CLINICAL GUIDELINES PROGRAM
NEW YORK STATE DEPARTMENT OF HEALTH AIDS INSTITUTE | HIV · HCV · SUBSTANCE USE · LGBT HEALTH

HIV Testing

Updates, Authorship, and Related Guidelines

Date of current publication	May 19, 2022
Highlights of changes, additions, and updates in the May 19, 2022 edition	• Box 1: NYS Public Health Law HIV Testing Requirements • Box 2: HIV Testing Services and Assistance Available Through the NYSDOH Wadsworth Center • HIV laboratory testing algorithm updated • "4th generation" is no longer used to refer to the HIV-1/2 antigen/antibody (Ag/Ab) immunoassay recommended as step 1 of the HIV laboratory testing algorithm • Available HIV Ag/Ab immunoassays • NYS exemption from CLIA-waived tests
Intended users	New York State care providers who do provide or should provide HIV testing to individuals who may be at risk of acquiring HIV or report a potential exposure
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Purpose of This Guideline

This guideline was developed by the New York State Department of Health (NYSDOH) AIDS Institute (AI) Clinical Guidelines Program to accomplish the following goals:

- Provide clinicians in NYS with up-to-date information on HIV testing policies and practices.
- Ensure awareness of and access to the standard 3-step HIV testing algorithm recommended by the Centers for Disease Control and Prevention (CDC) and the NYSDOH AI.
- Increase HIV testing in NYS to increase the number of people who know their HIV status.
- Ensure that clinicians recognize and respond to HIV testing as a gateway to care, such that an HIV diagnosis prompts a referral for HIV treatment and a negative HIV test result prompts a referral for HIV prevention services, including pre- and post-exposure prophylaxis (PrEP and PEP).
- Emphasize that rapid antiretroviral therapy (ART) initiation is the standard of care for all individuals diagnosed with HIV.
- Provide clinicians with information about the Wadsworth Center Bloodborne Viruses Laboratory services.

Accurate diagnosis or exclusion of HIV: This guideline provides an overview of the screening and diagnostic methods that are critical to accurate diagnosis or exclusion of HIV based on the CDC 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens and the Association of Public Health Suggested reporting language for the HIV laboratory diagnostic testing algorithm (see Figure 2: HIV Laboratory Testing Algorithm) [APHL 2019; CDC 2018; CDC 2014].

Widespread use of the HIV antigen (Ag)/antibody (Ab) immunoassay (formerly known as the "4th-generation" test) can increase the number of people aware of their HIV status, including those who may transmit HIV during acute infection. The 2018 CDC algorithm testing sequence for detecting HIV Ags, Abs, and nucleic acids differs from previous HIV testing recommendations based on Ab screening followed by Western blot confirmation [CDC 2018]. The updated algorithm features a specific sequence of tests to provide maximal sensitivity, specificity, and accuracy for HIV detection.

Rapid ART: The current standard of care for a newly diagnosed person is same-day ART initiation. If an individual cannot start ART on the day of diagnosis, then every effort should be made to initiate ART as soon as possible and no later than 30 days after diagnosis.

New York State Law and Testing Requirements

☆ NEW YORK STATE LAW

- Clinicians must perform diagnostic HIV laboratory tests in full compliance with New York State (NYS) HIV/AIDS Laws and Regulations.
- Clinicians must report confirmed cases of HIV according to NYS law (see NYSDOH Provider Reporting and Partner Services).
- Additional information regarding testing procedures and regulations is available from the NYSDOH Wadsworth Center (518-474-2163).

Accessible and routine HIV testing for all individuals ≥13 years old is intended to expand the number of people who know their HIV status and facilitate entry into the continuum of care or prevention. NYS public health law requires clinicians to offer HIV testing to all patients ≥13 years old who receive care in hospital or primary care settings. Performing an HIV test for all patients ≥13 years old is a critical clinical and public health intervention for people with or at risk of acquiring HIV.

HIV testing is not an isolated activity; it is the entry point to the continuum of care and prevention. When an HIV test result is reactive, NYS law specifies that the healthcare provider who ordered testing (or their representative) is responsible for providing or arranging immediate follow-up HIV care. A negative HIV screening test result affords a critical opportunity to assess whether routine prevention education, including information about post-exposure prophylaxis (PEP), or a referral for HIV pre-exposure prophylaxis (PrEP) are indicated.

Consent: In November 2016, amendments to NYS public health law removed the requirement for written or oral informed consent before an HIV test is ordered; see HIV Testing, Reporting and Confidentiality in New York State 2017-18 Update: Fact Sheet and Frequently Asked Questions.

Box 1, below, provides an overview of HIV testing requirements in NYS. See also: NYS Expanded HIV Testing.

Box 1: New York State (NYS) Public Health Law HIV Testing and Reporting Requirements	
Who to test	NYS law mandates that physicians offer an HIV test to all patients ≥13 years old (or younger with risk) if a previous test is not documented, even in the absence of symptoms consistent with acute HIV. For more information, see NYSDOH HIV Testing.
Consent	HIV testing remains voluntary, and patients have the right to refuse an HIV test, but obtaining written or oral consent for testing is no longer required in any setting. At a minimum, patients must be advised verbally that an HIV test is going to be performed.
Minor consent	Minors may consent to their own HIV testing, treatment, and/or prevention services (such as PrEP and PEP) without parent/guardian involvement.
Pre-test counseling	Before HIV testing is performed, information about HIV must be provided verbally, in writing, through signage, or in any other patient-friendly audio-visual format. Placing a NYSDOH HIV testing clinic poster in a visible location or providing patients with the NYSDOH patient brochure on HIV testing are easy and convenient ways to provide patients with this necessary information.

Box 1: New York State (NYS) Public Health Law HIV Testing and Reporting Requirements	
Post-test counseling	• *When testing indicates an HIV infection:* The clinician who ordered the HIV testing (or their representative) must provide the result to the patient, ensure the patient is scheduled for follow-up HIV care, and educate the patient on HIV transmission. • *When testing indicates no HIV infection:* The patient must be informed of the result and provided education on prevention options, including PrEP and PEP. This information may be in the form of written materials, such as the NYSDOH Information on Non-reactive (Negative) HIV Test Results. • *When testing indicates inconclusive or incomplete results:* The patient must be informed of the result and have an additional specimen collected to repeat the HIV testing algorithm.
Testing in pregnancy	HIV testing should be offered to pregnant individuals as early as possible during pregnancy and again during the third trimester for those who previously tested negative.
Reporting requirements	• NYS Public Health Law Article 21 (Chapter 163 of the Laws of 1998) requires the reporting of individuals with HIV as well as AIDS to the NYSDOH. The law also requires that reports contain the names of sex or needle-sharing partners known to the medical provider or whom the infected individual wishes to have notified. For more information, see NYSDOH Provider Reporting and Partner Services; also see NYC: How to Report a Diagnosis of HIV or AIDS. • See NYSDOH 2023 Changes to Provider Reporting of Human Immunodeficiency Virus (HIV) in New York State (November 2023) for updated reporting requirements, timelines, reporting methods, including instructions for accessing the HIV/AIDS Provider Portal. • Per the 2023 NYS Public Health Law update, the Medical Provider Report HIV/AIDS and Partner/Contact Report Form (DOH-4189) must be completed: – **Within 24 hours of an acute HIV infection diagnosis** – Within 7 days of receipt of laboratory results or diagnosis of HIV infection that is not acute or AIDS – HIV testing conducted in the context of insurance institution underwriting decisions is required to be reported by clinicians under whose medical license the HIV testing is ordered. Electronic reporting using the DOH-4189 form on the provider portal of the NYS Health Commerce System is preferred. • The DOH-4189 form can be completed electronically through the provider portal on the NYSDOH Health Commerce System. For information regarding Provider Portal access or to obtain printed copies of the PRF, call 518-474-4284.
Partner services	Clinicians must explain to all individuals with a new diagnosis of HIV the importance of notifying any sex or needle-sharing partners. Throughout the notification process, names or personal identifiers, including the dates of exposure, are never revealed to partners. The anonymity and privacy of the original patient is the highest priority. For more information, see NYSDOH Information on Partner Services.
Nomenclature	In NYS, the terms "clinical/symptomatic HIV illness or AIDS," "AIDS or HIV-related illness," and other similar terms shall mean laboratory-confirmed HIV diagnosis (source: NYSDOH June 2016 Policy Statement: Defining Program Eligibility by HIV Status).
Resources	• NYSDOH 2023 Changes to Provider Reporting of Human Immunodeficiency Virus (HIV) in New York State • NYSDOH: HIV Testing, Reporting and Confidentiality in New York State 2017-18 Update: Fact Sheet and Frequently Asked Questions • HIV/AIDS Laws and Regulations: Reporting and Partner Services • What Health Care Providers Need to Know about Partner Services • Occupational Exposure and HIV Testing: Fact Sheet and Frequently Asked Questions • CEI PEP and PrEP Line: 866-637-2342
Abbreviations: PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.	

