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Current HIV Testing Guidelines and Additional Considerations

Testing for Human Immunodeficiency Virus (HIV) has evolved over the last two decades to reflect changes in testing technology. This has resulted in changes in test algorithms recommended for HIV diagnosis, and additional testing for monitoring patients diagnosed with HIV. The most recent HIV testing guidelines from the Centers for Disease Control and Prevention (CDC) can be found in the following link, titled '2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens': https://stacks.cdc.gov/view/cdc/50872

The CDC recommended HIV test combinations and interpretations are in Appendix A and Appendix B, respectively, of this document. The following sections are applicable for HIV testing considerations in persons of age ≥ 13 years:

A. Typical immune response to HIV infection and timelines for detection
B. Diagnosing acute versus non-acute HIV infection
C. Interpretation of negative HIV test results

A. Typical immune response to HIV infection and timelines for detection

The time between initial HIV infection, and when tests can detect the virus is the window period, also known as the eclipse phase. Current detection tests cannot determine HIV positivity during the eclipse phase. It is not until eight to ten days after initial infection that the HIV nucleic acid test, known as a qualitative HIV-1 RNA/DNA, can detect the presence of HIV infection. This test is also known as a qualitative HIV-1 RNA NAT or HIV-1 RNA NAAT. The HIV protein antigen specific to HIV-1, also known as p24 antigen, is detectable earlier than the antibodies to HIV, and typically between 11 to 15 days post infection. It takes about three weeks for the HIV antibodies to be detectable.

Per the CDC recommended HIV testing algorithm, the initial screening test is the HIV antigen-antibody detection test, HIV-1/2 combination immunoassay (HIV 1/2 Ag/Ab), which can be reactive/positive as early as 11 to 15 days after initial infection. This test detects the presence of both HIV antibodies and the p24 antigen, but cannot differentiate between HIV-1 and HIV-2 infection. The confirmatory HIV antibody detection test, HIV-1/HIV-2 differentiating immunoassay, can differentiate between the type of HIV antibodies, that is, HIV-1 and HIV-2, and is detectable about three weeks after infection.

B. Diagnosing Acute versus Non-acute HIV infection

a. <u>Acute HIV infection with antigen only and no antibody detection</u>
In newly acquired HIV infection, the HIV 1/2 Ag/Ab screening test may be reactive/positive, while the HIV 1/2 differentiating immunoassay is nonreactive/negative or indeterminate. This can indicate that the infection is very new and positivity cannot be confirmed using the differentiating test. This **does not** necessarily mean that the individual is HIV negative, considering the HIV antibodies are **not** detectable until about three weeks after infection. A reactive/positive HIV 1/2 Ag/Ab screening test and a non-reactive/negative HIV-1/HIV-2 differentiating immunoassay result may indicate **acute** HIV infection. The specimen needs

additional testing to confirm HIV infection. In this case, to confirm the presence of HIV infection, the CDC recommends using the **qualitative** HIV-1 RNA NAT test (see Appendix A).

If the qualitative HIV-1 RNA NAT is non-reactive/ negative, then it is unlikely to be HIV-1 infection, and the HIV 1/2 Ag/Ab reactivity could be a false positive test result. The provider can investigate and/or consider potential causes of the false positive result, and perform repeat testing in one to two weeks. Appendix C enlists possible causes of false positive and false negative test results.

The qualitative HIV-2 RNA NAT detection test can rule out HIV-2 infection if there are epidemiologic risk factors and/or suspected exposure to HIV-2. Appendix D enlists HIV-2 endemic countries and Appendix E provides HIV-2 testing laboratory considerations, including list of laboratories performing qualitative HIV-2 NATs.

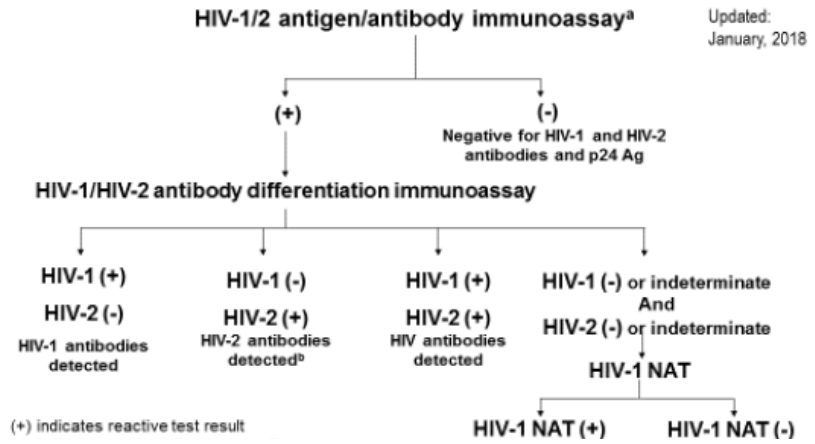
b. Non-acute HIV infection with antibody detection
If the initial screening test and the confirmatory antibody-differentiating test are both reactive/positive, there is no need for further HIV diagnostic testing. Detectable HIV antibodies on both tests suggest established HIV infection. Appendix B provides CDC's guidance for interpreting results from the HIV testing algorithm in a one-page table-format.

