CLINICAL GUIDELINES PROGRAM
NEW YORK STATE DEPARTMENT OF HEALTH AIDS INSTITUTE | HIV • HCV • STIs • SUBSTANCE USE • LGBTQ+ HEALTH

HIV Testing

Updates, Authorship, and Related Resources

Date of current publication	August 4, 2026
Highlights of changes, additions, and updates in the August 4, 2026 edition	• Comprehensive update with new text and references • HIV Testing With the Standard 3-Step Algorithm section: – Added new recommendations on repeat testing based on risk factors. – Added new recommendation: When acute HIV infection is suspected, clinicians should order both an HIV-1/2 Ag/Ab immunoassay and an HIV-1/HIV-2 RNA NAT at the same time, without waiting for the Ag/Ab immunoassay result. (A2) – Updated recommendations, Figure 2: HIV Laboratory Testing Algorithm, and supplementary text with addition of HIV-1/HIV-2 RNA NAT • Added new section: NYSDOH Wadsworth Center Bloodborne Viruses Laboratory Services • Added Table 2: HIV Test Detection Window and Table 3: FDA-Approved HIV Nucleic Acid Tests for Diagnostic Use • Added Box 2: Diagnosing Acute HIV Infection
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Author and writing group conflict of interest disclosures	There are no author or writing group conflict of interest disclosures.
Date of original publication	October 3, 2018
Committee	Medical Care Criteria Committee
Developer and funder	New York State Department of Health AIDS Institute (NYSDOH AI)
Development process	See Supplement: Guideline Development and Recommendation Ratings
Related NYSDOH AI resources	**Guidelines** • Diagnosis and Management of Acute HIV Infection • Diagnosis and Management of HIV-2 in Adults • HIV Testing During Pregnancy, at Delivery, and Postpartum • PrEP to Prevent HIV and Promote Sexual Health • Rapid ART Initiation • Virologic and Immunologic Monitoring in HIV Care **Podcast** • Viremic—Cases in HIV

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Committee: Medical Care Criteria Committee

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All Recommendations

☑ ALL RECOMMENDATIONS: HIV TESTING

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

- For initial HIV testing (“screening”), clinicians should use an FDA-approved 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).
  - Point-of-care tests are not recommended for step 1 of the standard HIV laboratory testing algorithm; however, using the FDA-approved point-of-care HIV-1/2 Ag/Ab immunoassay may be appropriate when venipuncture is not feasible or when rapid clinical decision-making is required (e.g., rapid PrEP initiation), particularly in the absence of symptoms of acute HIV infection.
- 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 reactive results of any in-home HIV testing. (A3)

☑ ALL RECOMMENDATIONS: HIV TESTING

- 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)
- **New York State Law:** For individuals without identified HIV exposure risk factors, clinicians are required to offer HIV testing at least once in a lifetime.
- For sexually active individuals and individuals with other potential HIV exposure risk factors, including injection drug use, clinicians should conduct an HIV risk assessment at least annually and offer HIV testing based on findings of that assessment or at the patient's request. (A3)
- For individuals with ongoing or repeated HIV exposure risk factors, including multiple sex partners, an STI diagnosis in the past year, injection drug use, and exchange of sex for money or drugs, clinicians should offer HIV testing every 3 to 6 months, or sooner if acute HIV infection is suspected, for as long as risk remains. (A3)
- For individuals currently taking PrEP, clinicians should perform HIV testing every 3 months (for oral PrEP) or at each injection visit while on injectable PrEP to rule out HIV infection. (A3)
  - See NYSDOH AI guideline PrEP to Prevent HIV and Promote Sexual Health.
- When acute HIV infection is suspected, clinicians should order both an HIV-1/2 Ag/Ab immunoassay and an HIV-1 or HIV-1/HIV-2 RNA NAT at the same time [a], without waiting for the Ag/Ab immunoassay result. (A2)

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 Ab differentiation immunoassay (step 2) is positive for HIV-1 or HIV-2 Abs, the clinician should recommend rapid ART initiation and prioritize counseling to prevent HIV transmission. (A1)
  - Note: If the 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.
  - See NYSDOH AI guidelines Rapid ART Initiation and Diagnosis and Management of HIV-2 in Adults.

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

- If the Ab differentiation immunoassay (step 2) result is nonreactive (negative) or indeterminate (neither positive nor negative for HIV-1 or HIV-2), and the laboratory does not perform reflex testing, the clinician should immediately order an FDA-approved qualitative HIV-1 or HIV-1/HIV-2 RNA NAT (step 3) to detect the presence of HIV RNA and confirm or exclude HIV infection. (A*)
  - See guideline section Step 3: HIV RNA Nucleic Acid Testing for detailed guidance on the use of quantitative RNA assays for the presumptive diagnosis of acute HIV infection.
- If HIV RNA is detected, the clinician should inform the patient of the acute HIV diagnosis, recommend ART initiation, and prioritize counseling to prevent HIV transmission. (A1)
  - See NYSDOH AI guidelines Rapid ART Initiation and Diagnosis and Management of HIV-2 in Adults.
- Clinicians should not wait for serologic confirmation of HIV to initiate ART when pregnant individuals are diagnosed with acute HIV infection by HIV 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, clinicians should perform an HIV-1 or HIV-2 RNA NAT based on the individual's HIV type. (A1)

Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; 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; STI, sexually transmitted infection.

Note:

a. Because HIV-2 is rare and difficult to transmit, an HIV-1 RNA NAT will provide an accurate result if an HIV-1/HIV-2 RNA NAT is not available.

Purpose of This Guideline

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

- Provide clinicians in New York State with information on HIV testing policies and practices and NYSDOH Wadsworth Center Bloodborne Viruses Laboratory services.
- 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.
- 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, when appropriate.
- Emphasize that rapid antiretroviral therapy (ART) initiation is the standard of care for all individuals diagnosed with HIV.

Diagnosing HIV: This guideline provides an overview of the screening and diagnostic methods used to accurately diagnose or exclude HIV infection, as outlined in the CDC 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens and Technical update for HIV nucleic acid tests approved for diagnostic purposes and the Association of Public Health Laboratories Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm (also see Figure 2: HIV Laboratory Testing Algorithm in this guideline) [APHL 2025; CDC 2023; CDC 2018].

Widespread use of the HIV-1/2 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 that were based on Ab screening followed by Western blot confirmation [CDC 2018]. The 2018 algorithm features a specific sequence of tests to provide maximal sensitivity, specificity, and accuracy for HIV detection. This algorithm was further updated in 2023 with the approval of the HIV-1/HIV-2 nucleic acid test for diagnosis [CDC 2023].

Initiating ART: The current standard of care for an individual newly diagnosed with HIV 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 HIV/AIDS Laws and Regulations.
- Clinicians must report confirmed cases of HIV according to New York State law (see NYSDOH HIV Reporting and Partner Services).