Time to HIV Detection

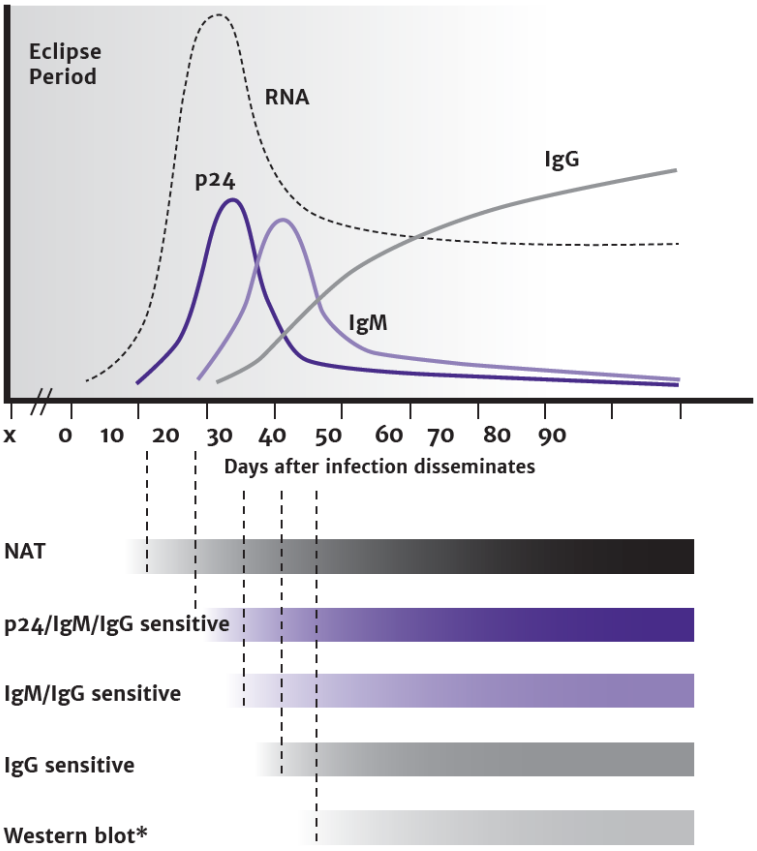
Establishing the exact window period for an initial HIV test is challenging because the precise time of exposure is rarely known. Among individuals on PrEP who have breakthrough infections, the exact window period may be even more difficult to define due to partial suppression of viral replication and possible blunted or delayed immune response. PEP may also alter the time to detection of HIV.

Eclipse period refers to the time following an HIV exposure during which no available HIV test can detect the virus (see Figure 1, below). The duration of the eclipse period varies depending on characteristics of the infecting virus and the person infected [Fiebig, et al. 2003]. A study that applied modeling methods to estimate the time from exposure with subsequent infection to HIV RNA detection for people not receiving PrEP reported the median length of the eclipse period as 11.5 days [Delaney, et al. 2017].

Window period refers to the period between an HIV exposure and when a test can detect HIV. The duration varies by person, test, and use of antiretrovirals (ARVs) as PrEP or PEP. It starts at the earliest time a test can accurately detect HIV and ends when the test consistently detects the biomarker quantified by the test. The antigen (Ag)/antibody (Ab) immunoassays detect HIV-1 and HIV-2 Abs and HIV-1 p24 Ag, which is present during acute HIV before Ab seroconversion (Ab production).

PrEP use can delay time from infection to seroconversion [Spinelli, et al. 2021; Lee, et al. 2020]. Very early treatment of acute HIV infection can, uncommonly, lead to delayed seroconversion or even seroreversion [Stekler 2022; Hare, et al. 2006; Kassutto, et al. 2005].

Figure 1: HIV Test Window of Detection [a,b]



* Western blot is no longer used for HIV.

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; NAT, nucleic acid test.

Notes:

a. Figure reproduced from CDC: HIV Diagnostic Tests.
b. Without PrEP or PEP exposure; PrEP or PEP exposure may delay seroconversion. Very early treatment of acute HIV infection may also alter the serologic response [Stekler 2022; Hare, et al. 2006; Kassutto, et al. 2005].

HIV Testing With the Standard 3-Step Algorithm

☑ RECOMMENDATIONS

Step 1: HIV-1/2 Antigen/Antibody Immunoassay

- For initial HIV testing (aka "screening"), clinicians should use an HIV-1/2 Ag/Ab immunoassay (formerly known as the "4th-generation" test). (A2)
- For initial testing of newborns or individuals who are in labor, being evaluated for PEP, or unlikely to return for test results, clinicians should use an FDA-approved HIV screening test that provides results within 60 minutes (A2); otherwise, rapid tests are not recommended for step 1 of the standard HIV laboratory testing algorithm.
- Because all initial HIV tests are subject to false positive results, clinicians should consider all reactive initial test results preliminary and perform appropriate laboratory diagnostic testing to confirm a patient's HIV status. (A1)
- Clinicians should educate patients about the limitations of in-home testing and emphasize that a laboratory should repeat both nonreactive and reactive results of any in-home HIV testing. (A3)
- In the case of a nonreactive result, the clinician should discuss goal-oriented, harm-reduction strategies, including PrEP and emergency PEP, with any patient who reports recent or likely ongoing HIV risk exposures or refer the patient for prevention services. (A3)
- Clinicians should offer repeat HIV testing every 3 months, or sooner if acute HIV is suspected, for as long as an individual remains at high risk of HIV exposure. (A3)

Step 2: HIV-1/HIV-2 Antibody Differentiation Immunoassay

- Per the standard HIV laboratory testing algorithm, if a reactive result is obtained with an HIV-1/2 Ag/Ab immunoassay test (step 1), clinicians should perform supplemental testing (step 2) with an FDA-approved HIV-1/HIV-2 Ab differentiation immunoassay. (A1)
- If the result of the HIV Ab differentiation immunoassay (step 2) is positive for HIV-1 or HIV-2 Abs, the clinician should provide or refer the patient for rapid ART initiation and transmission prevention counseling. (A1)
  - Note: If the HIV Ab differentiation immunoassay result is positive but undifferentiated (i.e., reactive for both HIV-1 and HIV-2), repeat testing may determine if the patient has HIV-1 or HIV-2 infection.

Step 3: HIV-1 Nucleic Acid Testing (qualitative or quantitative HIV RNA testing)

- If the HIV-1/2 Ab differentiation immunoassay (step 2) result is nonreactive (negative) or indeterminate (neither positive nor negative for HIV-1 or HIV-2), and the lab does not perform reflex testing, the clinician should immediately order HIV-1 RNA NAT (step 3) to detect the presence of HIV-1 RNA and confirm or exclude HIV-1 infection. (A*)
- If HIV-1 RNA is detected, the clinician should inform the patient of the acute HIV-1 diagnosis, recommend ART initiation, and prioritize counseling to prevent HIV transmission. (A1)
- Clinicians should not wait for serologic confirmation of HIV to initiate ART when pregnant individuals are diagnosed with acute HIV infection by HIV-1 NAT; initiation of ART is strongly recommended for pregnant individuals. (A2)
  - See DHHS: Recommendations for the Use of Antiretroviral Drugs During Pregnancy and Interventions to Reduce Perinatal HIV Transmission in the United States.
- To determine the HIV status of an infant born to an individual with HIV-1, clinicians should perform HIV-1 RNA NAT. (A1)

Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CEI, Clinical Education Initiative; DHHS, U.S. Department of Health and Human Services; FDA, U.S. Food and Drug Administration; NAT, nucleic acid testing; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

