

Human Immunodeficiency Virus (HIV) - HIV-1/2 Antigen/Antibody Screen with Reflex to HIV-1/2 Antibody Differentiation

Use

This test is used to screen for and confirm HIV-1/HIV-2 infection, including acute infection and to differentiate HIV-1 from HIV-2 infection.

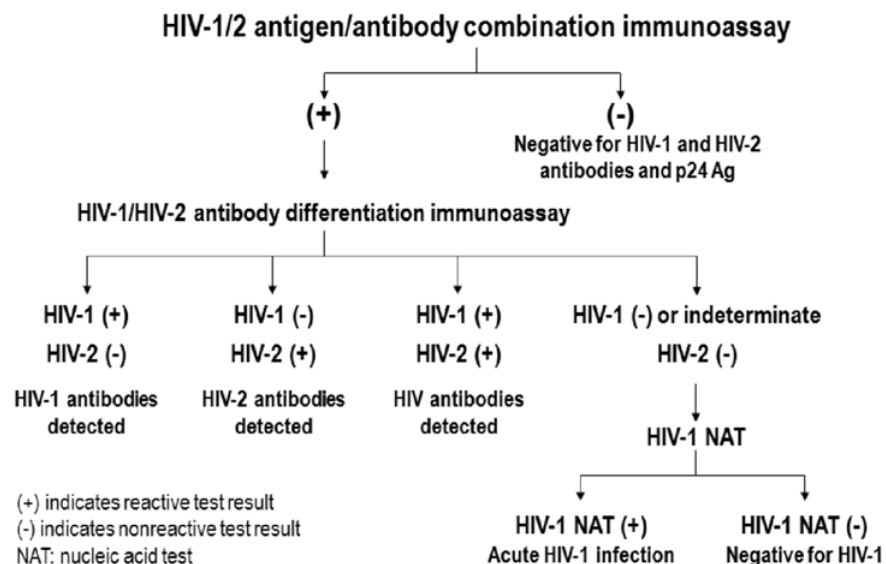
Clinical Significance

Two HIV serotypes, designated as HIV-1 and HIV-2, have been identified based on the results of serologic and molecular studies. Both viruses have the same morphology, lymphotropism, and modes of transmission. Following infection with HIV, an individual rapidly (within 4 weeks) develops antibodies to viral proteins, a process known as seroconversion. After seroconversion, HIV-specific antibodies can be readily detected in the blood specimen. Patient serum is initially screened for the presence of HIV-1 p24 antigen and HIV-1/HIV-2 antibodies, which allows for earlier detection of HIV in individuals who have not undergone seroconversion. Screen-reactive samples are then subject to a differentiation test to confirm the presence of HIV-1 or HIV-2 antibodies, which is important in treatment management, since HIV-2 does not respond to some anti-retroviral agents. In acute infections, where patients have not yet seroconverted, the initial screen may be positive while the differentiation test may be negative; patients with negative or indeterminate results for the differentiation test require follow-up HIV nucleic acid testing to resolve their infection status.

Further background information, fact sheets, statistics and educational resources may be found at the OSDH Infectious Disease Services [website](#).

Methodology

Sera are initially screened for HIV-1 p24 antigen and HIV-1 and HIV-2 specific antibodies using a 5th generation, qualitative, multiplex flow microbead immunoassay (MFIA). Specimens with an MFIA-reactive screen result are repeated in duplicate using the same MFIA. If either of the repeated samples is reactive, the specimen is reflexed to a supplemental HIV-1/HIV-2 antibody differentiation test. This testing algorithm aligns with the 2014 CDC recommended algorithm for [Laboratory Testing for the Diagnosis for the Diagnosis of HIV Infection](#), as illustrated below:



Specimen Type

- Whole blood collected and separated in serum separator tube (SST), or
- Separated serum poured into sterile, plastic, screw-cap tube

Minimum Volume/Size

2 mL serum. Draw enough whole blood (~4 mL) to yield the necessary serum volume. 1 mL of serum is acceptable, but this may be QNS if reflexive testing is needed.

Collection Instructions

Each facility should follow its guidelines for venipuncture collection of blood/serum. See guidance for collection of [Blood in a Serum Separator Tube](#).