Accessible and routine HIV testing for all individuals aged 13 years and older is a crucial 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 HIV care and prevention. Table 1, below, provides an overview of HIV testing requirements in New York State. Additional information regarding testing procedures and regulations is available from the NYSDOH Wadsworth Center Bloodborne Viruses Laboratory (phone: 518-474-2163).

Table 1: New York State Public Health Law HIV Testing and Reporting Requirements	
Who to test	New York State law mandates that physicians offer an HIV test to all individuals aged ≥13 years (or younger with risk) who receive care in hospital or primary care settings if a previous test is not documented, even in the absence of symptoms. See NYSDOH HIV Testing and HIV Testing, Reporting and Confidentiality in New York State 2024 Update: Fact Sheet and Frequently Asked Questions.

Table 1: New York State Public Health Law HIV Testing and Reporting Requirements	
Consent	HIV testing is voluntary. Although written or oral informed consent to HIV testing is not required in New York State, individuals must be given the opportunity to decline. In June 2024, amendments to New York State public health law expanded the various methods allowed for providing required notice that an HIV-related test will be performed. Healthcare providers must advise patients orally, in writing, with prominently displayed signage, or via electronic means or other appropriate form of communication that an HIV test will be performed. If the patient declines, it must be noted in the medical record. For more information, see NYSDOH HIV Testing, Reporting and Confidentiality in New York State 2024 Update: Fact Sheet and Frequently Asked Questions and New York State Senate Bill S7809.
Minor consent	Minors may consent to their own HIV testing, treatment, and/or prevention services (such as PrEP and PEP) without parent/guardian involvement. For more information, see NYSDOH HIV Testing, Reporting and Confidentiality in New York State 2024 Update: Fact Sheet and Frequently Asked Questions and New York State Confidentiality Law and HIV: Public Health Law, Article 27-F.
Pre-test counseling	Formal pre-test counseling is no longer expected. The required notice to patients about HIV testing can include placing an informational poster in a visible location or providing a patient brochure, with staff available to answer any patient questions.
Post-test counseling	• *When testing indicates 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. For more information, see New York State Public Health Law Section 63.3. • *When testing indicates no HIV infection:* The patient must be informed of the result and provided education on prevention options, including PrEP and PEP. Providing written materials, such as the NYSDOH patient brochure Information on Non-reactive (Negative) HIV Test Results, satisfies this requirement. • *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. See the NYSDOH AI guideline HIV Testing During Pregnancy, at Delivery, and Postpartum.
Reporting requirements	• New York State Public Health Law Article 21 (Chapter 163 of the Laws of 1998) requires the reporting of individuals with HIV and 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. • See NYSDOH HIV Reporting and Partner Services and NYC Health Reporting a Diagnosis of HIV or AIDS. Updated reporting requirements, timelines, and reporting methods, including instructions for accessing the HIV/AIDS Provider Portal, are available at NYSDOH 2023 Changes to Provider Reporting of Human Immunodeficiency Virus (HIV) in New York State. • 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 institutions 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 New York State Health Commerce System is preferred. For information regarding provider portal access or to obtain printed copies of the DOH-4189 form, 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 on the clinician's role in this process, see NYSDOH Information on Partner Services.

Table 1: New York State Public Health Law HIV Testing and Reporting Requirements	
Nomenclature	In New York State, the terms "clinical/symptomatic HIV illness or AIDS," "AIDS or HIV-related illness," and other similar terms shall mean laboratory-confirmed HIV diagnosis. For more information, see NYSDOH June 2016 Policy Statement: Defining Program Eligibility by HIV Status.
Additional NYSDOH resources	• 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.	

NYSDOH Wadsworth Center Bloodborne Viruses Laboratory Services

The NYSDOH Wadsworth Center Bloodborne Viruses Laboratory provides the following testing services and assistance to clinicians in New York State. All testing for New York State patients and providers is free of charge.

HIV-1/HIV-2 diagnostic testing:

- HIV-1/2 antigen/antibody testing; plasma and serum
- HIV-1/HIV-2 supplemental antibody testing of specimens that are reactive on the initial testing assay
- HIV-1 RNA testing on plasma, serum, and dried blood spots
- HIV-2 nucleic acid testing to detect HIV-2 RNA
- Assistance with inconclusive HIV test results
- Confirmatory testing of dried blood spots using an alternative algorithm for enrolled community-based HIV testing sites unable to collect venous blood
- Website | Phone: 518-474-2163

Pediatric HIV testing:

- HIV-1 RNA testing for all newborns exposed to HIV-1 in New York State
- If the individual submitting the sample indicates that the newborn has been exposed to HIV-2, the Pediatric HIV Testing Service will perform an RT-PCR (reverse transcription polymerase chain reaction) test for qualitative detection of HIV-2 RNA in all samples.
- Website | Phone: 518-486-9605

HIV-2 nucleic acid testing:

- Qualitative HIV-2 RNA testing for diagnosis and quantitative detection of HIV-2 RNA (viral load) 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 the patient's pregnancy to discuss the protocol and timing for testing.
- HIV-2 phenotypic and genotypic resistance testing is not offered at the Wadsworth Center or commercially available in the United States.
- Website | Phone: 518-473-6007

Time to HIV Detection

The time from HIV infection to detection of the virus depends on the test that is used. Figure 1, below, illustrates the window of detection of HIV infection according to antibody (Ab), antigen (Ag)/Ab combination, and HIV RNA tests. Establishing the exact window period for an initial HIV test is challenging because the precise time of exposure is rarely known. Among individuals using HIV pre-exposure prophylaxis (PrEP) who have breakthrough infections, the exact window period may be

