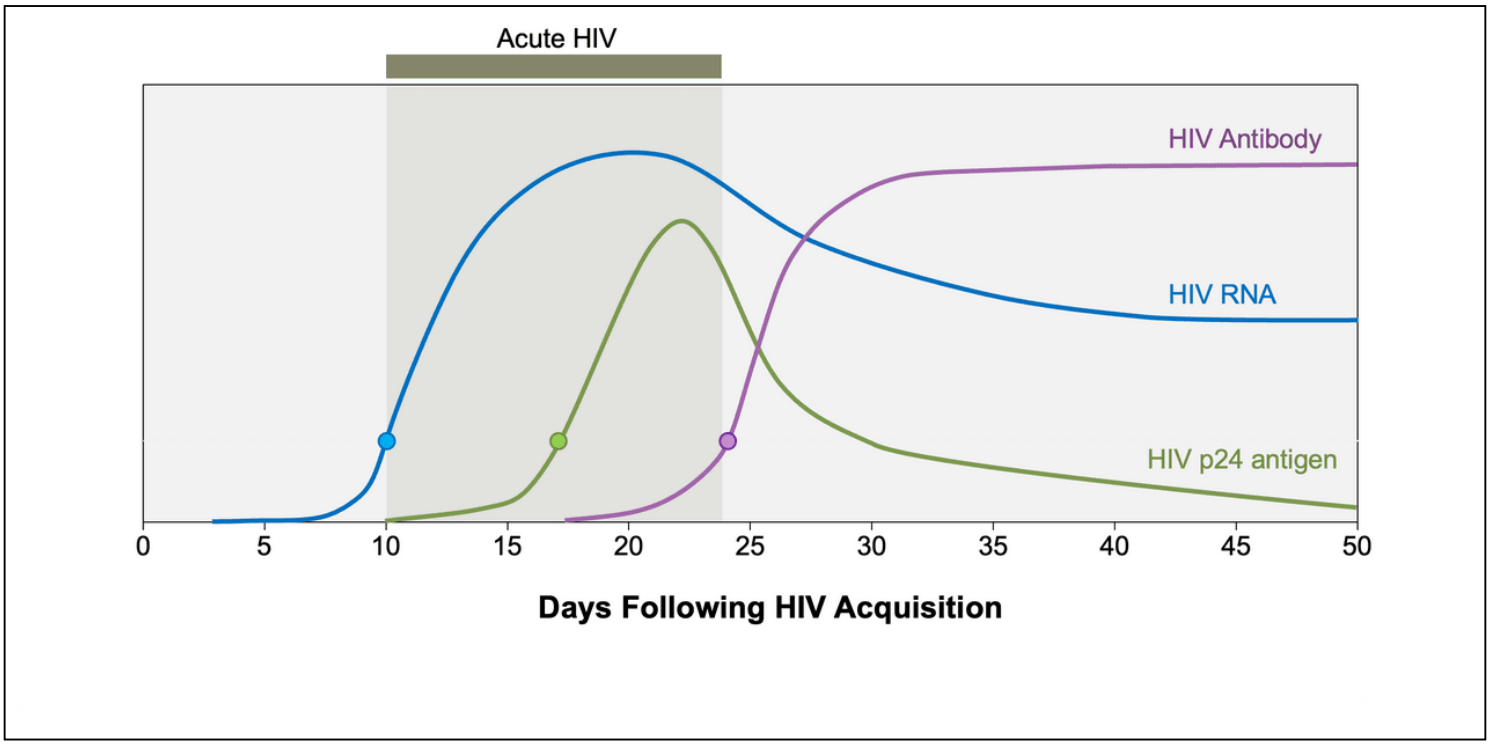

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition
Image 3D: Recent HIV

Illustration: David H. Spach, MD

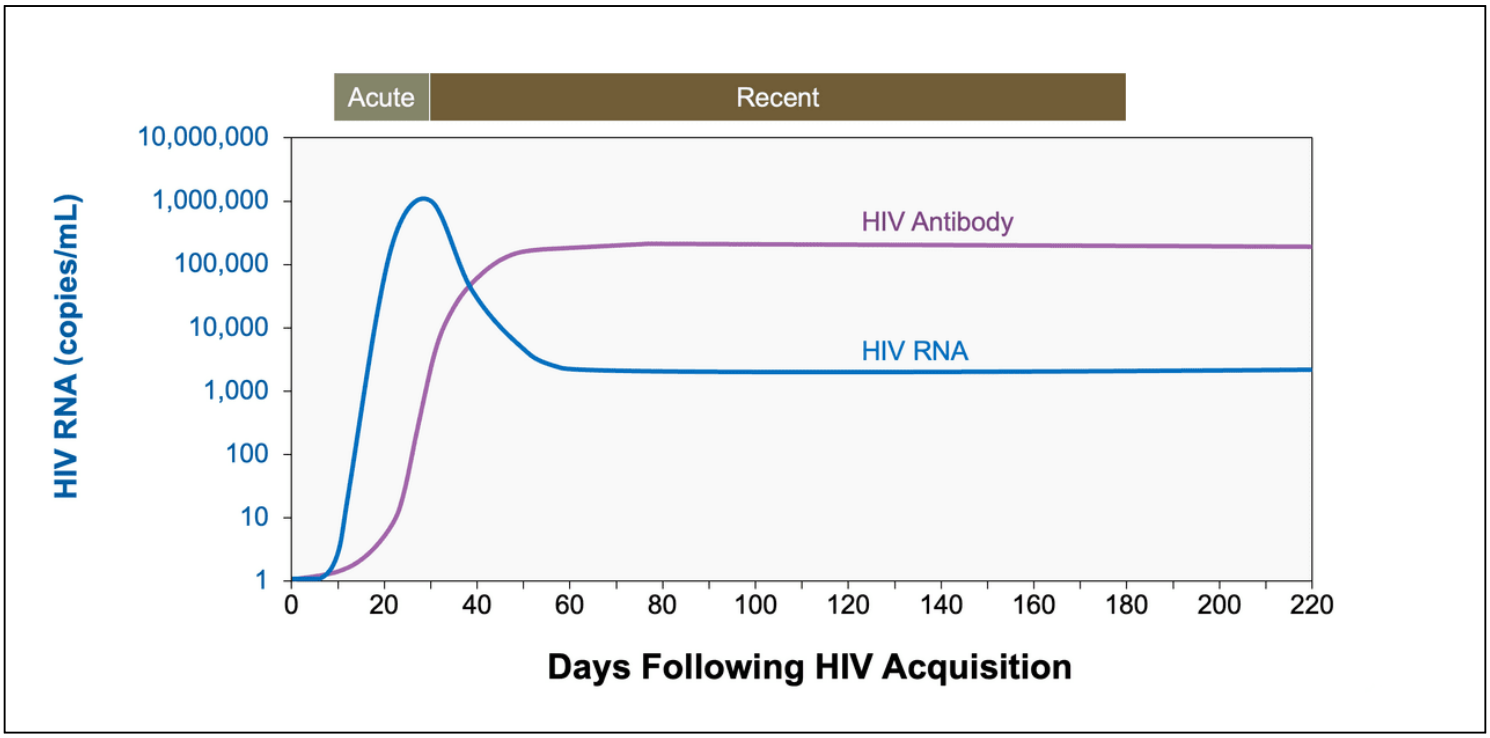

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition
Image 3E: Early HIV

Illustration: David H. Spach, MD

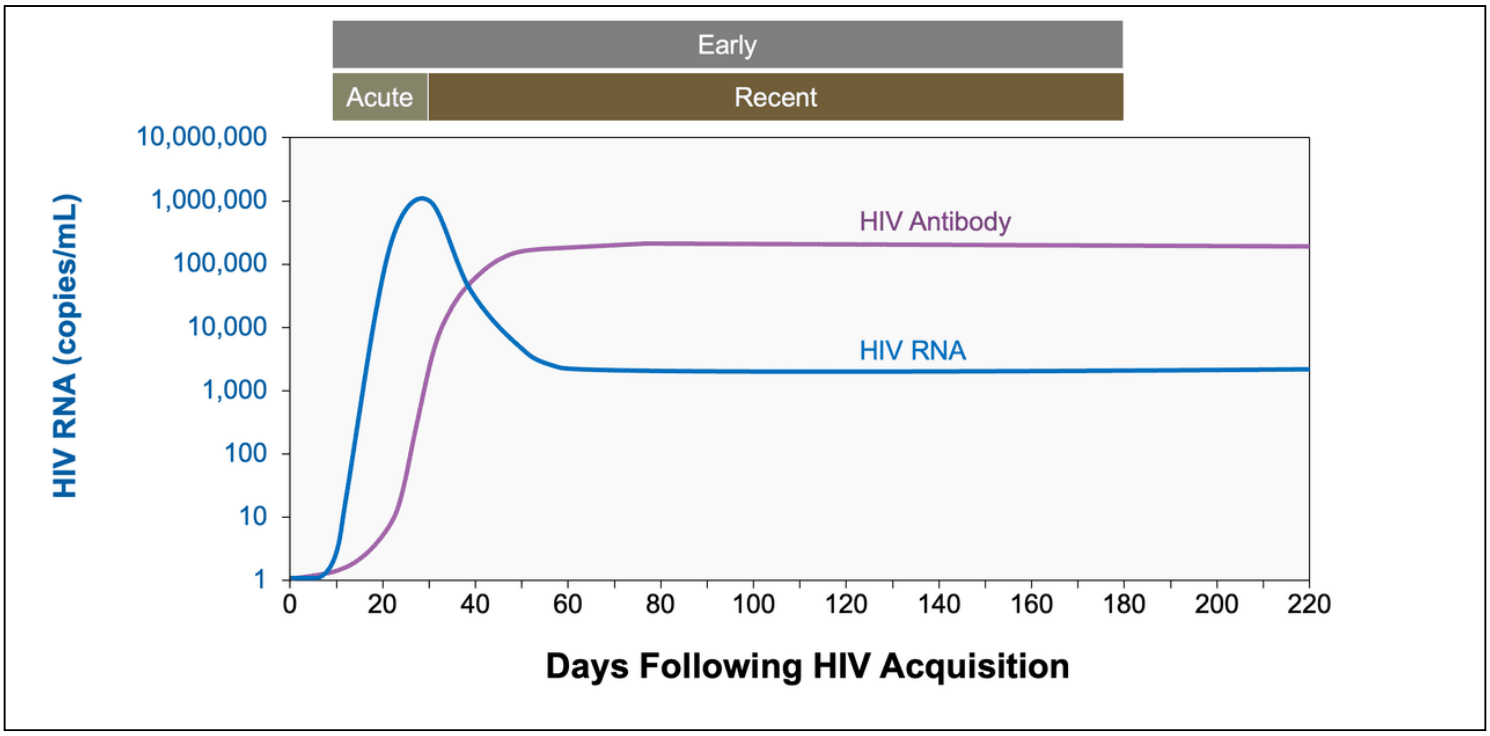