C. Interpretation of negative HIV test results

a. No HIV infection: If the initial screening test is non-reactive/negative, a confirmatory antibody-differentiating test is not necessary. This testing interpretation is valid and reliable when the provider performs the screening test at least 11 days from suspected HIV exposure. This is in alignment with the CDC's HIV test interpretation guidelines (see Appendices A and B).
b. Recent HIV exposure: The initial screening test is positive/reactive, but the antibody-differentiating test is non-reactive/negative or indeterminate may indicate recent exposure, or acute infection. The CDC recommends additional testing using the qualitative HIV-1 RNA NAT to confirm or rule-out HIV infection.
c. False negative test result: A false negative result can occur if the individual tests during the window period or eclipse phase. Current tests cannot determine positivity during the window period. The CDC recommends repeat testing on a new specimen (see Appendices A and B), and/or to investigate on potential causes of a false negative test result (see Appendix C).
d. Virally suppressed infection: Individuals on long-term use of antiretroviral medication (ARV) can have virally suppressed infection, that is, have undetectable result on a **quantitative** HIV-1 RNA/PCR/DNA viral load test. On occasion, such individuals may also have a negative result on a qualitative HIV RNA NAT. This can also be true for break-through HIV infections in individuals taking pre-exposure prophylaxis (PrEP). Despite viral suppression, the antibody tests are likely reactive/positive. Hence, it is important to note that the CDC recommended HIV testing algorithm is for diagnosing or confirming HIV infection, and the quantitative HIV-1 RNA/PCR/DNA viral load test is for monitoring the infection.

Appendix A: CDC Recommended HIV Testing Algorithm (Updated 2018)

Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens



1. Laboratories should conduct initial testing for HIV with an FDA-approved antigen/antibody immunoassay[a] that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to test for established HIV-1 and HIV-2 infection and for acute HIV-1 infection, respectively. No further testing is required for specimens that are non-reactive on the initial immunoassay. However, if there is a possibility of very early infection leading to a non-reactive initial antigen/antibody immunoassay, such as when recent HIV exposure is suspected or reported, then conduct an HIV-1 nucleic acid test (NAT), or request a new specimen and repeat the algorithm according to CDC guidance (1,4,5,6).
2. Specimens with a reactive antigen/antibody 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 antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies. Reactive results on the initial antigen/antibody immunoassay and the HIV-1/HIV-2 antibody differentiation immunoassay should be interpreted as positive for HIV-1 antibodies, HIV-2 antibodies[b], or HIV antibodies, untypable (undifferentiated).
3. Specimens that are reactive on the initial antigen/antibody immunoassay and non-reactive or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with an FDA-approved HIV-1 NAT.
   - A reactive HIV-1 NAT result and non-reactive or indeterminate HIV-1/HIV-2 antibody differentiation immunoassay result indicates laboratory evidence of acute HIV-1 infection.
   - A negative HIV-1 NAT result and non-reactive or HIV-1 indeterminate antibody differentiation immunoassay result indicates an HIV-1 false-positive result on the initial immunoassay.
   - A negative HIV-1 NAT result and repeatedly HIV-2 indeterminate or HIV indeterminate antibody differentiation immunoassay result should be referred for testing with a different validated supplemental HIV-2 test (antibody test or NAT) or repeat the algorithm in 2 to 4 weeks, starting with an antigen/antibody immunoassay (3).
4. Laboratories should use this same testing algorithm, beginning with an antigen/antibody immunoassay on all serum or plasma specimens submitted for testing after a preliminary positive result from any rapid HIV test conducted in a CLIA-waived setting (7).