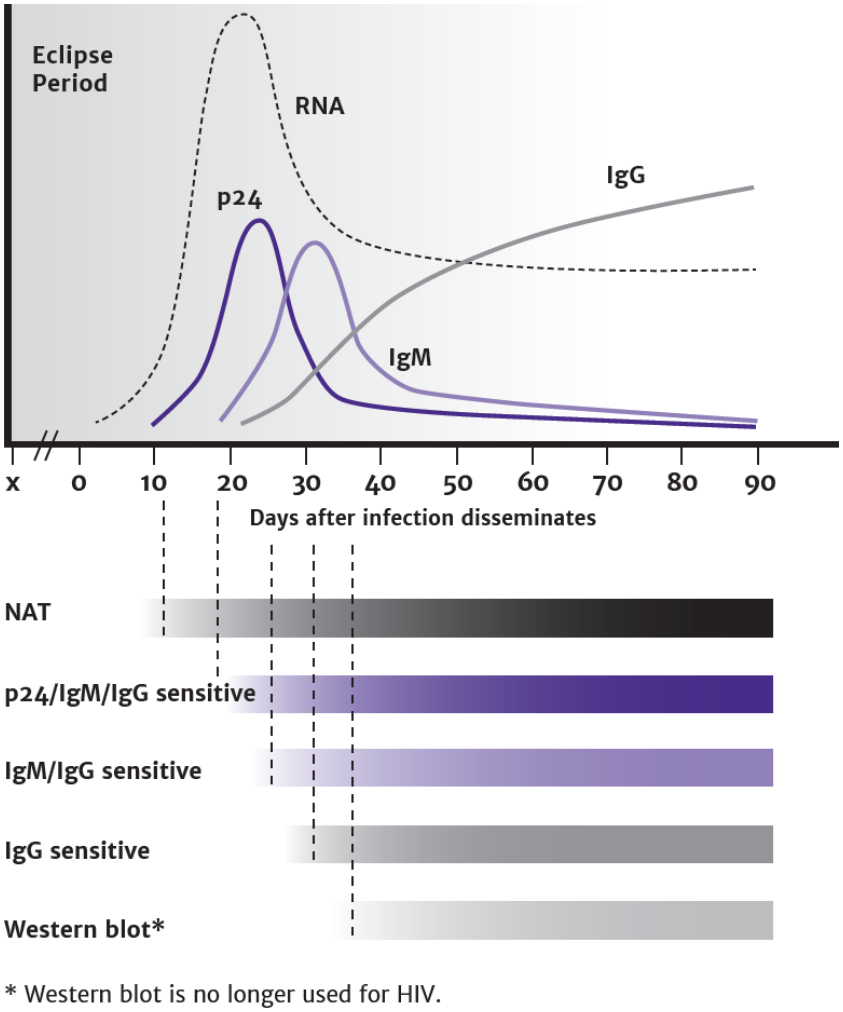
even more difficult to define because of partial suppression of viral replication and possible blunted or delayed immune response. Post-exposure prophylaxis (PEP) after an exposure may also alter the time to detection of HIV.

Eclipse period refers to the time following an HIV exposure during which no available HIV test can detect the virus (see Figure 1, below) [Stekler, et al. 2023; Spinelli, et al. 2021; CDC 2018; Delaney, et al. 2017; Hoenigl, et al. 2016]. The duration of the eclipse period varies depending on characteristics of the infecting virus and the individual [Fiebig, et al. 2003]. In a study that applied modeling methods to estimate the time from exposure, with subsequent infection, to HIV RNA detection among people not receiving PrEP, the median length of the eclipse period was 11.5 days [Delaney, et al. 2017].

Window period refers to the time between HIV exposure and when a test can accurately and consistently detect quantifiable HIV biomarkers. The duration is often given as a range because the time needed for a test to first become positive can vary due to host factors, how much virus was transmitted, transmission route, specimen type, test platform, and use of antiretroviral medication such as HIV PrEP or PEP. In general, HIV tests performed on plasma or serum specimens detect early HIV infection earlier than tests performed on whole blood [Masciotra, et al. 2017; Stekler, et al. 2016], and HIV tests performed on whole blood detect early HIV infection earlier than tests performed on oral fluid [Stekler, et al. 2016; Stekler, et al. 2013]. Instrumented laboratory-based HIV-1/2 Ag/Ab immunoassays are more sensitive for early HIV detection than point-of-care tests. Ag/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).

In individuals who acquire HIV while taking PrEP (oral or long-acting injectable), antiretroviral (ARV) medications may partially suppress HIV replication and alter the normal progression of HIV biomarkers. As a result, levels of HIV RNA, HIV DNA, HIV p24 Ag, and HIV Abs may remain near the limits of assay detection, making HIV diagnosis challenging [Spinelli, et al. 2021; Lee, et al. 2020]. This diagnostic challenge is particularly relevant to individuals who acquire HIV while receiving cabotegravir as part of long-acting injectable PrEP and has been described as long-acting early viral inhibition (LEVI) syndrome [Parikh, et al. 2026; Landovitz, et al. 2024]. Diagnostic delays may persist for weeks to months after discontinuation of long-acting injectable PrEP. Similarly, very early treatment of acute HIV infection can, uncommonly, result in delayed seroconversion or seroreversion, which may also complicate HIV diagnosis [Stekler, et al. 2023; Hare, et al. 2006; Kassutto, et al. 2005].

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



* Western blot is no longer used for HIV.

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; NAT, nucleic acid test; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

Notes:

a. Figure reproduced from CDC Clinical Testing Guidance for HIV.
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, et al. 2023; Hare, et al. 2006; Kassutto, et al. 2005].
c. The eclipse period is the time from the onset of HIV infection until the virus is detectable by virologic tests.

Each type of HIV test has a different detection window. Choosing the appropriate test and accurately interpreting its results requires an understanding of when each test becomes reliably reactive. A negative result before the detection window has passed cannot rule out HIV infection. To confidently exclude HIV infection, the test must be performed after the end of its detection window (see Table 2, below).

Table 2: HIV Test Detection Window	
HIV Test	**Post-Exposure Detection Window Period**
Antibody test (includes most point-of-care antibody tests and self-tests)	23 to 90 days
Point-of-care antigen/antibody test (fingerstick whole blood)	18 to 90 days
Antigen/antibody laboratory test (venipuncture)	18 to 45 days
Nucleic acid test (NAT)	10 to 33 days
Source: CDC Getting Tested for HIV	

HIV Testing With the Standard 3-Step Algorithm

☑ RECOMMENDATIONS

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

- For initial HIV testing ("screening"), clinicians should use an FDA-approved 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).
  - Point-of-care tests are not recommended for step 1 of the standard HIV laboratory testing algorithm; however, using the FDA-approved point-of-care HIV-1/2 Ag/Ab immunoassay may be appropriate when venipuncture is not feasible or when rapid clinical decision-making is required (e.g., rapid PrEP initiation), particularly in the absence of symptoms of acute HIV infection.
- 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 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)
- **New York State Law:** For individuals without identified HIV exposure risk factors, clinicians are required to offer HIV testing at least once in a lifetime.
- For sexually active individuals and individuals with other potential HIV exposure risk factors, including injection drug use, clinicians should conduct an HIV risk assessment at least annually and offer HIV testing based on findings of that assessment or at the patient's request. (A3)
- For individuals with ongoing or repeated HIV exposure risk factors, including multiple sex partners, an STI diagnosis in the past year, injection drug use, and exchange of sex for money or drugs, clinicians should offer HIV testing every 3 to 6 months, or sooner if acute HIV infection is suspected, for as long as risk remains. (A3)
- For individuals currently taking PrEP, clinicians should perform HIV testing every 3 months (for oral PrEP) or at each injection visit while on injectable PrEP to rule out HIV infection. (A3)
  - See NYSDOH AI guideline PrEP to Prevent HIV and Promote Sexual Health.
- When acute HIV infection is suspected, clinicians should order both an HIV-1/2 Ag/Ab immunoassay and an HIV-1 or HIV-1/HIV-2 RNA NAT at the same time [a], without waiting for the Ag/Ab immunoassay result. (A2)

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 Ab differentiation immunoassay (step 2) is positive for HIV-1 or HIV-2 Abs, the clinician should recommend rapid ART initiation and prioritize counseling to prevent HIV transmission. (A1)
  - Note: If the 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.
  - See NYSDOH AI guidelines Rapid ART Initiation and Diagnosis and Management of HIV-2 in Adults.