Figure 3 (Image Series) - HIV Definitions Involving Initial Year After HIV Acquisition
Image 3F: Established HIV

Illustration: David H. Spach, MD

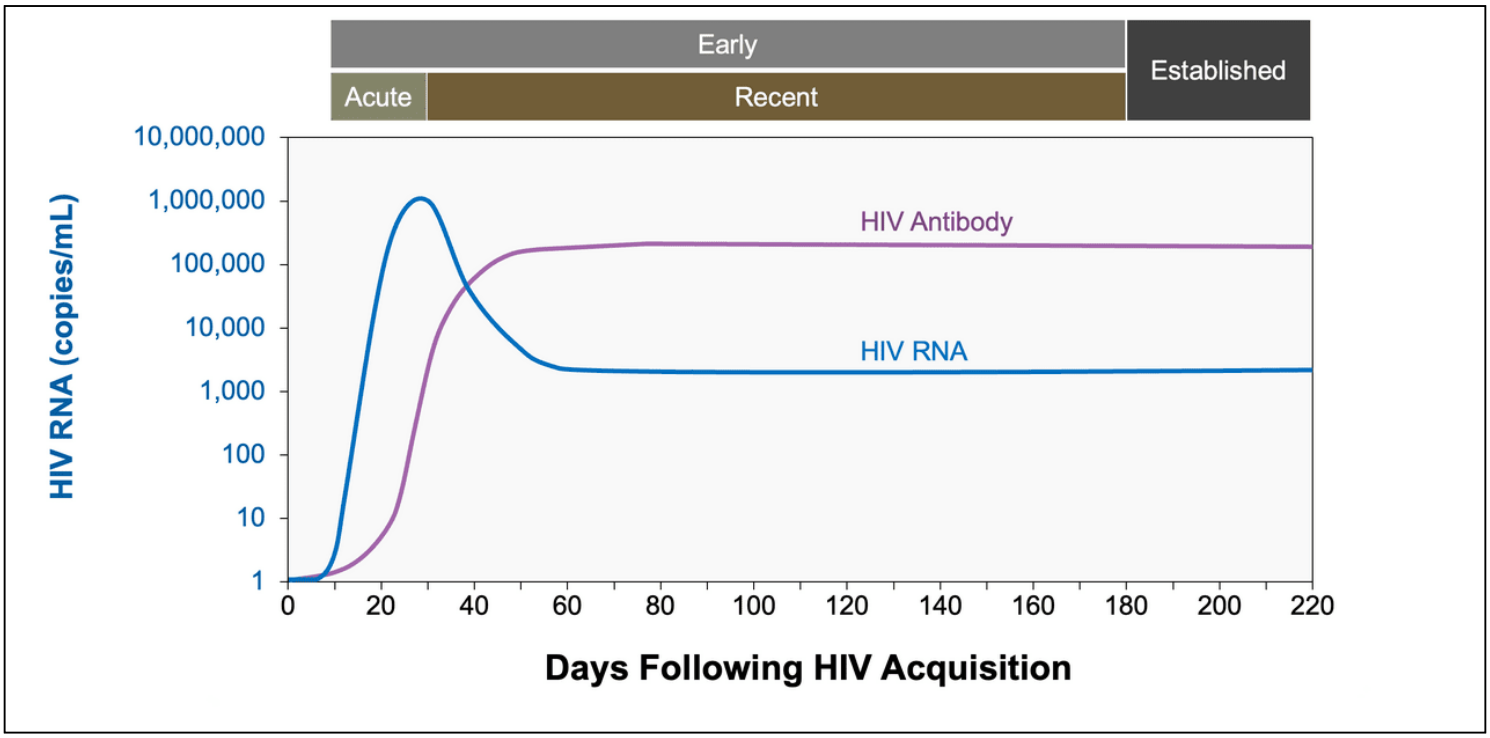

Figure 4 Timing of Positivity for HIV Diagnostic Tests

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

Source: modified from Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. Published June 27, 2014.