[a]The FDA-approved single-use rapid HIV-1/HIV-2 antigen/antibody immunoassay can be used as the initial assay in the laboratory HIV testing algorithm for serum or plasma. If any instrumented antigen/antibody test is available, it is preferred due to its superior sensitivity for detecting HIV during acute infection (1,2).
[b]This includes specimens reported as HIV-2 positive with HIV-1 cross-reactivity (3).
[c]Refer to last bullet, item 3 above.

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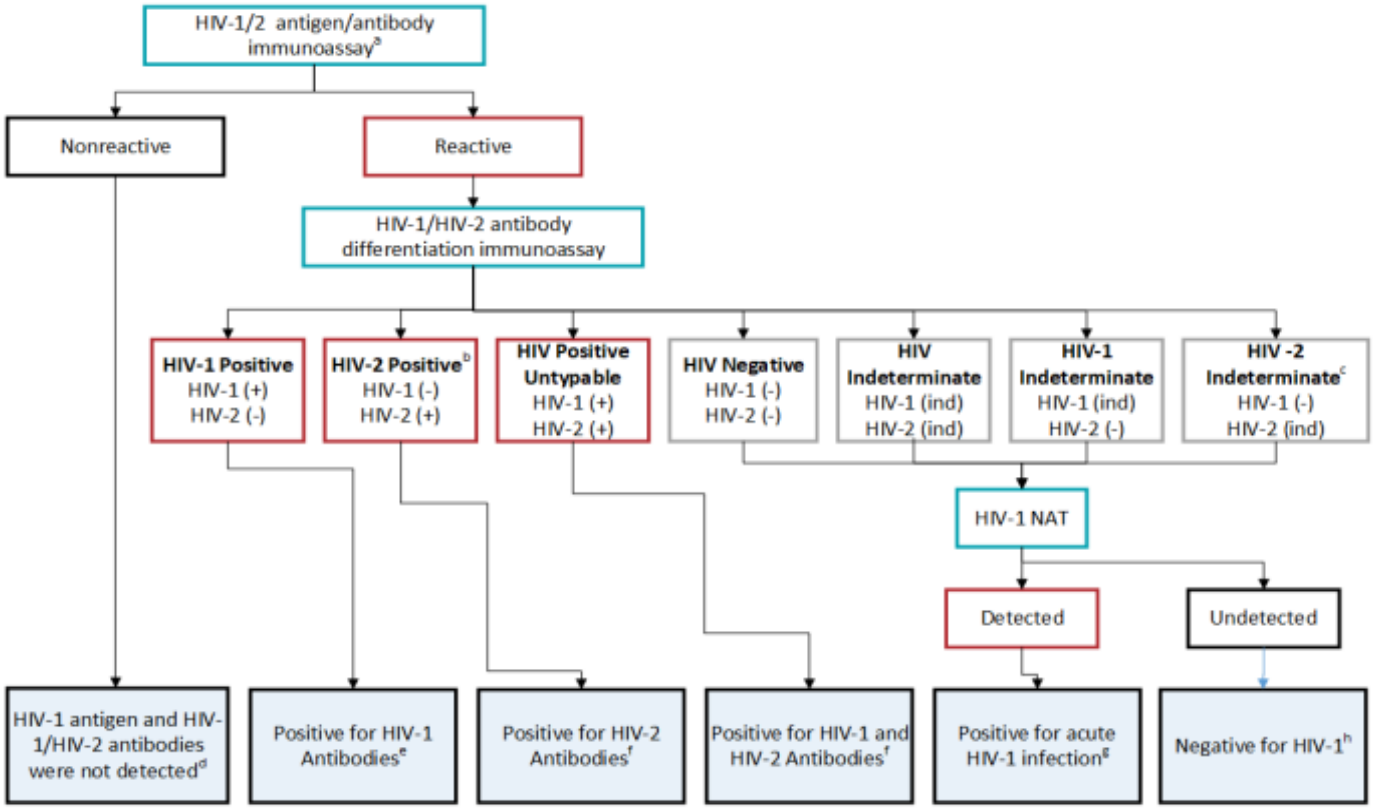
1) Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations https://stacks.cdc.gov/view/cdc/23447 2) Use of the Determine HIV 1/2 Ag/Ab Combo Test with Serum or Plasma in the Laboratory Algorithm for HIV Diagnosis https://stacks.cdc.gov/view/cdc/48472 3) Technical Update on HIV-1/2 Differentiation Assays https://stacks.cdc.gov/view/cdc/40790 4) Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm https://stacks.cdc.gov/view/cdc/45930 5) Updated Guidelines for Antiretroviral Postexposure Prophylaxis After Sexual, Injection Drug Use, or Other Nonoccupational Exposure to HIV-United States, 2016 https://stacks.cdc.gov/view/cdc/38856 6) Web content: How Soon Can Clinicians Rule Out Infection? https://www.cdc.gov/hiv/testing/clinical/index.html 7) Web content: Clinical Laboratory Improvement Amendments https://wwwn.cdc.gov/clia/