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

- If the Ab differentiation immunoassay (step 2) result is nonreactive (negative) or indeterminate (neither positive nor negative for HIV-1 or HIV-2), and the laboratory does not perform reflex testing, the clinician should immediately order an FDA-approved qualitative HIV-1 or HIV-1/HIV-2 RNA NAT (step 3) to detect the presence of HIV RNA and confirm or exclude HIV infection. (A*)
  - See guideline section Step 3: HIV RNA Nucleic Acid Testing for detailed guidance on the use of quantitative RNA assays for the presumptive diagnosis of acute HIV infection.

☑ RECOMMENDATIONS

- If HIV RNA is detected, the clinician should inform the patient of the acute HIV diagnosis, recommend ART initiation, and prioritize counseling to prevent HIV transmission. (A1)
  - See NYSDOH AI guidelines Rapid ART Initiation and Diagnosis and Management of HIV-2 in Adults.
- Clinicians should not wait for serologic confirmation of HIV to initiate ART when pregnant individuals are diagnosed with acute HIV infection by HIV 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, clinicians should perform an HIV-1 or HIV-2 RNA NAT based on the individual's HIV type. (A1)

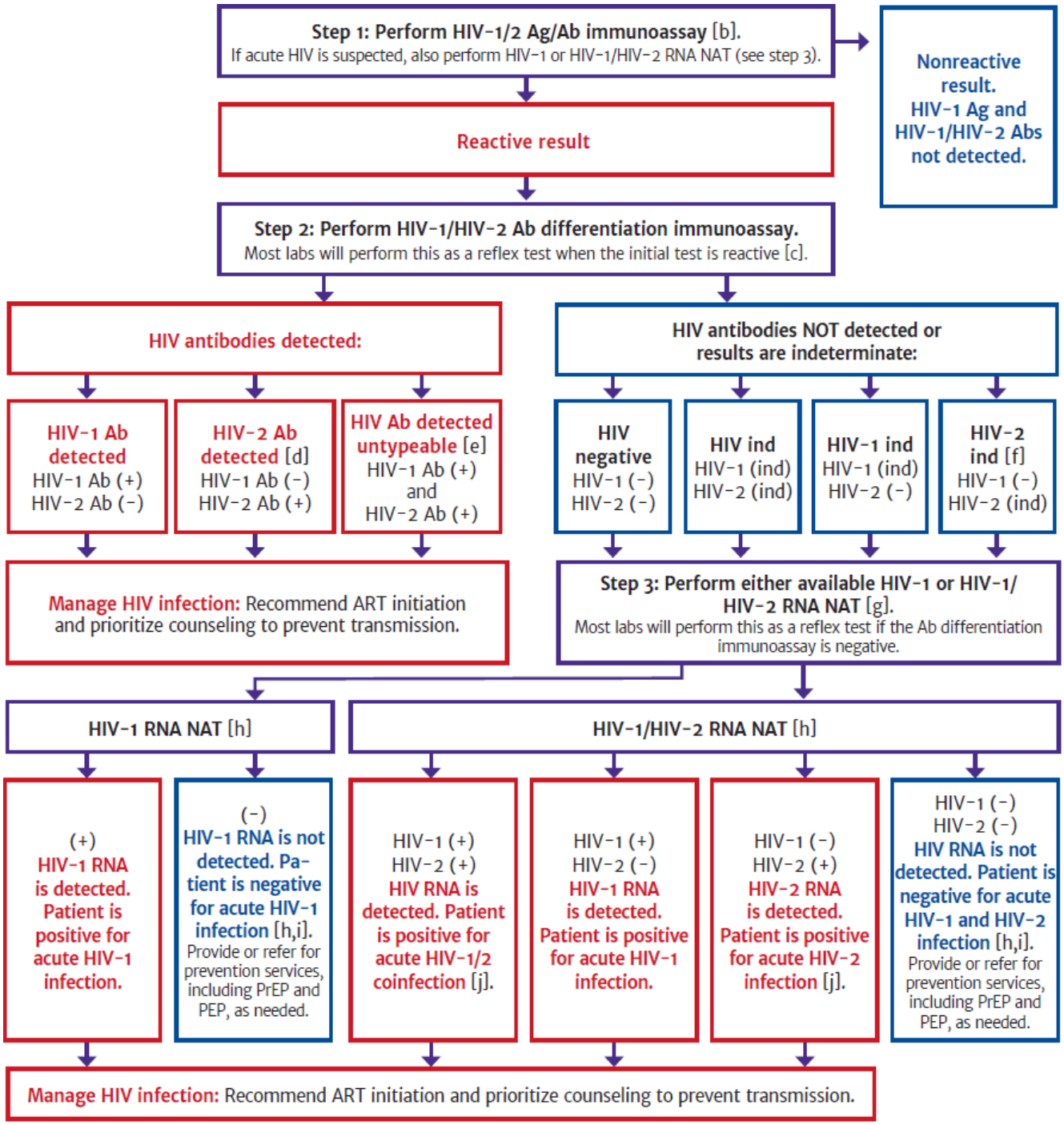
Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; DHHS, U.S. Department of Health and Human Services; FDA, U.S. Food and Drug Administration; NAT, nucleic acid testing; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis; STI, sexually transmitted infection.

Note:

a. Because HIV-2 is rare and difficult to transmit, an HIV-1 RNA NAT will provide an accurate result if an HIV-1/HIV-2 RNA NAT is not available.

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). 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], and this algorithm was further updated in 2023 with the approval of the HIV-1/HIV-2 RNA NAT for diagnosis of acute HIV [CDC 2023]. Figure 2, below, outlines the standard 3-step HIV testing algorithm recommended by this committee and the CDC [CDC 2023]. For information on the testing services and assistance available in New York State, see the guideline section NYSDOH Wadsworth Center Bloodborne Viruses Laboratory Services.