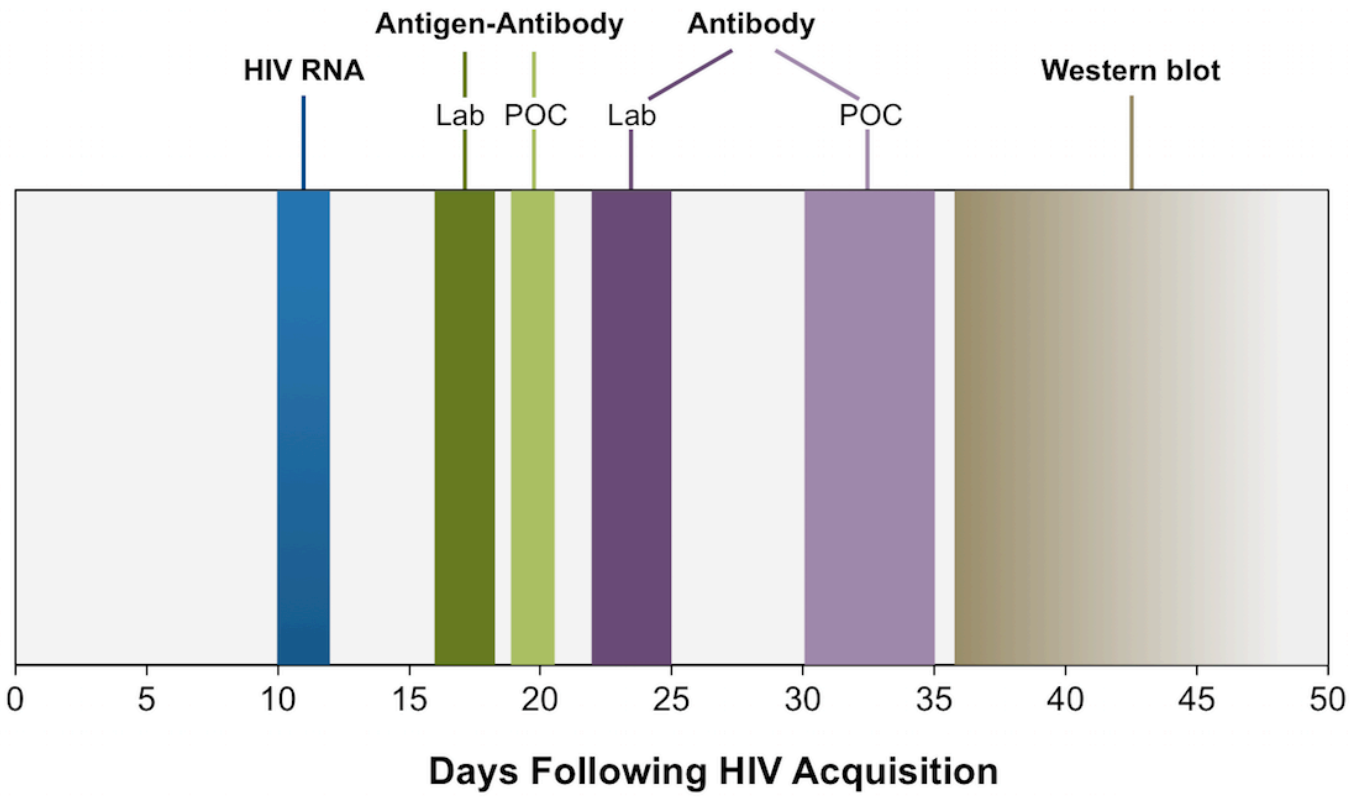

Figure 5 HIV Serologic Tests: HIV Antibody and HIV Antigen-Antibody

Illustration: David H. Spach, MD

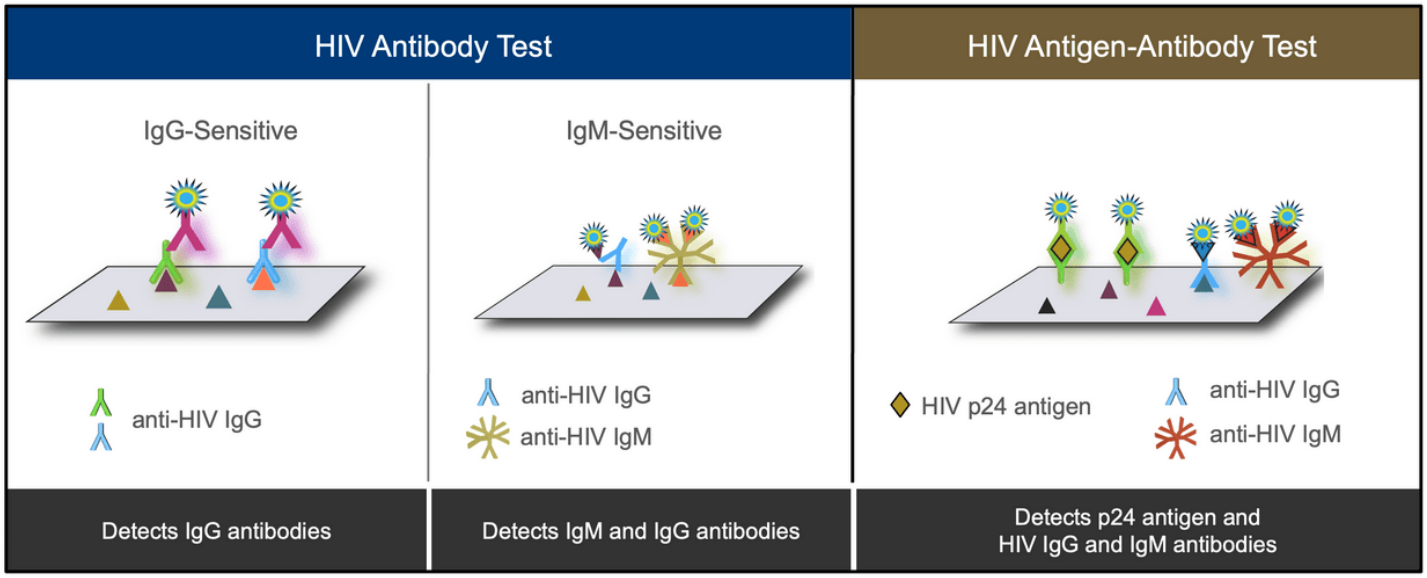

Figure 6 (Image Series) - Laboratory-Based HIV-1/2 Antigen-Antibody Immunoassays (Image Series) - Figure 6 (Image Series) - Laboratory-Based HIV-1/2 Antigen-Antibody Immunoassays Image 6A: Components of HIV-1/2 Antigen-Antibody Immunoassay

The HIV-1/2 antigen-antibody immunoassay contains 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