Appendix A (contd.): CDC Recommended HIV Testing Algorithm (Updated 2019)



a. APHL and CDC continue to recommend that laboratories use an FDA-approved instrumented HIV-1/HIV-2 antigen/antibody immunoassays as the initial assay 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 antigen/antibody immunoassay may be used as the initial assay the initial assay in the laboratory HIV testing algorithm for serum or plasma if an instrumented assay is not available. **b.** This includes specimens reported as HIV-2 positive with HIV-1 cross reactivity. **c.** 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. **d.** If recent HIV exposure is suspected or reported, conduct HIV-1 NAT or request a new specimen and repeat the algorithm according to CDC Guidance. **e.** Link patient to HIV medical care and provide appropriate prevention counseling. **f.** Link patient to HIV medical care and provide appropriate prevention counseling. Provider may consider additional testing. **g.** Link patient to HIV medical care and provide appropriate prevention counseling immediately to expedite prevention practices. **h.** A negative HIV-1 NAT result and repeatedly HIV-2 indeterminate or HIV indeterminate antibody 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 2-4 weeks to assess HIV-2 infection.

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Appendix B Guidance for reporting results from the HIV laboratory diagnostic algorithm for use with serum and plasma specimens (4)

Guidance for Reporting Results from the HIV Laboratory Diagnostic Testing Algorithm for Serum and Plasma Specimens[a]

Test Outcomes

Test Sequence			Final Algorithm Interpretation[d]	Interpretation for Provider[e] (Sample should be reported as:)	Further Actions[f]
Step 1	Step 2	Step 3			
HIV-1/HIV-2 Ag/Ab IA[b]	HIV-1/HIV-2 Antibody Differentiation IA[c]	HIV-1 NAT			
Nonreactive	n/a	n/a	HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection.	HIV negative	If recent HIV exposure is suspected or reported, conduct HIV-1 NAT or request a new specimen and repeat the algorithm according to CDC guidance. [g]
Reactive	HIV-1 Positive	n/a	Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present.	HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
Reactive	HIV-2 Positive	n/a	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	HIV-2 Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
Reactive	HIV-2 Positive with HIV-1 Cross reactivity	n/a	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	HIV-2 Positive. This result is distinct from HIV positive untypable (undifferentiated).	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
Reactive	HIV Positive untypable (undifferentiated)	n/a	Positive for HIV-1 and HIV-2 antibodies. Laboratory evidence of HIV-1 and/or HIV-2 infection is present.	HIV Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h] Provider may consider additional testing for HIV-1 RNA or DNA and HIV-2 RNA or DNA to verify or rule out HIV-1/HIV-2 dual infection. Request additional specimen if original specimen volume is insufficient.
Reactive	HIV-1 indeterminate, HIV-2 indeterminate[i], HIV indeterminate	Detected	Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an **acute** HIV-1 infection.	**Acute** HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling immediately[h] to expedite prevention practices.
Reactive	HIV-1 indeterminate	Not detected	HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected.	HIV Negative	If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance. [g]
Reactive	HIV-2 indeterminate[i]	Not detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.	HIV-1 Negative, HIV-2 inconclusive	Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.
Reactive	HIV Indeterminate	Not detected	HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.	HIV-1 Negative, HIV-2 inconclusive	Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.
Reactive	Negative	Detected	Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an **acute** HIV-1 infection.	**Acute** HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling immediately[h] to expedite prevention practices.
Reactive	Negative	Not detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected.	HIV Negative	If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance. [g]
Reactive	Negative or Indeterminate	Invalid or not performed	Inconclusive	Inconclusive	Request an additional specimen and repeat the algorithm. Ensure HIV-1 NAT is performed, if indicated by results of HIV-1/HIV-2 Ag/Ab IA and HIV-1/HIV-2 Ab differentiation IA.