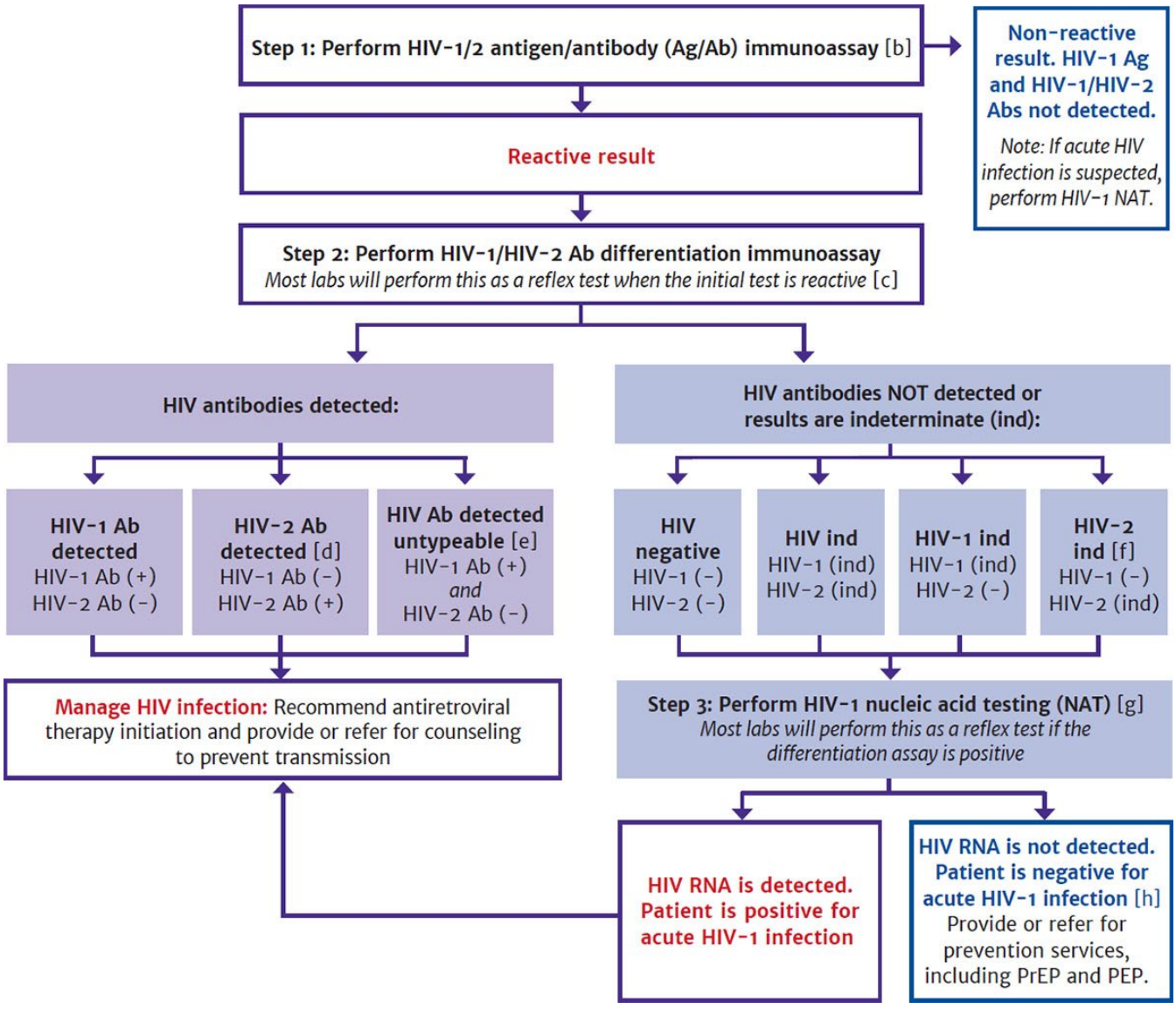
HIV-1/2 Antigen/Antibody Immunoassay (Step 1)

In 2018, the CDC issued a revised 3-step HIV testing algorithm for HIV diagnostic testing performed on serum and plasma specimens [CDC 2018; CDC 2014]. Advances in immunoassay technology have improved the sensitivity and specificity of HIV screening and diagnostic tests, which detect specific infection markers that may be virologic (viral proteins or nucleic acids) or immunologic (Abs produced in response to HIV infection). When requesting HIV diagnostic testing of anyone ≥2 years old, testing should be ordered from a laboratory that offers an FDA-approved HIV Ag/Ab immunoassay as an initial HIV test [CDC

2018; CDC 2014]. If the initial Ag/Ab immunoassay is reactive, the laboratory will usually follow the recommended algorithm steps to confirm or exclude laboratory evidence of HIV (see Appendix: HIV Immunoassays Available in New York State).

Figure 2, below, represents the 3-step standard HIV testing algorithm recommended by this committee and the CDC [CDC 2018]. Box 2, below, describes the testing services and assistance available through the NYSDOH Wadsworth Center.

Figure 2: HIV Laboratory Testing Algorithm [a]



Abbreviations: Ab, antibody; Ag, antigen; APHL, Association of Public Health Laboratories; CDC, Centers for Disease Control and Prevention; ind, indeterminate; FDA, U.S. Food and Drug Administration; NAT, nucleic acid test; NYSDOH, New York State Department of Health; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

Notes:

a. Adapted from CDC 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens and APHL Suggested reporting language for the HIV laboratory diagnostic testing algorithm.
b. APHL and CDC continue to recommend that laboratories use an FDA-approved instrumented HIV-1/HIV-2 Ag/Ab immunoassay as the initial assay in the laboratory HIV testing algorithm for serum or plasma due to their superior sensitivity for detecting acute HIV infection. However, the FDA-approved single-use rapid HIV-1/HIV-2 Ag/Ab immunoassay may be used as the initial assay in the laboratory HIV testing algorithm for serum or plasma if an instrumented assay is not available.
c. Become familiar with the laboratory's internal testing algorithm and results-reporting policies. Many labs will reflex additional screening steps (such as HIV Ab differentiation immunoassay and HIV RNA) on the original sample without supplemental orders. Other labs may require additional samples or supplemental orders to complete all steps in the algorithm.
d. This includes specimens reported as HIV-2 positive with HIV-1 cross-reactivity.
e. Further testing may be performed to determine type.

Figure 2: HIV Laboratory Testing Algorithm [a]

f. Per the Geenius package insert, specimens with this final assay interpretation should be retested with a new cartridge. If the final assay interpretation is again HIV-2 indeterminate, it should be reported as such and followed with an HIV-1 NAT.
g. Most laboratories reflex directly to an HIV-1 RNA test without requiring an additional test order or new specimen, either by performing the test in-house or referring the specimen to another laboratory. If the laboratory is unable to or does not automatically reflex directly to the RNA test, clinicians should order an HIV-1 RNA test as soon as possible. To reflex directly to an HIV-1 RNA test, a test kit approved by either the FDA or NYSDOH to aid in diagnosing HIV-1 infection is required. If HIV-1 RNA is detected, acute HIV-1 is present, and clinicians should proceed with clinical evaluation. If no HIV-1 RNA is detected, the initial immunoassay result is presumed false positive.
h. A negative HIV-1 NAT result and repeatedly HIV-2 indeterminate or HIV indeterminate Ab differentiation immunoassay result should be referred for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2 to 4 weeks to assess HIV-2 infection.

Box 2: HIV Testing Services and Assistance Available Through the NYSDOH Wadsworth Center

HIV-1/HIV-2 Diagnostic Testing (Phone: 518-474-2163)

- HIV-1/2 Ag/Ab testing; plasma and serum
- HIV-1/HIV-2 supplemental Ab testing of specimens that are reactive on the initial testing assay
- HIV-1 RNA testing on plasma, serum, and dried blood spots
- HIV-2 NAT to quantify HIV-2 RNA
- Assistance with inconclusive HIV test results
- Confirmatory testing of dried blood spots for HIV testing sites that cannot collect venous blood
- More information

HIV Testing for Newborns (Phone: 518-486-9605)

- HIV testing for all newborns exposed to HIV (HIV-1 and HIV-2) in NYS, free of charge
- If a sample is reactive for HIV-2 antibodies, the Pediatric HIV Testing Service will perform an RT-PCR test for qualitative detection of HIV-2 RNA (Phone: 518-486-9605)
- NYSDOH strongly recommends that all NYS birth facilities use the Pediatric HIV Testing Service at the Wadsworth Center, which is free of charge for NYS clinicians providing care for HIV-exposed infants
- More information

HIV-2 Viral Load Testing (Phone: 518-473-6007)

- Quantitative and qualitative HIV-2 viral load testing (free of charge), and quantitative detection of HIV-2 RNA in plasma samples for baseline and subsequent monitoring of response to ART in patients with confirmed HIV-2 infection
- HIV-2 RNA viral load testing during pregnancy. Contact the lab early in the patient's pregnancy to discuss the protocol and timing for testing
- More information

Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; NAT, nucleic acid testing; RT-PCR, reverse transcription polymerase chain reaction.

Screening for acute or established infection: In following the standard HIV laboratory testing algorithm, laboratories should perform initial HIV testing with an FDA-approved Ag/Ab immunoassay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen. Initial testing screens for established HIV-1 or HIV-2 infection and acute HIV-1 infection. Confirming the presence of Abs to HIV-1 and HIV-2 identifies the majority of new HIV infections.