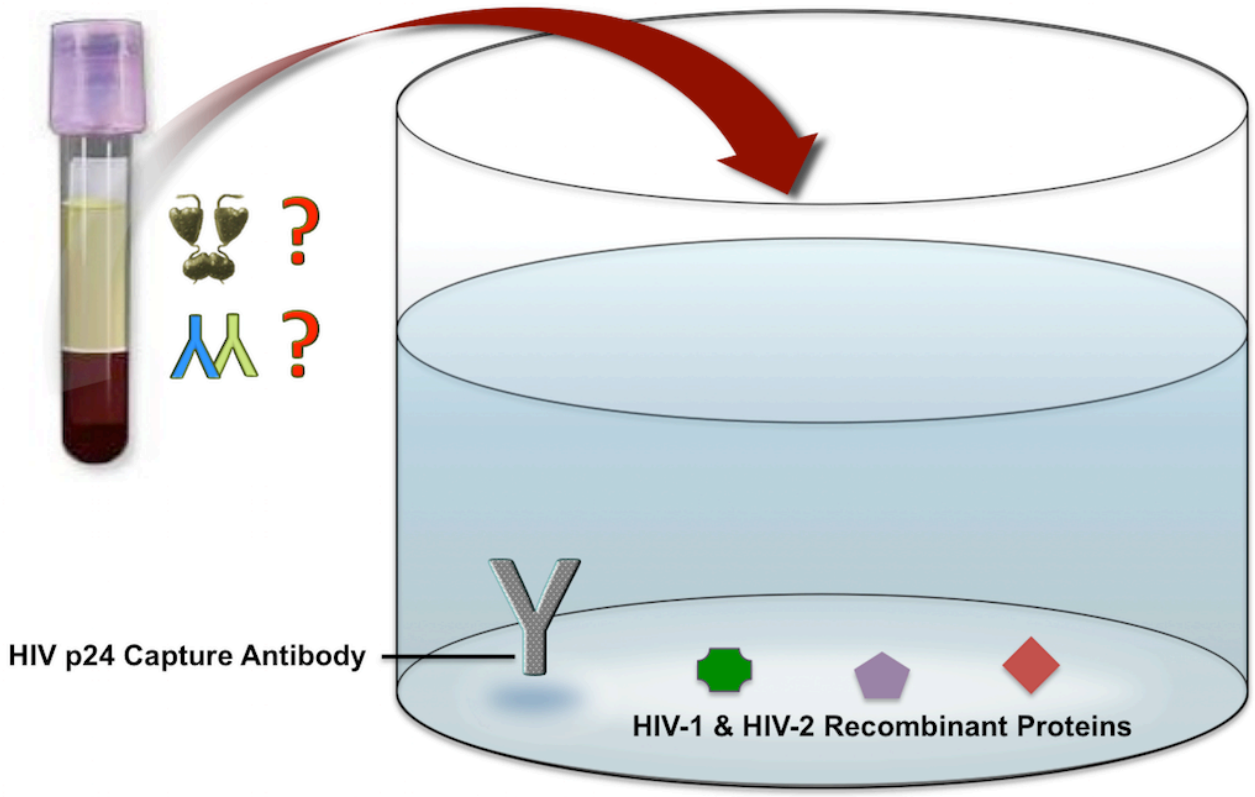

Figure 6 (Image Series) - Laboratory-Based HIV-1/2 Antigen-Antibody Immunoassays
Image 6B: Patient Sample Reacting with Components in HIV-1/2 Antigen-Antibody Immunoassay

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

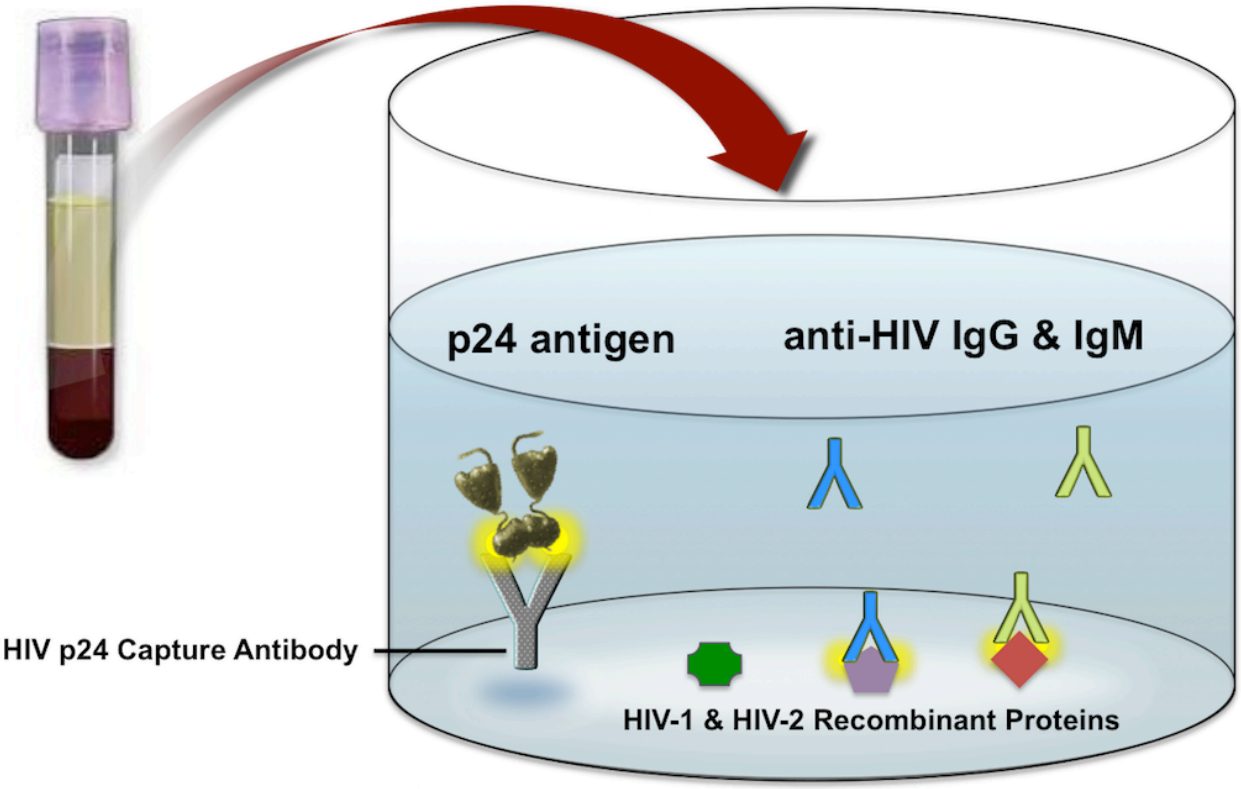

Figure 6 (Image Series) - Laboratory-Based HIV-1/2 Antigen-Antibody Immunoassays
Image 6C: Reactive HIV-1/2 Antigen-Antibody Immunoassay

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

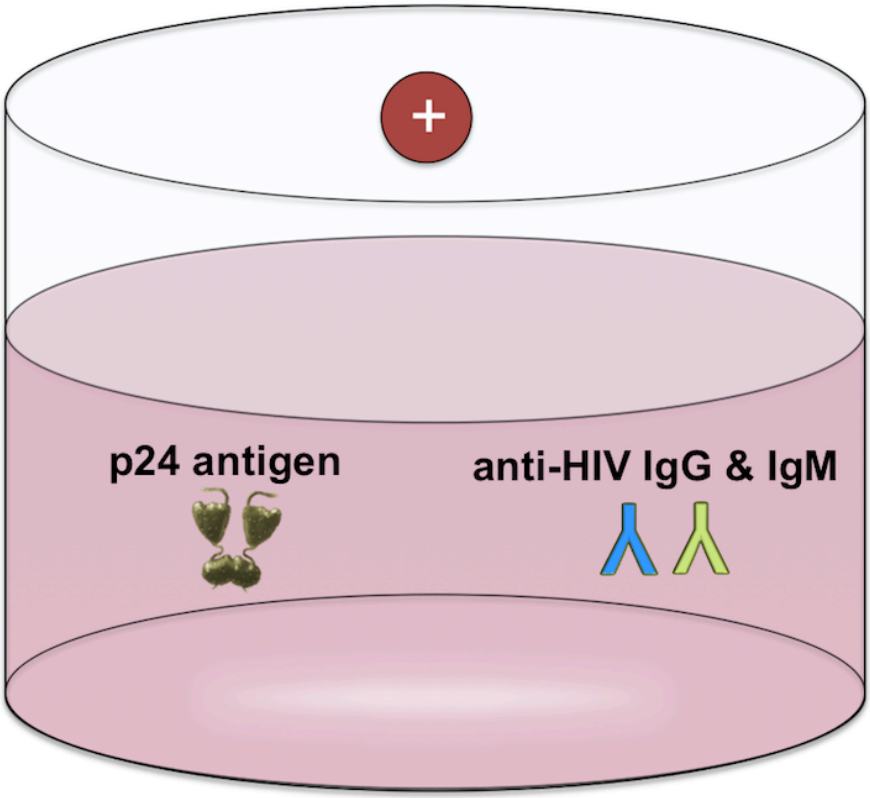

Figure 7 Geenius HIV 1/2 Supplemental Assay

Source: modified from Fernández McPhee C, Álvarez P, Prieto L, et al. HIV-1 infection using dried blood spots can be confirmed by Bio-Rad Geenius™ HIV 1/2 confirmatory assay. J Clin Virol. 2015;63:66-9.