Figure 2: HIV Laboratory Testing Algorithm [a]



Abbreviations: Ab, antibody; Ag, antigen; APHL, Association of Public Health Laboratories; ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; ind, indeterminate; FDA, U.S. Food and Drug Administration; NAT, nucleic acid test; 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 Technical update for HIV nucleic acid tests approved for diagnostic purposes, 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-1/2 Ag/Ab immunoassay as the initial assay in the laboratory HIV testing algorithm for serum or plasma because of their superior sensitivity for detecting acute HIV infection. However, the FDA-approved single-use point-of-care HIV-1/2 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 unavailable.

c. Become familiar with the laboratory's internal testing algorithm and results-reporting policies. Many laboratories will reflex additional screening steps (such as HIV Ab differentiation immunoassay and HIV RNA) on the original sample without supplemental orders. Other laboratories 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.
f. Per the Geenius instructions for use, 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 or HIV-1/HIV-2 NAT.
g. Most laboratories reflex directly to an HIV RNA NAT 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 FDA-approved HIV-1 or HIV-1/HIV-2 RNA NAT as soon as possible. To reflex directly to an HIV-1 or HIV-1/HIV-2 RNA NAT, a test kit approved by either the FDA or NYSDOH to aid in diagnosing HIV-1 infection or HIV-1/2 coinfection, respectively, is required. If HIV-1 or HIV-2 RNA is detected, acute HIV-1 or HIV-2 is present, respectively, and clinicians should proceed with clinical evaluation. If no HIV RNA is detected, the initial immunoassay result is presumed false positive.
h. A specimen with 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, starting with an HIV-1/2 Ag/Ab immunoassay. Samples with a negative HIV-1/HIV-2 RNA NAT result do not need additional HIV-2 testing.
i. NATs approved by the FDA to have a dual diagnostic claim should be used, which allows them to determine both the qualitative diagnosis of HIV-1 infection and quantitative clinical management of HIV-1 infection.
j. Data on interpreting acute HIV-2 infection are limited and subject to test instructions for use.

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

The first test in the algorithm screens for established HIV-1 or HIV-2 infection and acute HIV-1 infection. When requesting HIV diagnostic testing of anyone aged 2 years and older, testing should be ordered from a laboratory that offers an FDA-approved HIV-1/2 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 Figure 2, above).

HIV-1/2 Ag/Ab immunoassays, formerly known as "4th-generation" immunoassays, detect both immunoglobulin G and M Abs to HIV-1 and HIV-2 plus HIV-1 p24 Ag. These Ag/Ab immunoassays have a distinct advantage over screening tests that detect only Abs. Although Ag/Ab immunoassays cannot detect HIV during the eclipse period, when HIV Ab, Ag, and RNA are not 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.

→ KEY POINTS

- Become familiar with the laboratory's internal testing algorithm and results-reporting policies. Many laboratories will reflex additional screening steps (such as an HIV Ab differentiation immunoassay and HIV RNA NAT) on the original sample without supplemental orders. Other laboratories 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.

Laboratory HIV-1/2 Ag/Ab immunoassays (e.g., Abbott ARCHITECT, Bio-Rad Bioplex, Roche Elecsys, and others) are preferred for use in step 1 of the algorithm because of their superior sensitivity in detecting HIV during acute infection. The Determine HIV-1/2 Ag/Ab Combo (Abbott Diagnostics) is the only FDA-approved HIV-1/2 Ag/Ab immunoassay point-of-care screening test. The Determine HIV-1/2 Ag/Ab Combo may be used with serum or plasma (not fingerstick whole blood) when instrumented laboratory testing is not feasible [CDC 2017].

No HIV test is perfect at both detecting infection and excluding false results. Because of the serious consequences of missed HIV infection, this HIV testing algorithm begins with a highly sensitive screening test to minimize missed infections, including during early HIV infection. As a result, reactive results are considered preliminary positive and may include false positives; all reactive results require supplemental testing for confirmation. In populations with lower HIV prevalence, a greater proportion of reactive screening tests may represent false positive results.

For more information on laboratory testing in New York State, including FDA-approved tests, see NYSDOH 2024 Guidelines Update for Use of the HIV Diagnostic Testing Algorithm for Laboratories.

Results and next steps: When used for routine HIV screening, a negative initial HIV-1/2 Ag/Ab immunoassay result is referred to as *nonreactive*. If the initial test result is *reactive* (preliminary positive), supplemental testing with an HIV-1/HIV-2 Ab differentiation immunoassay (see step 2, below) is performed. When the standard HIV laboratory testing algorithm is followed, laboratory reporting may include the initial HIV test result and, if it is reactive, supplemental testing results.

A sequence of tests, rather than a single test, is recommended to establish a laboratory diagnosis of HIV infection. Full results confirm reactivity and include an HIV-1/HIV-2 Ab differentiation immunoassay that detects and discriminates between HIV-1 and HIV-2 Abs with high specificity. If the supplemental Ab test result is negative or indeterminate, an HIV-1 or HIV-1/HIV-2 RNA NAT (see Step 3: HIV RNA Nucleic Acid Testing, below) can verify acute HIV-1 infection (see Box 1: Reasons for False Positive, False Negative, or Indeterminate HIV Test Results, below).

Point-of-care HIV and supplemental testing: Screening with a point-of-care (POC) HIV test, including when used in community-based or outreach settings, allows individuals to quickly learn their HIV status. A reactive (preliminary positive) result should be provided as soon as possible. Because POC HIV tests can sometimes produce false positive results (see Box 1, below), particularly in populations not at high risk of HIV infection, supplemental testing is required to confirm all reactive results.

If a POC HIV test performed on oral fluid or fingerstick whole blood produces a reactive result, a venipuncture blood specimen should be collected. The CDC recommends confirming all reactive POC HIV test results using the HIV testing algorithm [CDC 2018], which begins with a laboratory-based HIV-1/2 Ag/Ab immunoassay. If the Ag/Ab immunoassay result is nonreactive, the POC HIV test result is interpreted as false positive (see Box 1, below), and no further testing is needed. If the immunoassay is reactive, specimen testing should continue according to the HIV testing algorithm.

Instrumented laboratory-based HIV-1/2 Ag/Ab immunoassays are preferred for the first step of the HIV testing algorithm because they are more sensitive for detecting early HIV infection than the Determine HIV-1/2 Ag/Ab Combo POC test. When instrumented laboratory testing is not available, the Determine HIV-1/2 Ag/Ab Combo test performed using serum or plasma specimens may serve as an alternate first step HIV-1/2 Ag/Ab test [CDC 2017]. In this setting, a reactive result may be followed directly by a supplemental HIV-1/2 Ab differentiation immunoassay. However, when the Determine HIV-1/2 Ag/Ab Combo test is performed on whole blood and yields a reactive result, the CDC recommends confirmatory testing with a laboratory-based HIV-1/2 Ag/Ab immunoassay.

Alternative specimen types may be used for supplemental testing if collecting a venous blood specimen is not possible or practical. The NYSDOH Wadsworth Center Bloodborne Viruses Laboratory offers confirmatory testing of dried blood spot specimens using an alternative algorithm for enrolled community-based HIV screening sites unable to collect venous blood.

For more information, see Appendix > Point-of-Care (Rapid) Screening Tests.

HIV self-testing: The CDC encourages health departments to support HIV self-testing, in which HIV testing is conducted by individuals outside the healthcare setting rather than by trained clinicians, as a means to expand access, especially for populations who face barriers to HIV testing.