Results and next steps: A nonreactive initial Ag/Ab immunoassay is a negative HIV test result when used during routine HIV screening. If the initial test result is reactive, then supplemental testing with an HIV-1/HIV-2 Ab differentiation immunoassay is performed to rule out a false positive result. When the standard HIV laboratory testing algorithm is followed, laboratory reporting may include the initial HIV test result and supplemental testing results if it is reactive. A single test for establishing a laboratory diagnosis of HIV infection is not recommended; interpreting the complete set of results from a specific sequence of initial and supplemental tests is now recommended to establish laboratory evidence of HIV. Full results confirm reactivity and include an HIV-1/2 Ab differentiation immunoassay that detects and discriminates between HIV-1 and HIV-2 Abs with

high specificity. If the supplemental antibody test (step 2) is negative or indeterminate, an HIV-1 RNA NAT (step 3) can verify acute HIV-1 infection (see Box 3, below).

HIV Ag/Ab immunoassay advantage: HIV-1/2 Ag/Ab immunoassays, formerly known as "4th-generation" immunoassays, detect both immunoglobulin G and M antibodies to HIV-1 and HIV-2 plus HIV-1 p24 antigen. These Ag/Ab immunoassays have a distinct advantage over screening tests that detect only Abs. Although these immunoassays cannot detect HIV during the eclipse period, when neither Ag nor RNA is detectable, the ability to identify both HIV-1 p24 Ag and HIV-1/2 Abs in a single screening test enables detection of HIV early in the acute period and throughout established infection, including in HIV controllers (see Table A.1: FDA-Approved HIV-1/2 Ag/Ab Immunoassays for Step 1 of the CDC Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens).

→ KEY POINTS

- Become familiar with the laboratory's internal testing algorithm and results-reporting policies. Many labs will reflex additional screening steps (such as HIV Ab differentiation immunoassay and HIV RNA) on the original sample without supplemental orders. Other labs may require additional samples or supplemental orders to complete all steps in the algorithm.
- When possible, collect blood by venipuncture for laboratory submission.
- Consult the specimen collection and handling instructions provided by the laboratory to ensure the specimen will be suitable for all tests in the algorithm.
- As seroconversion proceeds and HIV Abs are produced, p24 is bound in an Ag-Ab complex and becomes more difficult to detect by standard laboratory assays. Serologic assays that detect only p24 Ag are *not* recommended for any diagnostic purpose and are not available at most clinical laboratories.
- NYSDOH strongly recommends that all NYS birth facilities use the Pediatric HIV Testing Service at the Wadsworth Center, which is free of charge for NYS clinicians providing care for HIV-exposed infants. For information about this service, contact the Wadsworth Center at 518-486-9605.

Available HIV Ag/Ab immunoassays: As of February 11, 2022, 7 FDA-approved HIV Ag/Ab immunoassays are available; all are approved for use in step 1 of the recommended HIV laboratory testing algorithm (see CDC: Advantages and Disadvantages of FDA-Approved HIV Assays Used for Screening). The Abbott Determine HIV-1/2 Ag/Ab Combo is the only FDA-approved HIV-1/2 Ag/Ab immunoassay rapid screening test. The CDC states that laboratory HIV-1/2 Ag/Ab immunoassays are preferred for use in step 1 of the algorithm due to their superior sensitivity for detecting HIV during acute infection. The Abbott Determine HIV-1/2 Ag/Ab Combo may be used with serum or plasma (not fingerstick whole blood) in this step when laboratory testing is not feasible [CDC 2017].

Although Ag/Ab immunoassays are recommended for laboratories performing HIV testing, some laboratories may still use less sensitive assays for HIV screening. If an Ag/Ab immunoassay is not available, a laboratory-based HIV Ab immunoassay (3rd generation) test may be used. HIV Ab differentiation immunoassay offers the next best sensitivity for early detection; however, early acute HIV-1 infections may not be detected by an Ab differentiation immunoassay and it is only approved for supplemental testing to differentiate HIV-1/2 [CDC 2018; CDC 2014]. Several studies have shown that the HIV-1/2 Ab differentiation immunoassay and HIV-1 RNA test (NAT) performed according to the recommended laboratory algorithm are better than the Western blot for confirming a reactive HIV Ab differentiation immunoassay result [Nasrullah, et al. 2013; Delaney, et al. 2011; Styer, et al. 2011; Wesolowski, et al. 2011].

Confirming a rapid screening test result: A reactive result to a rapid screening test should be given to the patient as soon as possible. Rapid screening tests may produce false positive results, particularly in populations not at high risk of HIV infection, and supplemental testing must be performed to confirm a reactive screening result.

If a rapid HIV screening test was performed on oral fluid or fingerstick whole blood, a blood specimen should be collected by venipuncture and handled according to the laboratory's instructions.

The CDC advises laboratories to use the recommended HIV diagnostic testing algorithm to confirm all reactive rapid screening test results, including the Abbott Determine HIV-1/2 Combo rapid test when conducted on whole blood. An exception may be made when serum or plasma is screened with the Abbott Determine.

If reactive, the specimen may be tested directly with the supplemental HIV Ab differentiation immunoassay. The laboratory should test the confirmatory specimen with an HIV-1/2 Ag/Ab immunoassay approved for step 1 of the HIV diagnostic algorithm. If the specimen is not reactive, the rapid screening test result is interpreted as false positive, and no further testing is needed. If the immunoassay is reactive, specimen testing should continue according to the algorithm.

Collecting a tube of blood following a reactive rapid screening test may not always be possible or practical; supplemental testing of alternative specimen types may be necessary. The NYSDOH Wadsworth Center offers confirmatory testing of dried blood spots using an alternative algorithm for enrolled community-based HIV screening sites unable to collect venous blood.

Box 3: Reasons for False Positive, False Negative, or Indeterminate HIV Test Results [a]

False Positive Results	False Negative Results	Comments
• Reduced specificity associated with increased assay sensitivity • Technical errors, including specimen mix-up or mislabeling, contamination, improper handling, and misinterpretation of results • Presence of HIV Abs in recipients of HIV-1 trial vaccines • Other rare possibilities: Hypergammaglobulinemia/Abs reactive to cellular components; cross-reactivity with influenza vaccine that causes cross-reactivity with HIV Ab assays (time course for such cross-reactivity remains uncertain)	• Test is performed during any of the following periods: Eclipse period before detection of Ag or HIV RNA is possible; during acute infection (before seroconversion) when using a method that detects Abs only; during the early stage of seroconversion when using a method that does not detect early (IgM) Abs • Technical errors, including specimen mix-up or mislabeling, contamination, improper handling, and misinterpretation of results • Other possibilities: Delayed seroconversion in infants, in those who have concurrent acute HCV infection, due to PEP or PrEP use, or due to ART initiation very early during acute HIV; diminished immune response in patients receiving intensive or long-term immunosuppressive therapy; congenital or drug-induced hypogammaglobulinemia or agammaglobulinemia; insufficient host Ab response (e.g., advanced HIV disease); unavailability of Abs due to the formation of Ag-Ab complexes	• Individual laboratories may have different internal protocols for reporting preliminary HIV test results. Indeterminate, inconclusive, nondiagnostic, and pending confirmation are among the terms used when preliminary results cannot be classified definitively • A reactive result on the initial screening test with inconclusive supplemental serologic testing may represent either a false or true positive. The laboratory should be contacted to determine the significance of the nondefinitive results and determine supplemental testing • Determining the significance of nondefinitive results is particularly important when testing pregnant individuals, newborn children, and patients with suspected acute HIV or HIV-2

Abbreviations: Ab, antibody; Ag, antigen; HCV, hepatitis C virus; IgG, immunoglobulin G; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

Note:

a. See Centers for Disease Control and Prevention: False-Positive HIV Test Results for more information.

HIV-1/HIV-2 Antibody Differentiation Immunoassay (Step 2)

Specimens with a reactive Ag/Ab immunoassay result (or repeatedly reactive, if repeat testing is recommended by the manufacturer or required by regulatory authorities) should be tested with an FDA-approved supplemental immunoassay that differentiates HIV-1 Abs from HIV-2 Abs. If the initial Ag/Ab immunoassay is reactive and the HIV-1/2 Ab differentiation immunoassay is positive for HIV-1 Abs, HIV-2 Abs, or HIV Abs, this should be interpreted as positive for HIV. If the specimen is positive for HIV Abs but cannot be differentiated as HIV-1 or HIV-2, clinicians should evaluate for HIV and contact the NYSDOH Wadsworth Center to obtain HIV-1 and HIV-2 RNA testing.

Geenius HIV 1/2 supplemental assay: On October 24, 2014, the Geenius HIV 1/2 Supplemental Assay (Bio-Rad Laboratories) received FDA approval for the confirmation and differentiation of individual HIV-1 and HIV-2 Abs. It is currently the only FDA-approved test for use in step 2 of the standard HIV laboratory testing algorithm.