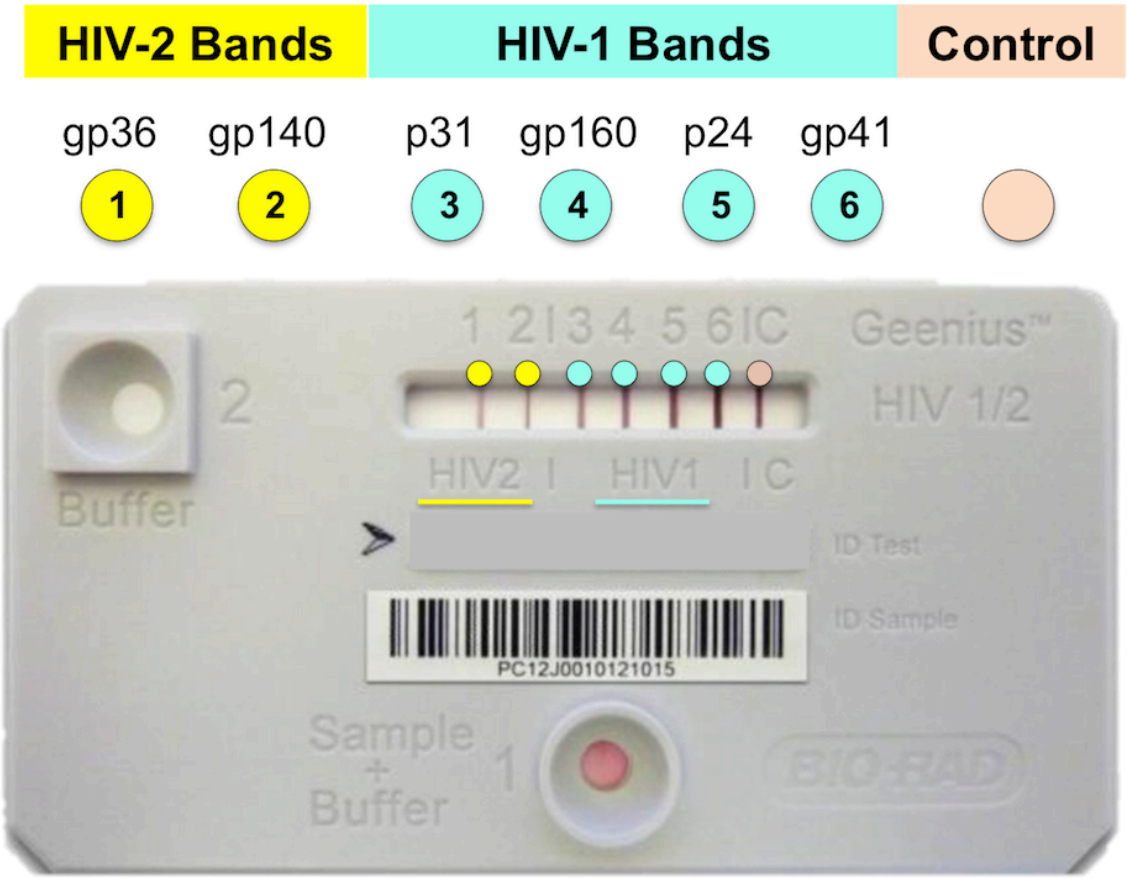

Figure 8 HIV-1 Western blot

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

HIV-1 Gene and Product	Band on Western blot
env	
Precursor Protein	gp160
External Glycoprotein	gp120
Transmembrane Protein	gp41
pol	
Reverse Transcriptase	p66
Reverse Transcriptase	p51
Endonuclease	p31
gag	
Gag Precursor	p55
Core	p24
Matrix	p17
Nucleocapsid Precursor	p15

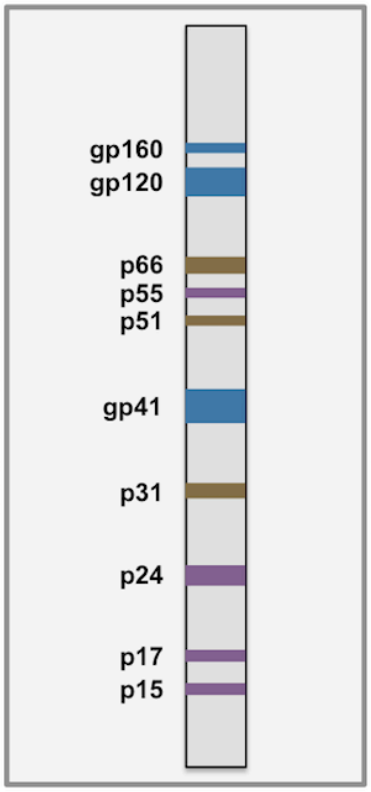

Figure 9 CDC and APHL Recommended Laboratory Testing Algorithm for the Diagnosis of HIV Infection

Source: Centers for Disease Control and Prevention and Association of Public Health Laboratories. 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens. Published January 27, 2018.

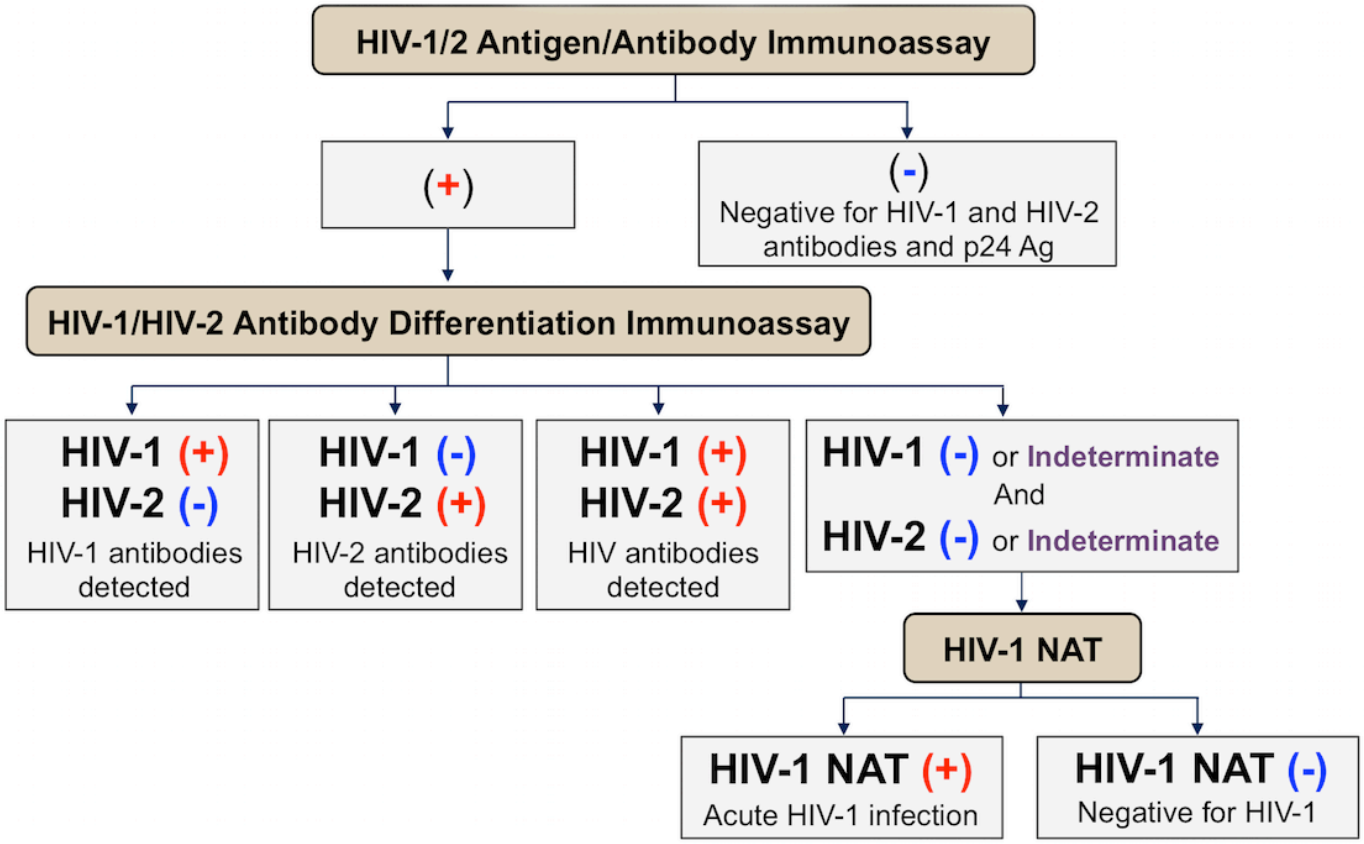

Figure 10 Timing of HIV-1/2 Antigen-Antibody Immunoassay Reactivity Following HIV Acquisition

This graphic shows the time course for test HIV-1/2 antigen-antibody immunoassay positivity after HIV acquisition: 25% at day 14, 50% at day 18, 75% at day 24, and 99% after day 44. Thus, a negative test at day 45 after an exposure virtually excludes HIV infection from that exposure.