Common Causes for Rejection

- Facility not approved for testing
- Patient younger than 2 years of age
- Blood collected in tube other than SST
- SST received unspun
- Specimen received at ambient temperature and > 48 hours from collection
- Specimen received at 2-8°C and > 7 days from DOC
- SST frozen
- Serum (in transport tube) received frozen and > 14 days from DOC
- QNS
- Bacterial contamination
- Extensive hemolysis
- Extensive lipemia

Shipping and Storage

- **Storage:** Serum samples may be stored in an SST or in a secondary transfer tube. Samples can be held at 2–8°C for up to 7 days or at room temperature (18–30°C) for up to 48 hours prior to screen testing. For long-term storage, serum specimens must be transferred to a secondary transfer tube and frozen at –20°C or colder.
Please note: Delays in delivering the sample to the PHL may interfere with the laboratory’s ability to perform secondary (i.e., confirmatory) testing if the screen result is positive.
- **Shipping:** Refrigerated samples should be shipped with sufficient cold packs to keep the sample at 2–8°C. Frozen samples should be shipped on dry ice to maintain a frozen state. Place each specimen in an individually sealed bag.

Turn-around Time

Within 10 working days from receipt

Reference Range

- HIV-1 Antibody: Non-reactive
- HIV-2 Antibody: Non-reactive
- HIV-1 Antigen: Non-reactive

Reportable Results

- HIV-1 Antibody Non-reactive

- HIV-2 Antibody Non-reactive
- HIV-1 Reactive
- HIV-2 Reactive
- HIV Reactive (Undifferentiated)
- HIV-1 and/or HIV-2 Indeterminate

Interpretation

- Non-Reactive, HIV-1 antigen and HIV-1/HIV-2 antibodies not detected: A non-reactive test result does not exclude the possibility of infection with HIV. Levels of HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2 may be undetectable in early infection. If a recent HIV exposure is suspected, consider re-testing.
- HIV-1 Reactive: Specimens that are HIV-1 reactive with differentiation test (following a reactive HIV-1/HIV-2 screening results are considered HIV-1 positive, and no further testing is necessary.
- HIV-2 Reactive: Specimens that are HIV-2 reactive with differentiation test (following a reactive HIV-1/HIV-2 screening result) are considered HIV-2 positive, and no further testing is necessary.
- HIV Reactive, Undifferentiated: Specimens that are HIV-1 and HIV-2 reactive with differentiation test (following a reactive HIV-1/HIV-2 screening result) have evidence of HIV infection, but the test is unable to differentiate antibodies as HIV-1 or HIV-2, i.e., undifferentiated. HIV-1 RNA testing and HIV-2 RNA or DNA testing are recommended to verify or rule-out dual infection.
- Inconclusive: Specimens that are HIV, HIV-1 or HIV-2 indeterminate (i.e., incomplete pattern of antibodies) or are non-reactive with the differentiation test (following a reactive HIV-1/HIV-2 screening result) could either indicate either an acute or early infection or false positive reaction. Recommend submission of additional specimens for repeat HIV antigen/antibody testing and HIV-1 RNA testing or HIV-2 RNA or DNA testing, as indicated.

Limitations/Interferences

- A person who has antibodies to HIV-1 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of an HIV infection is accurate.
- Detection of HIV antibodies in infants born to seropositive mothers is not adequate to diagnose HIV infection in the infant, since maternal IgG frequently persists in the infant's blood for as long as 18 months after birth. Supplemental assays designed specifically for neonatal specimens may be helpful in resolving such cases.
- Non-reactive, inconclusive and undifferentiated HIV-1/HIV-2 Differentiation Test results should be referred for HIV-1 and/or HIV-2 nucleic acid testing as per CDC guidelines.
- Bacterial contamination, icteric, lipemic, hemolyzed, or heat-inactivated samples may cause erroneous results and should be avoided.

CPT Code

87389 / 86689

Notes

This test is only available to County Health Departments in Oklahoma and sites approved through the OSDH HIV/STD Division. Follow-up nucleic acid testing for HIV-reactive, Undifferentiated and Inconclusive results, as recommended by CDC guidelines, must be approved by the [Sexual Health & Harm Reduction \(SHHR\) Service](#). Instructions for submitting specimens for HIV-1 RNA testing or HIV-2 DNA/RNA testing can be found on Health Hub (OSDH County Health Departments). These tests are approved for *in vitro* diagnostic use by the U.S. Food and Drug Administration.