This single-use immunochromatographic assay can confirm and differentiate individual HIV-1 and HIV-2 Abs in specimens found to be reactive by initial diagnostic screening. It is approved for use with whole blood, serum, or plasma specimens. Geenius uses 4 HIV-1 Ags derived from the core (p24), polymerase (p31), and envelope (gp41, gp160) proteins and 2 HIV-2 envelope Ags (gp36 and gp140). The test produces results within 30 minutes, and results must be read with the Geenius Reader system, which uses validated software to interpret the test results. The Geenius Reader can electronically transmit results to the laboratory's information system, eliminating subjective result interpretation and error-prone manual data transcription.

If the final assay interpretation of the Geenius HIV 1/2 Supplemental Assay is "HIV-1 Positive," "HIV-2 Positive," or "HIV Positive," then Abs are considered confirmed (see the package insert for additional information on the results reported in the final assay interpretation). The Geenius Reader may also produce a final assay interpretation that is indeterminate for either HIV-1, HIV-2, or untypable HIV.

If the test is nonreactive or indeterminate for any HIV type (HIV-1, HIV-2, or untypable HIV), the next step should be to test the specimens for HIV-1 RNA (qualitative or quantitative HIV RNA NAT), even if the result is HIV-2 indeterminate. Acute HIV-1 infection is much more common than HIV-2 infection, and nonspecific reactivity could cause an HIV-2 indeterminate result to occur in some cases. If HIV-1 RNA is not detected and the Geenius Reader result was HIV-2 indeterminate or HIV indeterminate, then an HIV-2 NAT may be warranted.

→ KEY POINTS

- If the Geenius HIV 1/2 Supplemental Assay interpretation is nonreactive or indeterminate for any HIV type (HIV-1, HIV-2, or untypable HIV), test the specimens for HIV-1 RNA, even if the result is HIV-2 indeterminate.
- Nonspecific reactivity could cause an HIV-2 indeterminate result to occur in some cases.
- If HIV-1 RNA is not detected and the Geenius Reader interpretation is HIV-2 indeterminate or HIV indeterminate, an HIV-2 NAT may be warranted.

HIV-1 RNA Nucleic Acid Testing (Step 3)

If the HIV-1/2 Ab differentiation immunoassay is nonreactive or indeterminate, then HIV-1 RNA NAT (qualitative or quantitative) should be performed immediately to confirm or exclude HIV-1 infection.

For specimens that are reactive on the initial Ag/Ab immunoassay and nonreactive or indeterminate on the HIV-1/2 Ab differentiation immunoassay, the standard HIV laboratory testing algorithm recommends HIV RNA testing with an FDA-approved HIV-1 NAT, with results interpreted as follows:

- A reactive HIV-1 NAT result and nonreactive HIV-1/2 Ab differentiation immunoassay result indicates laboratory evidence for acute HIV-1 infection.
- A reactive HIV-1 NAT result and indeterminate HIV-1/2 Ab differentiation immunoassay result indicates the presence of HIV-1 infection confirmed by HIV-1 Abs.
- A negative HIV-1 NAT result and nonreactive or indeterminate HIV-1/2 Ab differentiation immunoassay result indicates a false positive result on the initial immunoassay.

Most laboratories reflex directly to an HIV-1 RNA test without requiring an additional test order or new specimen, either by performing the test in-house or referring the specimen to another laboratory. To reflex directly to an HIV-1 RNA test, a test kit approved by either the FDA or NYSDOH to aid in diagnosing HIV-1 infection is required. If HIV-1 RNA is detected, acute HIV-1 is present, and clinicians should proceed with clinical evaluation. If no HIV-1 RNA is detected, the initial immunoassay result is presumed false positive.

→ KEY POINT

- If the person being tested is taking antiretroviral agents as PEP, PrEP, or for rapid ART initiation, a false negative result for the HIV-1 RNA test may occur.

Available HIV-1 RNA qualitative assay: Currently, the only NAT kit approved by the FDA for diagnostic use is the APTIMA HIV-1 RNA Qualitative Assay (Hologic Gen-Probe Inc). This NAT detects a specific region of the HIV-1 viral RNA genome by transcription-mediated amplification (TMA), a nucleic acid amplification method similar in principle to polymerase chain reaction (PCR). TMA differs from PCR in that the amplification occurs on a linear rather than logarithmic scale, and the

amplification product is composed of single-stranded RNA rather than double-stranded DNA. TMA is FDA-approved for use with serum or plasma specimens and produces a qualitative result (i.e., "Detected" or "Not Detected").

Data from analytical sensitivity studies presented in the package insert indicate that the APTIMA HIV-1 RNA Qualitative Assay achieved >98.5% detection for specimens containing 30 copies/mL of HIV-1 RNA and 100% detection for specimens containing 100 copies/mL. This detection level was also verified for HIV-1 specimen panels consisting of subtypes A, B, C, D, E, F, and G. The APTIMA HIV-1 RNA Qualitative Assay is performed by the NYSDOH and New York City Department of Health and Mental Hygiene public health laboratories and at several commercial laboratories; others are unable to support its use because of the expense and low volume of specimens that require qualitative RNA testing. Many laboratories already perform HIV-1 quantitative testing for viral load monitoring, and maintaining an additional qualitative test for diagnostic purposes may be impractical and not economically feasible.

The NYSDOH strongly recommends that all NYS birth facilities use the Pediatric HIV Testing Service at the Wadsworth Center. The Wadsworth Center uses the APTIMA HIV-1 RNA Qualitative Assay, which has been demonstrated to identify HIV earlier in non-breastfed infants than methods based on PCR amplification of proviral DNA.

Quantitative HIV-1 RNA tests: Quantitative HIV-1 RNA tests are widely available and approved by the FDA only to monitor the prognosis of HIV-1 infection and response to ART. Although regulatory restrictions may prevent laboratories from reflexing to a quantitative HIV-1 RNA test as part of the diagnostic testing algorithm, the NYSDOH recommends that clinicians order quantitative HIV-1 RNA for the presumptive diagnosis of acute HIV. With a quantitative HIV-1 RNA test, an HIV viral load ≥5,000 copies/mL is used to diagnose acute HIV when there is no antiretroviral exposure. A lower threshold of ≥200 copies/mL is appropriate for those receiving PrEP or PEP. The performance qualities of the HIV-1 viral load tests are discussed further in the NYSDOH AI guideline Virologic and Immunologic Monitoring in HIV Care.

For further guidance in the identification and management of acute HIV infection, see the NYSDOH AI guideline Diagnosis and Management of Acute HIV Infection.

→ A NEW HIV DIAGNOSIS IS A CALL TO ACTION

- In support of the NYSDOH AI January 2018 call to action for patients newly diagnosed with HIV, the Medical Care Criteria Committee stresses the following:
  - Immediate linkage to care is essential for any individual diagnosed with HIV.
  - For the individual with HIV, ART dramatically reduces HIV-related morbidity and mortality.
  - Viral suppression helps prevent HIV transmission to sex partners of people with HIV and prevents perinatal transmission of HIV.
- ART initiation is urgent if the newly diagnosed patient is pregnant, has acute HIV infection, is ≥50 years old, or has advanced HIV disease. Every effort should be made to initiate ART immediately for these patients, ideally on the same day as diagnosis.
- All clinical care settings should be prepared, either on-site or with a confirmed referral, to support patients in initiating ART as rapidly as possible after diagnosis.

Diagnosis of HIV-2 Infection

☑ RECOMMENDATION

Diagnosis of HIV-2 Infection

- When HIV-2 antibodies are detected, clinicians should perform a clinical evaluation for HIV-2 infection that is similar in scope to the evaluation of patients with HIV-1. (A1)

Box 4: HIV-2 Related Services Available Through the Wadsworth Center

The following services are available through the Wadsworth Center (phone: 518-474-2163):

- Quantitative detection of HIV-2 RNA in plasma samples for baseline and subsequent monitoring of response to antiretroviral therapy in patients with confirmed HIV-2 infection.
- HIV-2 RNA viral load testing during pregnancy. Contact the lab early in a patient's pregnancy to discuss the testing protocol and timing.
- HIV testing for all New York State newborns exposed to HIV (HIV-1 and HIV-2), free of charge. The Pediatric HIV Testing Service will perform a reverse transcription polymerase chain reaction test for qualitative detection of HIV-2 RNA in all samples positive for HIV-2.

Note: HIV-2 phenotypic and genotypic resistance testing is not offered at the Wadsworth Center or commercially available in the United States.

HIV-2 antibodies (Abs) are confirmed by a reactive result to an HIV-1/2 antigen (Ag)/Ab immunoassay (step 1) and detection of HIV-2 Abs on a supplemental HIV-1/HIV-2 Ab differentiation assay (step 2). See the NYSDOH AI guideline Diagnosis and Management of HIV-2 in Adults for management recommendations.