Advise patients to only use FDA-approved tests and emphasize that these tests are accurate when used correctly [Figueroa, et al. 2018] but that errors in specimen collection, processing, or report interpretation can occur. All individuals with a reactive (preliminary positive) self-test result should seek confirmatory laboratory testing and linkage to care as soon as possible.

For more information, see Appendix > Home-Based Tests.

Repeat testing: For sexually active individuals and those with other potential HIV exposure risk factors, including injection drug use, clinicians should conduct an HIV risk assessment at least annually and offer HIV testing based on findings of that assessment or at the patient's request. For individuals with ongoing or repeated HIV exposure risk factors, including multiple sex partners, an STI diagnosis in the past year, injection drug use, and exchange of sex for money or drugs, clinicians should offer HIV testing every 3 to 6 months, or sooner if acute HIV infection is suspected, for as long as risk remains. Risk factors can change over time and should be reassessed at each visit.

When acute HIV infection is suspected: When very early acute HIV infection is suspected, HIV RNA testing may be warranted. During early acute HIV infection, an HIV-1/2 Ag/Ab immunoassay may return a false negative result because HIV Ag and Abs are not yet present in sufficient quantities for detection, while HIV RNA may already be detectable. If available, clinicians should use an FDA-approved HIV-1/HIV-2 RNA NAT for diagnostic use (e.g., cobas HIV-1/HIV-2 Qualitative). If this test is not available, an FDA-approved HIV-1 RNA NAT should be used. HIV RNA testing is warranted when initiating PrEP and when an individual has:

- A recent high-risk exposure (within the past 10-33 days; median time to HIV RNA detectability is ~11.5 days), or
- Symptoms consistent with acute retroviral syndrome, such as fever, sore throat, rash, myalgia, and lymphadenopathy

Box 1: Reasons for False Positive, False Negative, or Indeterminate HIV Screening Test Results

Reasons for false positive HIV test results:

- 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: Pregnancy [Kaewpoowat, et al. 2025; Güler, et al. 2022]; hypergammaglobulinemia/Abs reactive to cellular components; cross-reactivity with influenza vaccine that causes cross-reactivity with HIV-1/HIV-2 Ab differentiation immunoassays (time course for such cross-reactivity remains uncertain) [Wazzi-Mkahal, et al. 2025]; autoimmune conditions, such as lupus [Lam, et al. 2022]; leukemia [Alali, et al. 2022]; malaria [Ainoda, et al. 2023]; Epstein-Barr virus infection [Chaudhary and Jha 2025]

Reasons for false negative HIV test results:

- Test is performed during any of the following periods: Eclipse period before detection of Ag or HIV RNA is possible; before the end of the detection window period; 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 individuals with concurrent acute HCV, in individuals using antiretroviral medications for PEP or PrEP (e.g., HIV detection may be delayed for weeks to months after discontinuation of long-acting injectable cabotegravir), 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

Comments:

- 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 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-1 or HIV-2.

Abbreviations: Ab, antibody; Ag, antigen; ART, antiretroviral therapy; HCV, hepatitis C virus; IgM, immunoglobulin M; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

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

When an initial HIV-1/2 Ag/Ab immunoassay (see step 1, above) result is reactive (or repeatedly reactive), the next step in the HIV testing algorithm is to perform a supplemental immunoassay that differentiates HIV-1 and HIV-2 antibodies (see Figure 2, above). There are currently 2 FDA-approved HIV-1/HIV-2 Ab differentiation immunoassays:

- Geenius HIV 1/2 Supplemental Assay (Bio-Rad Laboratories)
- VioOne HIV Profile Supplemental Assay (Avioq)

HIV-1/HIV-2 Ab differentiation immunoassays produce separate results for HIV-1 and HIV-2 antibodies, with each result categorized as reactive, nonreactive, or indeterminate. The individual HIV-1 and HIV-2 results are combined into an overall assay interpretation, such as HIV Antibody Negative, HIV-1 Positive, HIV-1 Indeterminate, etc. Laboratories are required to include the overall assay interpretation on the laboratory report, as per the assay instruction for use. Laboratories should not include individual HIV-1 and HIV-2 results, as they may lead to misinterpretation of the test results. Results listed below refer to the final assay interpretation provided by the HIV-1/HIV-2 Ab differentiation immunoassays.

Reactive: An HIV-1/HIV-2 Ab differentiation immunoassay (step 2) with positive results for HIV-1 or HIV-2 should be interpreted as positive for HIV-1 or HIV-2.

An HIV-1/HIV-2 Ab differentiation immunoassay (step 2) that detects HIV Abs but cannot distinguish between HIV-1 and HIV-2 (untypable) should be treated as presumptive HIV positive. HIV type can be determined by repeating the HIV testing algorithm, starting with step 1, in 2 to 4 weeks or performing HIV-1 or HIV-2 RNA testing. If assistance is needed with this testing, contact the NYSDOH Wadsworth Center Bloodborne Viruses Laboratory.

Nonreactive/indeterminate: If an HIV-1/HIV-2 Ab differentiation immunoassay (step 2) result is nonreactive or indeterminate for any HIV type (HIV, HIV-1, or HIV-2), the next step in the HIV testing algorithm is to perform HIV-1 or HIV-1/HIV-2 RNA testing (see step 3, below) using a test approved for diagnostic purposes (qualitative or quantitative; see Step 3: HIV RNA Nucleic Acid Testing, below).

Acute HIV-1 infection is much more common than HIV-2 infection. Some individuals with acute HIV-1 infection may have HIV indeterminate or HIV-2 indeterminate results on the HIV-1/HIV-2 Ab differentiation immunoassay (step 2), which is why HIV-1 RNA testing is recommended as the third step of the algorithm. If HIV-1 RNA is not detected and the HIV-1/HIV-2 Ab differentiation immunoassay result remains HIV-2 indeterminate or HIV indeterminate, HIV-2 RNA NAT testing should be considered. Alternatively, an HIV-1/HIV-2 RNA test may be performed. If nonreactive, no additional HIV-2 testing is needed.

For more information on laboratory testing in New York State, see NYSDOH 2024 Guidelines Update For Use of the HIV Diagnostic Testing Algorithm for Laboratories.

Step 3: HIV RNA Nucleic Acid Testing

For specimens that are reactive on the initial HIV-1/2 Ag/Ab immunoassay (see step 1, above) and nonreactive or indeterminate on the HIV-1/HIV-2 Ab differentiation immunoassay (see step 2, above), the next step in the HIV testing algorithm is to perform HIV RNA testing using an HIV-1 or HIV-1/HIV-2 RNA NAT that is FDA-approved for diagnostic purposes (see Figure 2, above).

Most laboratories reflex directly to an HIV RNA NAT without requiring an additional test order or new specimen, either by performing the test in-house or referring the specimen to another laboratory.