Source: Delaney KP, Hanson DL, Masciotra S, Ethridge SF, Wesolowski L, Owen SM. Time Until Emergence of HIV Test Reactivity Following Infection With HIV-1: Implications for Interpreting Test Results and Retesting After Exposure. Clin Infect Dis. 2017;64:53-9.

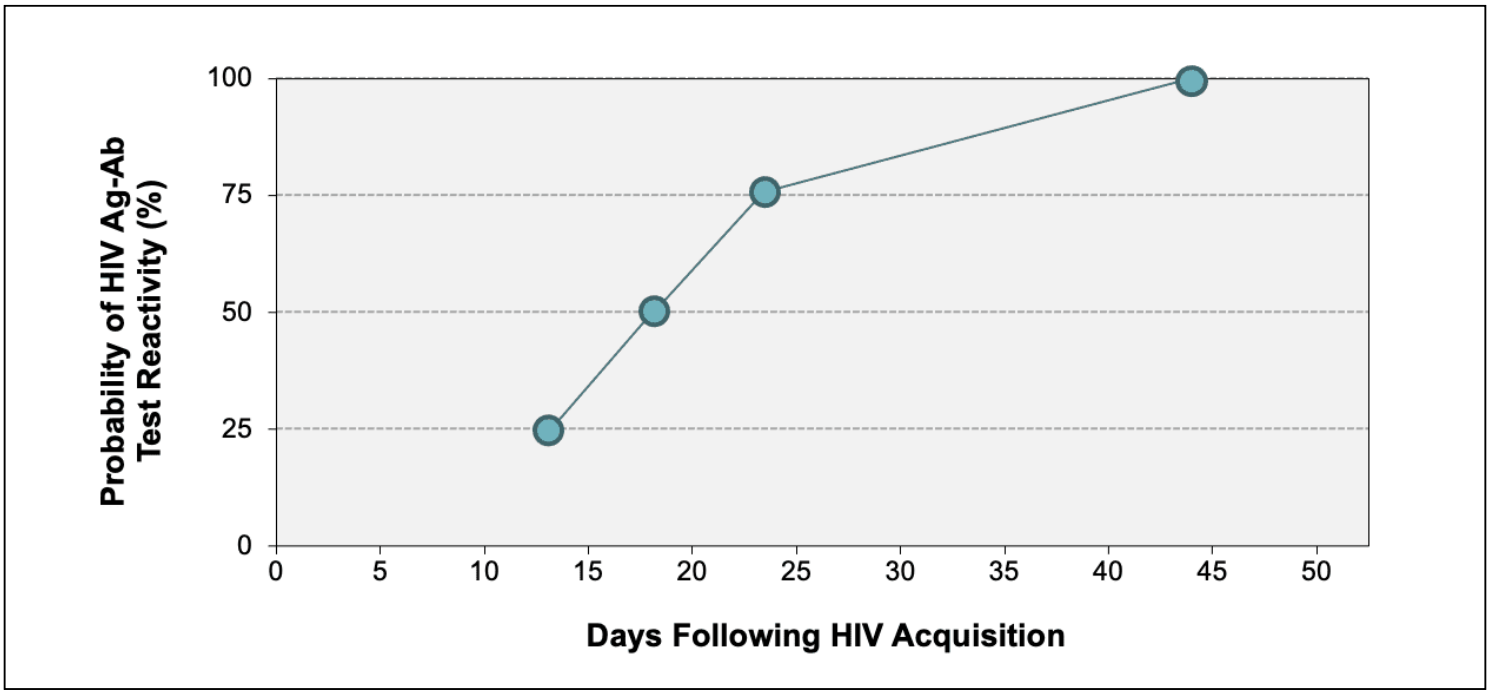

Figure 11 (Image Series) - False-Negative HIV Diagnostic Test (Image Series) - Figure 11 (Image Series) - False-Negative HIV Diagnostic Test
Image 11A: Test Results for Persons with HIV Infection

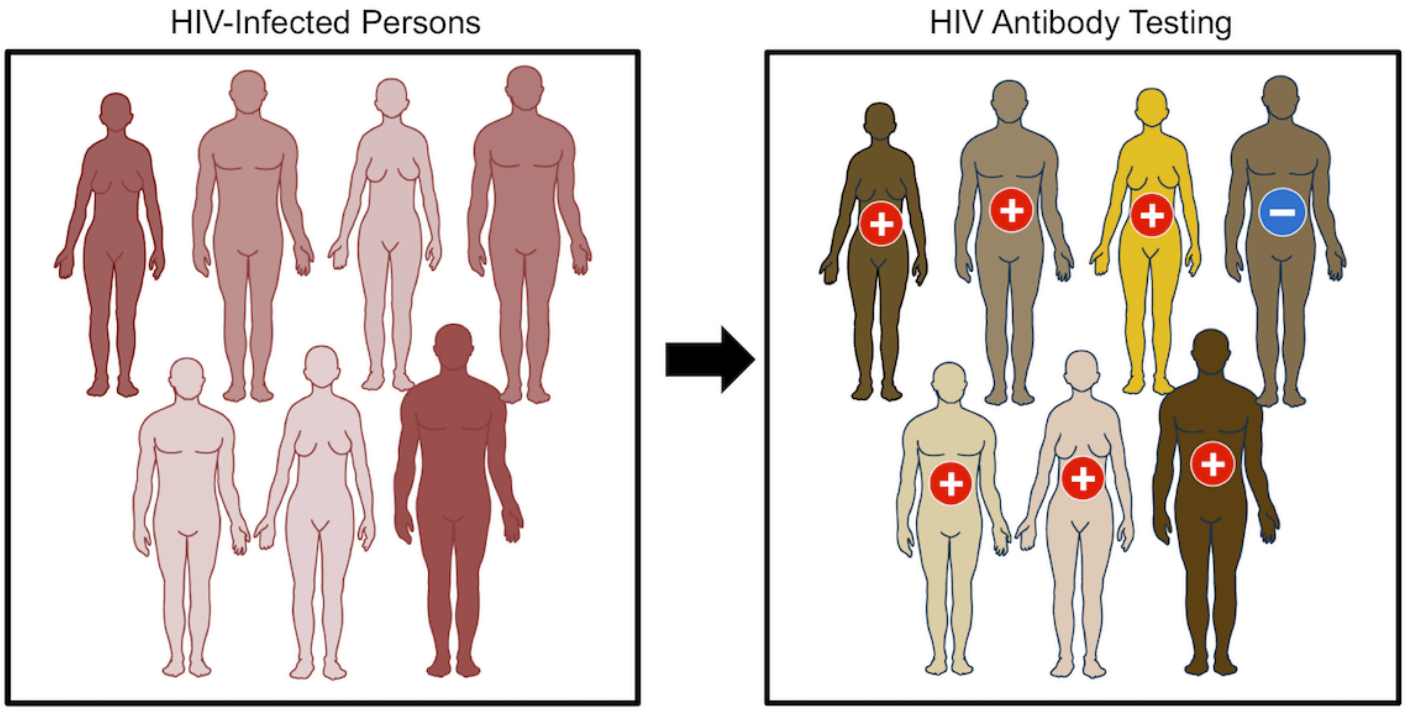

Figure 11 (Image Series) - False-Negative HIV Diagnostic Test
Image 11B: False-Negative Identified