Before the HIV-1/2 Ag/Ab and HIV-1/HIV-2 Ab differentiation immunoassays for HIV testing became widely available, clinicians suspected chronic HIV-2 infection in certain clinical scenarios, such as a declining CD4 cell count in an HIV-1–seropositive, untreated individual with an undetectable HIV-1 plasma viral load, or an opportunistic infection in an individual from West Africa who is not HIV-1 seropositive.

Currently, all HIV testing performed according to the Centers for Disease Control and Prevention (CDC) HIV testing algorithm begins with a U.S. Food and Drug Administration-approved HIV-1/2 Ag/Ab combination immunoassay [CDC 2018], which detects HIV-1 p24 antigen and HIV-1 and HIV-2 Abs but not HIV-2 Ag. If the combination immunoassay is reactive, a supplemental HIV-1/HIV-2 Ab differentiation assay is performed. There are 4 scenarios, described below, in which clinicians should consider HIV-2 infection.

- **HIV-1/HIV-2 differentiation assay is reactive for HIV-2 antibody:** The individual is considered HIV-2 antibody positive, and a clinical evaluation for HIV-2 infection should be performed.
- **HIV-1/HIV-2 differentiation assay is reactive for HIV-1 and HIV-2 antibody:** The individual is considered HIV positive, undifferentiated, and HIV-1 RNA and HIV-2 RNA or DNA testing should be performed to confirm or exclude HIV-1/HIV-2 coinfection. A minority of individuals with HIV-2 are coinfected with HIV-1. Qualitative and quantitative HIV-2 viral load testing is available by contacting the Wadsworth Center (see Box 4, above).
- **HIV-1/HIV-2 differentiation assay is nonreactive or indeterminate for HIV-1 and/or HIV-2 antibody:** Plasma HIV-1 RNA testing should be performed to confirm or exclude acute HIV-1 infection [CDC 2018].
  - If the Ab differentiation assay is nonreactive or HIV-1 indeterminate and HIV-1 RNA is not detected, the individual is considered negative for HIV-1 and HIV-2.
  - If the Ab differentiation assay is either HIV-2 indeterminate or HIV indeterminate and HIV-1 RNA is not detected, then HIV-2 RNA testing may be used if there is a suspicion for HIV-2 infection. However, because HIV-2 RNA levels can be low or undetectable in a person with HIV-2 infection, the absence of HIV-2 RNA does not exclude HIV-2 infection. Therefore, in a person at high risk for HIV-2 infection who has undetectable HIV-2 RNA, clinicians should consider testing for HIV-2 DNA or repeating the HIV testing algorithm in 2 to 4 weeks, starting with the HIV-1/2 Ag/Ab immunoassay. If results remain unclear, clinicians may consider obtaining other HIV-2–specific tests through public health or commercial laboratories or the CDC.
- **Nonreactive HIV-1/2 Ag/Ab immunoassay and suspected recent exposure to HIV-2** (e.g., exposure from a sex partner from an HIV-2 endemic area)**:** HIV-2 RNA testing may be required or the HIV testing algorithm may be repeated, beginning with the HIV-1/2 Ag/Ab immunoassay, 4 weeks (and not later than 12 weeks) after the first test.

Appendix: HIV Immunoassays Available in New York State

HIV screening and diagnostic tests are designed to detect specific infection markers, which may be virologic, such as viral proteins or nucleic acids, or immunologic, such as antibodies produced in response to HIV infection. HIV testing should begin with an immunoassay approved by the U.S. Food and Drug Administration (FDA) as an initial test to detect HIV-1 and HIV-2 infection. Advances in immunoassay technology have led to improved immunoassay sensitivity and/or specificity. See CDC: Advantages and Disadvantages of FDA-approved HIV Assays Used for Screening.

Clinical Laboratory Improvement Amendments (CLIA)-waived point-of-care HIV screening tests: The Centers for Medicare and Medicaid Services (CMS) regulates all laboratory testing in the United States performed on humans through the CLIA. The FDA categorizes tests that can use unprocessed specimens (whole blood or oral fluid), are easy to use, and have little risk of an incorrect result as "CLIA-waived" tests. These tests, which can be used at or near the patient care or nonclinical settings, are sometimes also used in laboratory settings and often provide results within 60 minutes (they are also referred to as "rapid tests").

Laboratories licensed in New York State are exempt from CLIA. However, this exemption does not apply physicians' office laboratories, which are required to have a CLIA certificate. In all other settings, NYS requirements meet or exceed CLIA requirements. In NYS, facilities that have to obtain a clinical laboratory permit must obtain a CLIA registration number from the Wadsworth Center Clinical Laboratory Evaluation Program (CLEP).

Rapid HIV screening tests: Rapid HIV screening tests can provide results within 60 minutes. Almost all rapid tests in the United States are CLIA-waived and can be used outside a laboratory. Rapid tests are an alternative option when HIV testing according to the standard algorithm is not possible or practical.

Laboratory HIV screening tests: Laboratory screening tests require serum or plasma specimens and are more complex than CLIA-waived tests. They require clinical laboratorians and instrumentation to perform the test or read results. Some laboratory screening tests provide results within 60 minutes.

In addition to improving sensitivity and specificity, several manufacturers have also developed HIV screening tests used with random access instrument systems. These systems eliminate or reduce the need for laboratories to batch samples and produce HIV screening test results very quickly, in many cases within 60 minutes. Immunoassays used for initial HIV screening can be divided into 2 categories:

- **Enzyme or chemiluminescent immunoassays (EIAs, CIAs):** Conducted by licensed technologists in a clinical laboratory.
- **Rapid screening tests:** Simple, single-use devices that produce a result in 30 minutes or less.

Interpreting screening results: All HIV immunoassays designed for initial screening may produce false positive results. Regardless of the test method, all reactive results should be interpreted as preliminary. Further testing is required to verify the reactive screening result and confirm the presence or absence of HIV infection.

Table A.1: FDA-Approved HIV-1/2 Ag/Ab Immunoassays for Step 1 of the CDC Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens

Test	Method and Specimen
• Abbott ARCHITECT HIV Ag/Ab Combo (Abbott Laboratories) • FDA-approved 2010 • Package insert	• CMIA • Serum, plasma • Does not differentiate detection of Ag and Ab
• ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay (Siemens Healthcare Diagnostics) • FDA-approved 2015 • Package insert	• CMIA • Serum, plasma • Does not differentiate detection of Ag and Ab
• BioPlex 2200 HIV Ag-Ab Assay (Bio-Rad Laboratories) • FDA-approved 2015 • Package insert	• Multiplex flow immunoassay • Serum, plasma • Separately detects HIV-1 Ag, HIV-1 Ab, and HIV-2 Ab
• GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories) • FDA-approved 2011 • Package insert	• EIA • Serum, plasma • Does not differentiate detection of Ag and Ab

Table A.1: FDA-Approved HIV-1/2 Ag/Ab Immunoassays for Step 1 of the CDC Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens	
Test	**Method and Specimen**
• Elecsys HIV combi PT (Roche Diagnostics) • FDA-approved 2017 • Package insert	• ECLIA • Serum, plasma • Does not differentiate detection of Ag and Ab
• VITROS Immunodiagnostic Products HIV Combo (Ortho-Clinical Diagnostics) • FDA-approved 2017 • Package insert	• Immunometric assay • Serum, plasma • Does not differentiate detection of Ag and Ab
Abbreviations: Ab, antibody; Ag, antigen; CDC, Centers for Disease Control and Prevention; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; EIA, enzyme immunoassay. **Notes:** a. Laboratory Ag/Ab immunoassays are preferred over the Abbott Determine HIV-1/2 Ag/Ab Combo rapid test for detecting HIV during acute HIV. When instrumented Ag/Ab combination testing is not feasible, the Abbott Determine can be used with serum or plasma as the first step in the HIV laboratory testing algorithm [CDC 2017].	

Point-of-Care (Rapid) Screening Tests

The FDA has approved several HIV screening tests as CLIA-waived tests, which allows a test to be performed in non-laboratory, point-of-care (POC) settings. The FDA categorizes tests as CLIA-waived if they can use unprocessed specimens (whole blood or oral fluid), are easy to use, and have little risk of an incorrect result (see CDC: CLIA Certificate of Waiver). CLIA-waived tests are an option for initial HIV testing when it is not possible or practical to collect blood by venipuncture to submit to a clinical laboratory. These POC tests can produce results within 60 minutes and are usually also referred to as rapid tests. Currently, HIV rapid screening tests may be used only as initial screening tests; they are an alternative option when HIV testing according to the standard algorithm is not possible or practical.

HIV rapid screening tests are single-use test devices that produce results within 60 minutes but usually within 30 minutes. Table A.2, below, lists the characteristics of each of the 7 current FDA-approved rapid screening tests. Result interpretation is typically performed visually without instrumentation; the appearance of a line or circle in the appropriate area indicates a reactive result. The devices include a built-in procedural control that produces the expected appearance for a valid test result.