Interpreting NAT results:

- A reactive HIV-1 or HIV-1/HIV-2 NAT (step 3) result with a nonreactive or indeterminate HIV-1/HIV-2 Ab differentiation immunoassay (see step 2, above) result indicates laboratory evidence of acute HIV-1 or HIV-2 infection, respectively. Clinicians should proceed with clinical evaluation and management.
- A nonreactive HIV-1 or HIV-1/HIV-2 NAT (step 3) result with a nonreactive or indeterminate HIV-1/HIV-2 Ab differentiation immunoassay (see step 2, above) result generally indicates that the reactive initial HIV screening test (see step 1, above) was a false positive and that the patient does not have HIV infection.
  - This result pattern can also occur in individuals taking PrEP or in individuals with HIV who initiated ART early in infection, when seroconversion is delayed or inhibited [Smith, et al. 2018]. In these situations, history of HIV exposure risk, recent PrEP or ART use, and timing of potential HIV exposures should be assessed to interpret results appropriately and determine whether repeat testing with a fresh blood sample is necessary to rule out very early infection or technical issues. Data suggest that delays in HIV detection can be longer, due to delayed Ab formation, among individuals using injectable cabotegravir (4 to 26 weeks) than those using tenofovir disoproxil fumarate/emtricitabine (1 to 12 weeks) [Avelino-Silva, et al. 2024]. It is also possible that the viral load will be undetectable because of the antiretroviral activity of cabotegravir.
- If the HIV-1/HIV-2 Ab differentiation immunoassay (see step 2, above) result is repeatedly HIV-2 indeterminate or HIV indeterminate, and the HIV-1 NAT (step 3) is nonreactive, HIV-2 RNA NAT testing should be considered, or the algorithm should be repeated in 2 to 4 weeks starting with step 1. Additional HIV-2 testing is generally unnecessary if an HIV-1/HIV-2 RNA NAT (step 3) result is nonreactive.

→ KEY POINT

- If an individual is taking antiretroviral agents as PEP, PrEP, or for rapid ART initiation, a false negative result on the HIV RNA test may occur.

HIV-1 and HIV-1/HIV-2 RNA qualitative assays approved for diagnostic use: There are currently 3 FDA-approved NATs for diagnostic use (see Table 3, below). All have low limits of detection (<30 copies/mL) and the ability to detect low levels of all major HIV-1 and HIV-2 groups and HIV-1 subtypes.

Two assays, the Aptima HIV-1 RNA Quant Dx (Hologic) and Alinity m HIV-1RNA (Abbott Molecular), produce both qualitative and quantitative results, and can confirm HIV-1 infection and monitor disease prognosis. The cobas HIV-1/HIV-2 Qualitative (Roche Molecular Systems) assay, which produces a qualitative result only, detects and differentiates HIV-1 RNA and HIV-2 RNA.

Table 3: FDA-Approved HIV Nucleic Acid Tests for Diagnostic Use

Test (Manufacturer)	Limit of Detection	
	HIV-1 RNA [a]	HIV-2 RNA [b]
Qualitative and Quantitative Results		
Aptima HIV-1 RNA Quant Dx (Hologic) • **Detection:** HIV-1 RNA • **Specimens:** serum (qualitative), plasma (qualitative and quantitative)	• Plasma: 12 copies/mL • Serum: 8.9 copies/mL	—
Alinity m HIV-1 (Abbott Molecular) • **Detection:** HIV-1 RNA • **Specimens:** serum (qualitative), plasma (qualitative and quantitative)	• Plasma: 13.99 copies/mL • Serum: 15.94 copies/mL	—
Qualitative Results		
cobas HIV-1/HIV-2 Qualitative (Roche Molecular Systems) • **Detection:** HIV-1/HIV-2 RNA • **Specimens:** serum (qualitative), plasma (qualitative)	Plasma and serum: 12.8 copies/mL	Plasma and serum: 26.3 copies/mL

Notes:

a. Limit of detection determined based on 95% predicted detection limit against the third HIV-1 WHO International Standard (subtype B):
   – Hologic: 1 copy = 2.86 IU
   – Abbott Molecular: 1 copy = 1.63 IU
   – Roche Molecular Systems: 1 copy = 1.7 IU
b. Limit of detection determined based on 95% predicted detection limit against the HIV-2 WHO International Standard (1 copy = 0.2 IU).

HIV infection can be vertically transferred from pregnant individuals with HIV to their infants. Because infants acquire birth-parent antibodies to HIV, HIV RNA testing is performed to diagnose infection in infants. The NYSDOH strongly recommends that all New York State birth facilities use the NYSDOH Wadsworth Center Pediatric HIV Testing Service. The Wadsworth Center uses the Aptima HIV-1 RNA Quant Dx assay (Hologic) and has validated the use of lower specimen volumes and extended specimen stability.

→ KEY POINT

- NYSDOH strongly recommends that all New York State birth facilities use the NYSDOH Wadsworth Center Pediatric HIV Testing Service, which is free of charge for New York State clinicians providing care for HIV-exposed infants.

HIV-1 RNA quantitative assays (specifically assays without dual diagnostic and monitoring claims): Quantitative HIV-1 RNA NATs are widely available and approved by the FDA to monitor the prognosis of HIV-1 infection and response to ART. The performance characteristics of the quantitative HIV-1 tests are discussed further in the NYSDOH AI guideline Virologic and Immunologic Monitoring in HIV Care.

Regulatory restrictions may prevent laboratories from reflexing to a quantitative HIV-1 RNA test as part of the diagnostic testing algorithm. Quantitative HIV-1 RNA testing may be separately ordered when acute HIV infection is suspected or upon initiation of PrEP. Although these tests are highly sensitive, they have not been evaluated for HIV diagnosis to the same

extent as the 3 HIV RNA NATs that are FDA-approved for diagnostic use. As a result, quantitative HIV-1 RNA NATs may have lower specificity for diagnosing acute HIV infection. Therefore, when one of the 3 FDA-approved diagnostic HIV RNA NATs is available, it should be used instead of a quantitative HIV-1 RNA assay to diagnose acute HIV infection.

If a quantitative HIV-1 RNA is ordered, clinicians should be aware that a low-level viremia (<200 copies/mL) in the absence of serologic confirmation of HIV infection may indicate a false positive test result. Repeat HIV RNA and HIV Ag/Ab testing should be completed. See Box 2, below.

Box 2: Diagnosing Acute HIV Infection

Recommendations from the NYSDOH AI guideline Diagnosis and Management of Acute HIV Infection:

- Clinicians can presume the diagnosis of acute HIV and initiate treatment when HIV RNA levels ≥200 copies/mL are detected in plasma with sensitive NAT, and the result of the HIV screening or type-differentiation test is negative or indeterminate. (A2)
- When a low-level quantitative HIV RNA viral load result (<200 copies/mL) is obtained in the absence of serologic evidence of HIV infection, the clinician should repeat HIV RNA testing and perform an Ag/Ab combination immunoassay within 1 to 2 weeks to determine whether the result represents a false positive test or early HIV infection with rising HIV RNA levels. (A2)
- Clinicians should seek expert consultation for individuals taking PrEP or PEP because the diagnosis of acute HIV can be particularly challenging in those taking antiretroviral medications. (A3)
  - For expert consultation, call the Clinical Education Initiative (CEI) Line at 866-637-2342.