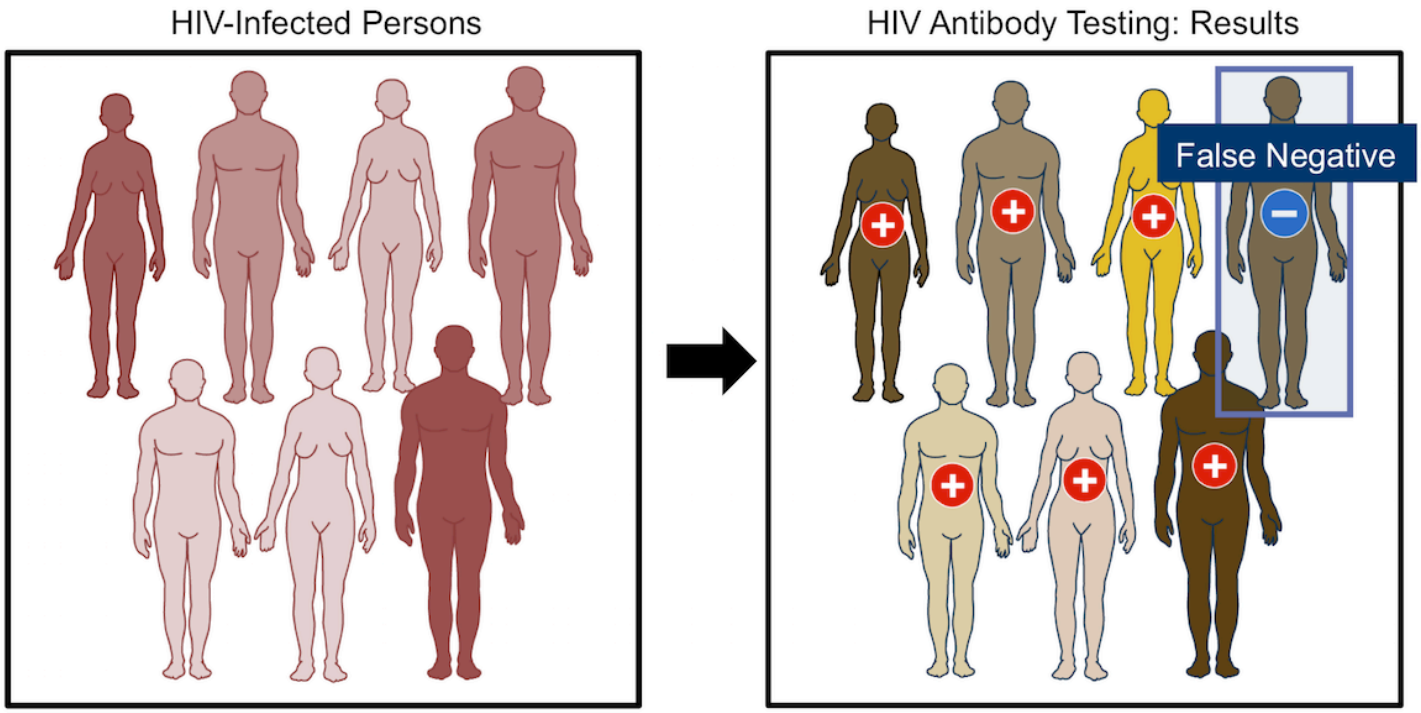

Figure 12 (Image Series) - False-Positive HIV Diagnostic Test (Image Series) - Figure 12 (Image Series) - False-Positive HIV Diagnostic Test
Image 12A: Test Results for Persons without HIV

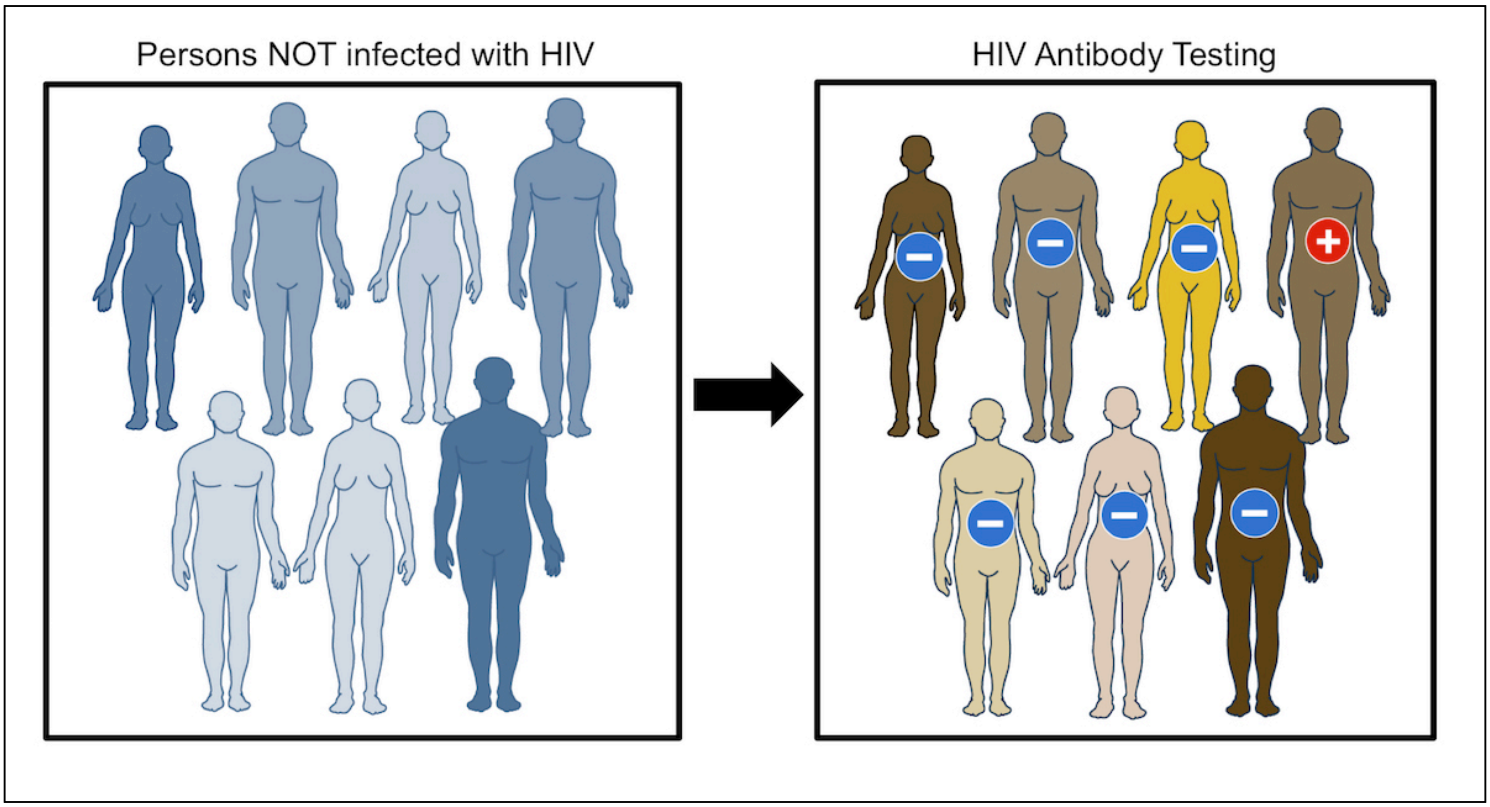

Figure 12 (Image Series) - False-Positive HIV Diagnostic Test
Image 12B: False-Positive Identified