Although many rapid screening tests have been designed for POC use, clinical laboratories also use rapid screening tests for HIV screening when a result is needed very quickly or the laboratory's overall testing volume is low. Depending on the device and its specific approval, laboratories may perform rapid screening tests using serum, plasma, or whole blood specimens collected by venipuncture. Each of the HIV rapid screening tests is restricted to the body fluid(s) that it was designed to analyze (see Table A.2, below). All CLIA-waived HIV screening tests may be used with plasma or serum specimens; however, laboratories processing these specimen types with CLIA-waived tests require a moderate- or high-complexity laboratory permit. The additional steps and instrumentation needed to process blood to plasma and serum add complexity to the test procedure; therefore, these tests are classified as moderate-complexity for serum and plasma specimens [CDC 2016].

Rapid screening tests employ various technologies, and some devices are more sensitive for early detection than others. The Abbott Determine HIV-1/2 antigen/antibody (Ag/Ab) Combo test is distinguished by its ability to detect both HIV-1 p24 Ag and HIV-1 and HIV-2 Abs. Studies conducted by the CDC show that the Abbott Determine is capable of detecting HIV infection 1 to 2 weeks earlier than all other FDA-approved rapid screening tests but is less sensitive than the laboratory HIV-1/2 Ag/Ab combination immunoassays [Masciotra, et al. 2013].

Aside from the Abbott Determine, all other FDA-approved rapid screening tests only detect HIV Abs and so are less sensitive for identifying acute HIV. These detect HIV Abs 6 to 12 days later than EIAs [Masciotra, et al. 2013; Masciotra, et al. 2011].

Table A.2: Characteristics of FDA-Approved Rapid HIV Tests		
Test (manufacturer)	**Sensitivity (95%) [a]**	**Specificity (95%)**
Chembio SURE CHECK HIV 1/2 Assay (Chembio Diagnostic Systems; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Whole blood, serum, plasma • **CLIA category [c]:** Waived; whole blood [b] only	• Whole blood [b]: 99.7% • Serum: 99.7% • Plasma: 99.7%	• Whole blood [b]: 99.9% • Serum: 99.9% • Plasma: 99.9%
Chembio DPP HIV 1/2 Assay (Chembio Diagnostic Systems; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Oral fluid, whole blood, plasma, serum • **CLIA category [c]:** Waived; oral fluid and whole blood [b]	• Oral fluid: 98.9% • Fingerstick whole blood: 99.8% • Venous whole blood: 99.9% • Plasma: 99.9% • Serum: 99.9%	• Fingerstick whole blood: 100% • Oral fluid: 99.9% • Venous whole blood: 99.9% • Plasma: 99.9% • Serum: 99.9%
Chembio HIV 1/2 STAT-PAK Assay (Chembio Diagnostic Systems; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Whole blood, serum, plasma • **CLIA category [c]:** Waived; whole blood [b] only	• Whole blood [b]: 99.7% • Serum: 99.7% • Plasma: 99.7%	• Whole blood [b]: 99.9% • Serum: 99.9% • Plasma: 99.9%
Abbott Determine HIV-1/2 Ag/Ab Combo (Abbott; package insert) • **Detection:** HIV-1 p24 Ag, HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Whole blood, serum, plasma • **CLIA category [c]:** Waived; fingerstick whole blood only	• Whole blood [b]: 99.9% • Serum: 99.9% • Plasma: 99.9%	• Fingerstick whole blood: 99.8% • Venipuncture whole blood: 99.7% • Plasma: 99.7% • Serum: 99.6%
INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Whole blood, plasma • **CLIA category [c]:** Waived; fingerstick whole blood only	• Fingerstick whole blood: 98.9% • Venipuncture whole blood: 99.9% • Plasma: 99.9%	• Fingerstick whole blood: 99.0% (people at low risk of HIV) and 99.9% (people at high risk of HIV) • Venipuncture whole blood: 100% • Plasma: 100%
OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Oral fluid, whole blood, plasma • **CLIA category [c]:** Waived; oral fluid and whole blood [b]	• Oral fluid: 99.3% • Whole blood: 99.6% • Plasma: 99.6%	• Oral fluid: 99.8% • Whole blood: 100% • Plasma: 99.9%
Uni-Gold Recombigen HIV-1/2 (Trinity Biotech; package insert) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** Whole blood, serum • **CLIA category [c]:** Waived; whole blood [b] only	• Whole blood [b]: 100% • Serum: 100% • Plasma: 100%	• Whole blood [b]: 99.7% • Serum: 99.8% • Plasma: 99.8%

Abbreviations: Ab, antibody; Ag, antigen; CLIA, Clinical Laboratory Improvements Act; POC, point-of-care.

Notes:

a. Data shown are for HIV-1 only. For HIV-2 data, see package inserts.
b. Fingerstick and venipuncture.
c. Information regarding CLIA waivers of HIV tests is available from the Wadsworth Center.

Home-Based Tests

The CDC encourages health departments to allow HIV self-testing (i.e., HIV testing not performed by trained professionals). Currently, only 2 in-home HIV tests are in use: Home Access HIV-1 Test System (Home Access Health) and OraQuick In-Home HIV Test (OraSure Technologies).

Home Access HIV-1 Test System: FDA-approved in 1996 for sale in the United States. The individual collects blood from a fingerstick and transfers the blood onto filter paper, which is mailed to a facility for analysis using FDA-approved tests to detect HIV-1 Abs. If a reactive result is obtained, a trained HIV counselor conducts post-test counseling by telephone. If a negative result is obtained, pre- and post-test counseling consists of a recorded message (a counselor is also available if requested). Results are available in either 3 or 7 days.

OraQuick In-Home HIV Test: FDA-approved in 2012 for over-the-counter sales for people ≥17 years old to use with oral fluid to obtain a result in 20 minutes. The user swipes an oral swab along the gums to collect a sample, which is inserted into a test tube provided in the kit. OraSure provides 24/7 support in English and Spanish by telephone for technical questions, interpretation of results, counseling, and referrals for follow-up support and care. For more information about HIV testing of oral specimens, see the guideline section Alternative HIV Tests: Oral and Urine Specimens, below.

When discussing the possible use of self-tests with patients, care providers should emphasize that although these tests are generally accurate [Figueroa, et al. 2018], errors in specimen collection, processing or interpreting results may occur, and should also stress the importance of using only tests that are FDA-approved. The possibility of these types of errors vary between the Home Access HIV-1 fingerstick mail-in test and the OraQuick in-home test kit.

Alternative HIV Tests: Oral and Urine Specimens

A limited number of FDA-approved assays may be performed on body fluids other than blood for HIV diagnostic testing. Advantages to oral fluid and urine specimens include noninvasive sample collection in settings where phlebotomy is not available and reduced risk of occupational exposure to infectious agents. Disadvantages include reduced sensitivity and specificity of the test methods [FDA(a) 2009; FDA(b) 2009].

Oral fluid specimens: Oral fluid is not saliva but oral mucosal transudate (OMT) obtained by swabbing the gums. Though Abs are detectable in OMT, they are present at concentrations 800- to 1,000-fold lower than those found in serum or plasma. The FDA has approved 1 EIA for use on oral fluid specimens. The Avioq HIV-1 Microelisa System may be performed on OMT specimens collected using the OraSure Oral Fluid Collection Device, and reactive results must be confirmed.

The OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies) is the only rapid screening test that has been FDA-approved for use with oral fluid (see Table A.2, above). The test detects both HIV-1 and HIV-2 Abs but cannot distinguish between them. This test is CLIA-waived and may be used with oral fluid specimens in POC and nonclinical testing sites.

Urine specimens: HIV-1 Abs may be detected in urine. One screening test and one Western blot are FDA-approved for use with urine specimens. Although urine specimens are commonly used for HIV testing in particular situations, such as insurance company testing, HIV tests for urine specimens do not offer adequate sensitivity or specificity for general diagnostic use and should be avoided. Both nonreactive and reactive results of urine HIV testing should be confirmed with standard serological testing, preferably an HIV-1/2 Ag/Ab immunoassay.

Not Recommended for the HIV Diagnostic Laboratory Testing Algorithm

Notably, the Western blot has been eliminated from the recommended testing algorithm (see Figure 2: HIV Laboratory Testing Algorithm) and is used only in the situations described previously. The Western blot is very specific, and false positive results are rare, but it has several important disadvantages. The HIV-1 test is less sensitive than Ag/Ab immunoassays and will produce false negative or indeterminate results on specimens collected before or during seroconversion [Masciotra, et al. 2011; Styer, et al. 2011; Owen, et al. 2008]. It also produces indeterminate results for various other reasons and misclassifies the majority of HIV-2 infections [Lasry, et al. 2014; Nasrullah, et al. 2011; Torian, et al. 2010].