For more information on laboratory testing in New York State, see NYSDOH 2024 Guidelines Update For Use of the HIV Diagnostic Testing Algorithm for Laboratories.

→ A NEW HIV DIAGNOSIS IS A CALL TO ACTION

- Rapid ART initiation is the standard of care for HIV treatment in New York; ideally, ART should be initiated on the same day in patients newly diagnosed with HIV.
- In support of the NYSDOH AI Ending the Epidemic key strategies, this committee stresses the following:
  - Increase the number of people who know their HIV status by diagnosing HIV as early as possible.
  - Prevent new HIV acquisition by increasing access to effective prevention interventions, such as PrEP and PEP, harm reduction, and supportive services.
  - Improve viral suppression in people with HIV by increasing access to immediate ART and optimizing medication adherence and access to care.
- For more information, see NYSDOH AI guidelines:
  - Rapid ART Initiation
  - Selecting an Initial ART Regimen
  - Diagnosis and Management of HIV-2 in Adults

Appendix: Point-of-Care, Home-Based, and Other Alternative HIV Tests Available in New York State

For more information on laboratory testing in New York State, see NYSDOH 2024 Guidelines Update For Use of the HIV Diagnostic Testing Algorithm for Laboratories.

Point-of-Care (Rapid) Screening Tests

The U.S. Food and Drug Administration FDA has approved several HIV screening tests as Clinical Laboratory Improvements Act (CLIA)-waived tests, which allows a test to be performed in nonlaboratory, 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 Centers for Disease Control and Prevention [CDC] CLIA Certificate of Waiver). CLIA-waived tests may be used 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 are single-use test devices that can produce results within 30 minutes and are often referred to as rapid tests. Currently, POC HIV screening tests may only be used as initial screening tests; they are an alternative option when HIV testing according to the standard algorithm is not possible or practical. Reactive results on these tests require follow-up confirmatory testing.

Table A1, below, lists the characteristics of each of the 7 current FDA-approved POC HIV 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 some POC HIV screening tests have been designed for POC use, clinical laboratories also use these tests for HIV screening when a quick result is needed or the laboratory's overall testing volume is low. Depending on the device and its specific approval, laboratories may perform POC screening tests using serum, plasma, or whole blood specimens collected by venipuncture. Each of the POC HIV screening tests is restricted to the body fluid(s) that it was designed to analyze (see Table A1, 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 [Hurt, et al. 2017].

POC screening tests employ various technologies, and some devices are more sensitive for early detection than others. The Determine HIV-1/2 antigen/antibody (Ag/Ab) Combo (Abbott Diagnostics) 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 Determine HIV-1/2 Ag/Ab Combo is capable of detecting HIV infection 1 to 2 weeks earlier than all other FDA-approved POC screening tests but is less sensitive than the laboratory HIV-1/2 Ag/Ab immunoassays [Masciotra, et al. 2013].

Aside from the Determine HIV-1/2 Ag/Ab Combo, all other FDA-approved POC 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 enzyme immunoassays (EIAs) [Masciotra, et al. 2013; Masciotra, et al. 2011].

Table A1: FDA-Approved Point-of-Care HIV Tests

Test (manufacturer)	Sensitivity (95%) [a]	Specificity (95%)
Chembio SURE CHECK HIV 1/2 Assay (Chembio Diagnostic Systems) • **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) • **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) • **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%

Table A1: FDA-Approved Point-of-Care HIV Tests		
Test (manufacturer)	**Sensitivity (95%) [a]**	**Specificity (95%)**
Determine HIV-1/2 Ag/Ab Combo (Abbott Diagnostics) • **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) • **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) • **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%
Reveal G4 Rapid HIV-1/2 Antibody Test (MedMira Laboratories) • **Detection:** HIV-1/2 Abs • **Use:** POC, lab • **Specimens:** whole blood [b], serum, plasma • **CLIA category [c]:** Waived; fingerstick whole blood only	• Whole blood: 99.64% • Serum: 99.8% • Plasma: 99.8%	• Whole blood: 99.71% • Serum: 99.1% • Plasma: 98.6%

Abbreviations: Ab, antibody; Ag, antigen; CLIA, Clinical Laboratory Improvements Act; FDA, U.S. Food and Drug Administration; POC, point-of-care.

Notes:

a. Data shown are for HIV-1 only. For HIV-2 data, see instructions for use.
b. Fingerstick and venipuncture.
c. Information regarding CLIA waivers of HIV tests is available from the NYSDOH Wadsworth Center Clinical Laboratory Evaluation Program.

Home-Based Tests

HIV self-testing allows individuals to collect a specimen and test themselves for HIV at home. Product instructions guide users through specimen collection and result interpretation, and manufacturers offer online support for questions about test use. Self-tests are screening tests; reactive results require confirmatory laboratory testing using the standard HIV diagnostic algorithm (see guideline section HIV Testing With the Standard 3-Step Algorithm).

Currently, 2 types of FDA-approved HIV self-tests are available for purchase over the counter:

Oral fluid self-tests:

- Use a mouth swab to collect a specimen.
- Provide results in approximately 20 minutes.
- Example: OraQuick HIV Self-Test (OraSure Technologies)
- For additional information about oral fluid testing, see guideline section Alternative HIV Tests: Oral and Urine Specimens, below.

Fingerstick whole blood self-tests:

- Use a small blood sample to collect a specimen.
- Provide results in approximately 1 minute.
- Example: INSTI HIV Self Test (bioLytical Laboratories)

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 2009; FDA 2004].

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, plasma, or fingerstick whole blood.

The only FDA-approved EIA for use on oral fluid specimens is the Avioq HIV-1 Microelisa System (Avioq), which may be performed on OMT specimens collected using the OraSure HIV-1 Oral Fluid Collection Device (OraSure Technologies). Reactive results must be confirmed.

The only FDA-approved POC screening tests for use on oral fluid specimens are the OraQuick ADVANCE POC HIV-1/2 Antibody Test (OraSure Technologies) and Chembio DPP HIV 1/2 Assay (Chembio Diagnostic Systems). The tests detect both HIV-1 and HIV-2 Abs but cannot distinguish between them. These tests are CLIA-waived and may be used with oral fluid specimens in POC and nonclinical testing sites. For more information, see Table A1, above.

Urine specimens: HIV-1 Abs may be detected in urine. Only 1 screening test and 1 Western blot are FDA-approved for use with urine specimens. Although urine specimens are commonly used for HIV testing in 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 serologic testing, starting with an HIV-1/2 Ag/Ab immunoassay.

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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.
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.

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
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.