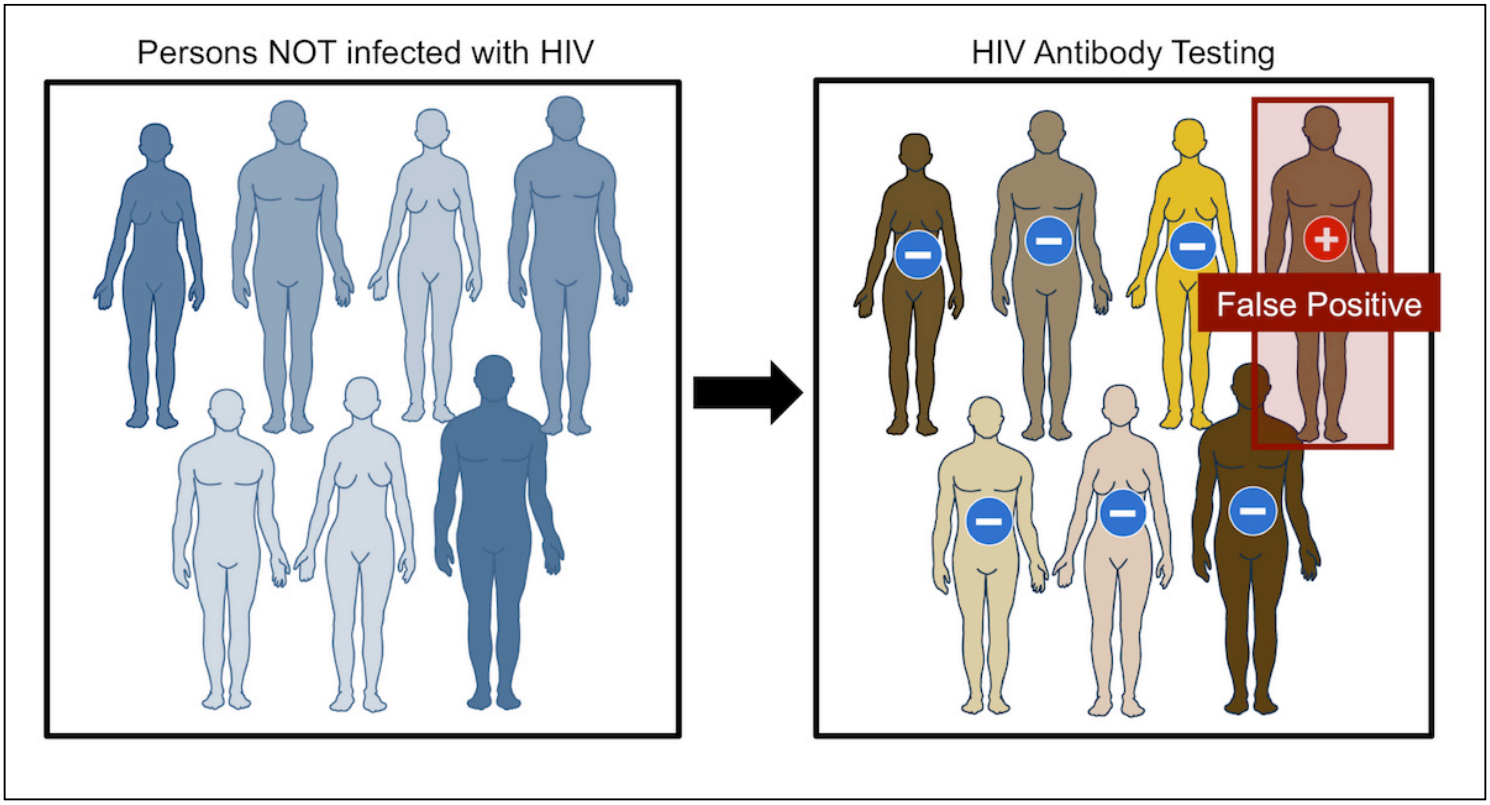

Figure 13 Diagnostic Test Performance in Persons with Acute HIV

During acute HIV (shown in shaded area), the typical pattern is positive HIV RNA, positive HIV p24 antigen, and negative anti-HIV antibodies. Note that with very early acute HIV, the HIV p24 antigen test may be negative. The colored circles indicate when the test typically becomes positive (blue for HIV RNA, green for HIV p24 antigen, and purple for HIV antibody).
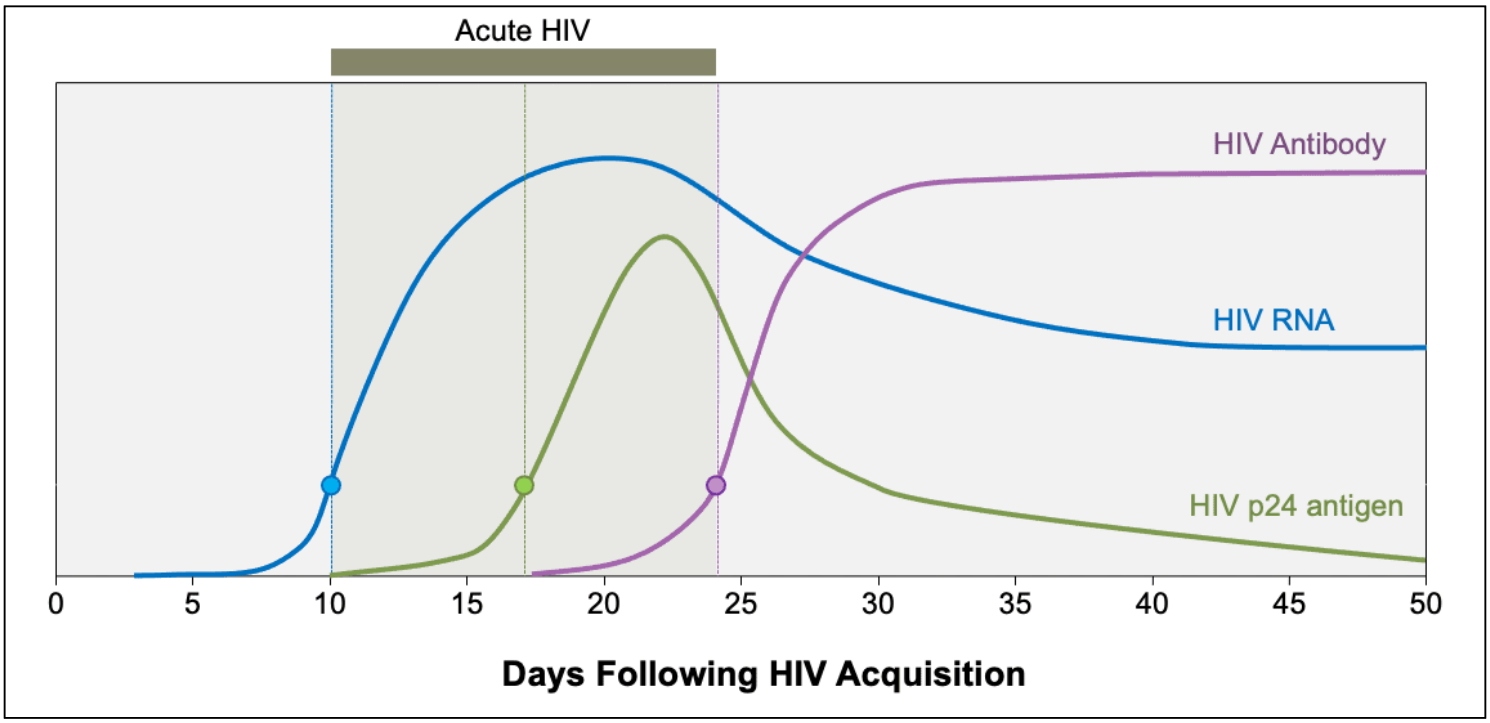
Illustration: David H. Spach, MD