The indirect immunofluorescence assay (IFA) is another supplemental test designed to confirm the presence of HIV-1 Abs. The IFA is not routinely performed for HIV diagnostic testing and is not recommended as a supplemental test for confirming the presence of HIV Abs.

All Recommendations

☑ ALL RECOMMENDATIONS

Step 1: HIV-1/2 Antigen/Antibody Immunoassay

- For initial HIV testing (aka “screening”), clinicians should use an HIV-1/2 Ag/Ab immunoassay (formerly known as the "4th-generation" test). (A2)
- For initial testing of newborns or individuals who are in labor, being evaluated for PEP, or unlikely to return for test results, clinicians should use an FDA-approved HIV screening test that provides results within 60 minutes (A2); otherwise, rapid tests are not recommended for step 1 of the standard HIV laboratory testing algorithm.
- Because all initial HIV tests are subject to false positive results, clinicians should consider all reactive initial test results preliminary and perform appropriate laboratory diagnostic testing to confirm a patient's HIV status. (A1)
- Clinicians should educate patients about the limitations of in-home testing and emphasize that a laboratory should repeat both nonreactive and reactive results of any in-home HIV testing. (A3)
- In the case of a nonreactive result, the clinician should discuss goal-oriented, harm-reduction strategies, including PrEP and emergency PEP, with any patient who reports recent or likely ongoing HIV risk exposures or refer the patient for prevention services. (A3)
- Clinicians should offer repeat HIV testing every 3 months, or sooner if acute HIV is suspected, for as long as an individual remains at high risk of HIV exposure. (A3)

Step 2: HIV-1/HIV-2 Antibody Differentiation Immunoassay

- Per the standard HIV laboratory testing algorithm, if a reactive result is obtained with an HIV-1/2 Ag/Ab immunoassay test (step 1), clinicians should perform supplemental testing (step 2) with an FDA-approved HIV-1/HIV-2 Ab differentiation immunoassay. (A1)
- If the result of the HIV Ab differentiation immunoassay (step 2) is positive for HIV-1 or HIV-2 Abs, the clinician should provide or refer the patient for rapid ART initiation and transmission prevention counseling. (A1)
  - Note: If the HIV Ab differentiation immunoassay result is positive but undifferentiated (i.e., reactive for both HIV-1 and HIV-2), repeat testing may determine if the patient has HIV-1 or HIV-2 infection.

Step 3: HIV-1 Nucleic Acid Testing (qualitative or quantitative HIV RNA testing)

- If the HIV-1/2 Ab differentiation immunoassay (step 2) result is nonreactive (negative) or indeterminate (neither positive nor negative for HIV-1 or HIV-2), and the lab does not perform reflex testing, the clinician should immediately order HIV-1 RNA NAT (step 3) to detect the presence of HIV-1 RNA and confirm or exclude HIV-1 infection. (A*)
- If HIV-1 RNA is detected, the clinician should inform the patient of the acute HIV-1 diagnosis, recommend ART initiation, and prioritize counseling to prevent HIV transmission. (A1)
- Clinicians should not wait for serologic confirmation of HIV to initiate ART when pregnant individuals are diagnosed with acute HIV infection by HIV-1 NAT; initiation of ART is strongly recommended for pregnant individuals. (A2)
  - See DHHS: Recommendations for the Use of Antiretroviral Drugs During Pregnancy and Interventions to Reduce Perinatal HIV Transmission in the United States.
- To determine the HIV status of an infant born to an individual with HIV-1, clinicians should perform HIV-1 RNA NAT. (A1)

Diagnosis of HIV-2 Infection

- When HIV-2 antibodies are detected, clinicians should perform a clinical evaluation for HIV-2 infection that is similar in scope to the evaluation of patients with HIV-1. (A1)

Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CEI, Clinical Education Initiative; DHHS, U.S. Department of Health and Human Services; FDA, U.S. Food and Drug Administration; NAT, nucleic acid testing; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

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Supplement: Guideline Development and Recommendation Ratings

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
Developer	New York State Department of Health AIDS Institute (NYSDOH AI) Clinical Guidelines Program
Funding source	NYSDOH AI
Program manager	Clinical Guidelines Program, Johns Hopkins University School of Medicine, Division of Infectious Diseases. See Program Leadership and Staff.
Mission	To produce and disseminate evidence-based, state-of-the-art clinical practice guidelines that establish uniform standards of care for practitioners who provide prevention or treatment of HIV, viral hepatitis, other sexually transmitted infections, and substance use disorders for adults throughout New York State in the wide array of settings in which those services are delivered.
Expert committees	The NYSDOH AI Medical Director invites and appoints committees of clinical and public health experts from throughout New York State to ensure that the guidelines are practical, immediately applicable, and meet the needs of care providers and stakeholders in all major regions of New York State, all relevant clinical practice settings, key New York State agencies, and community service organizations.
Committee structure	• Leadership: AI-appointed chair, vice chair(s), chair emeritus, clinical specialist(s), JHU Guidelines Program Director, AI Medical Director, AI Clinical Consultant, AVAC community advisor • Contributing members • Guideline writing groups: Lead author, coauthors if applicable, and all committee leaders
Disclosure and management of conflicts of interest	• Annual disclosure of financial relationships with commercial entities for the 12 months prior and upcoming is required of all individuals who work with the guidelines program, and includes disclosure for partners or spouses and primary professional affiliation. • The NYSDOH AI assesses all reported financial relationships to determine the potential for undue influence on guideline recommendations and, when indicated, denies participation in the program or formulates a plan to manage potential conflicts. Disclosures are listed for each committee member.
Evidence collection and review	• Literature search and review strategy is defined by the guideline lead author based on the defined scope of a new guideline or update. • A comprehensive literature search and review is conducted for a new guideline or an extensive update using PubMed, other pertinent databases of peer-reviewed literature, and relevant conference abstracts to establish the evidence base for guideline recommendations. • A targeted search and review to identify recently published evidence is conducted for guidelines published within the previous 3 years. • Title, abstract, and article reviews are performed by the lead author. The JHU editorial team collates evidence and creates and maintains an evidence table for each guideline.

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
Recommendation development	• The lead author drafts recommendations to address the defined scope of the guideline based on available published data. • Writing group members review the draft recommendations and evidence and deliberate to revise, refine, and reach consensus on all recommendations. • When published data are not available, support for a recommendation may be based on the committee's expert opinion. • The writing group assigns a 2-part rating to each recommendation to indicate the strength of the recommendation and quality of the supporting evidence. The group reviews the evidence, deliberates, and may revise recommendations when required to reach consensus.
Review and approval process	• Following writing group approval, draft guidelines are reviewed by all contributors, program liaisons, and a volunteer reviewer from the AI Community Advisory Committee. • Recommendations must be approved by two-thirds of the full committee. If necessary to achieve consensus, the full committee is invited to deliberate, review the evidence, and revise recommendations. • Final approval by the committee chair and the NYSDOH AI Medical Director is required for publication.
External reviews	• External review of each guideline is invited at the developer's discretion. • External reviewers recognized for their experience and expertise review guidelines for accuracy, balance, clarity, and practicality and provide feedback.
Update process	• JHU editorial staff ensure that each guideline is reviewed and determined to be current upon the 3-year anniversary of publication; guidelines that provide clinical recommendations in rapidly changing areas of practice may be reviewed annually. Published literature is surveilled to identify new evidence that may prompt changes to existing recommendations or development of new recommendations. • If changes in the standard of care, newly published studies, new drug approval, new drug-related warning, or a public health emergency indicate the need for immediate change to published guidelines, committee leadership will make recommendations and immediate updates and will invite full committee review as indicated.

Table S2: Recommendation Ratings and Definitions		
Strength	**Quality of Evidence**	
A: Strong B. Moderate C: Optional	1	Based on published results of at least 1 randomized clinical trial with clinical outcomes or validated laboratory endpoints.
	*	Based on either a self-evident conclusion; conclusive, published, in vitro data; or well-established practice that cannot be tested because ethics would preclude a clinical trial.
	2	Based on published results of at least 1 well-designed, nonrandomized clinical trial or observational cohort study with long-term clinical outcomes.
	2†	Extrapolated from published results of well-designed studies (including nonrandomized clinical trials) conducted in populations other than those specifically addressed by a recommendation. The source(s) of the extrapolated evidence and the rationale for the extrapolation are provided in the guideline text. One example would be results of studies conducted predominantly in a subpopulation (e.g., one gender) that the committee determines to be generalizable to the population under consideration in the guideline.
	3	Based on committee expert opinion, with rationale provided in the guideline text.