a. The tests outlined in this table are not FDA approved for oral fluid or dried blood spots. **b.** The need for repeating screening IA on an initial reactive test is assay dependent, refer to product package insert. **c**. This column contains the Final Assay interpretation per the Geenius package insert, the only FDA approved test for this step. We recommend excluding the individual HIV-1 and HIV-2 results on the laboratory report. If they are used, the final assay interpretation or final assay result should also be included. **d.** This column contains suggested language to be used for the laboratory report and it can be directly used for reporting from LIMS systems. **e**. This column contains simplified language of the previous column, "Final Algorithm Interpretation," and is included here for healthcare providers or other non-laboratorians that may also use this table as a reference document. This does not need to be included on the laboratory report. **f.** Comments under "Further Action" can be included as language in the laboratory report or can be used as guidance for laboratorians to discuss test results with healthcare providers or health deparment staff. **g**. Please refer to Centers for Disease Control and Prevention guidance. Available at: https://www.cdc.gov/hiv/testing/laboratorytests.html, https://stacks.cdc.gov/view/cdc/38856 and https://www.cdc.gov/hiv/testing/clinical/index.html **h.** Please refer to the Centers for Disease Control and Prevention HIV Guidelines and Recommendations to find the most appropriate information by age and risk group for the patient in question. Available at: http://www.cdc.gov/hiv/guidelines/ **i.** Follow Geenius package insert and refer to the CDC Technical Update. Available at: https://stacks.cdc.gov/view/cdc/40790

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Appendix C: Potential Causes of False Positive and False Negative HIV Test Results

Table 1:

Potential Causes of False-Negative and False-Positive Results in HIV Assays		
False Negative	**False Positive**	
	Any of the following conditions, infections, or findings	
Pre-analytical or analytical causes	Renal failure	Anti-lymphocyte, -collagen, -smooth, muscle, -nuclear, -mitochondrial, -parietal cell, -HAV IgM, -HBc IgM, or -polystyrene antibodies
Hemodilution	Alpha-interferon therapy in hemodialysis patients	Heat-treated, lipemic, hemolyzed, or icteric specimens
Mislabeling of samples or wells	Flu or flu vaccination	Normal human ribonucleoproteins
Variability in test kits, including the inability to detect HIV-1 subtype O individuals using an HIV kit that does not detect subtype O antibodies	HSV I or II	Visceral leishmaniasis
Masking of HIV antigenic determinants or insufficient antigenic determinants for detection	URI	Fungal infections, including TB and *Mycobacterium avium*
By the HIV assay	Recent viral infection or vaccination against HBV or with tetanus toxoid	
Pipetting error	Pregnancy in multiparous women	
	Malaria	
Assay inhibition due to:	RA	
Powder from powdered gloves	Blood transfusions	
Storing samples in serum separator tubes	Myeloma	
	Hemophilia	
Biological, pathologic, or pharmacologic determinants	Stevens-Johnson syndrome	
Window (preseroconversion) period status	Organ transplantation	
Delayed antibody synthesis in infants	ALD or AH	
Diminished immune response in patients	Autoimmune disease	
*With HIV 1-related immune dysfunction	Receptive anal sex	
*On immunosuppressive therapy		
*With concurrent EBV or CMV infection		
Congenital or drug-induced hypogammaglobulinemia		
Formation of antigen-antibody complexes		

*Reference: "Evaluation of Four Qualitative Third-Generation HIV Antibody Assays and the Fourth-Generation Abbott Ag/Ab Combo Test". http://labmed.ascpjournals.org/content/42/9/523.full.pdf

Appendix D: HIV-2 Endemic Countries

Table 2:

African countries with a high prevalence of HIV-2 infection

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West African nations

Mauritania *
Benin
Burkina Faso
Cape Verde *
Cote d'Ivoire *
Gambia *
Ghana
Guinea
Guinea-Bissau *
Mali *
Niger
Nigeria *
Sao Tome
Senegal
Sierra Leone *
Togo

Other African nations Liberia
Angola *
Mozambique *

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* Prevalence of HIV-2 reported to exceed 1% in the general population.

Appendix E: HIV-2 Laboratory Testing Considerations

Additional testing for HIV-2 needed with any of the following results from initial screening:

1. HIV Ag/Ab Reactive paired with Reactive HIV-2 Ab from an HIV-1/HIV-2 Antibody differentiating immunoassay (with HIV-1 Ab reactive, indeterminate or negative), and HIV-1 qualitative RNA *Not Detected*; HIV-2 RNA or DNA test Detected.
2. Reactive HIV-2 IA (performed by commercial reference laboratory) paired with detected HIV-2 qualitative RNA or DNA test performed by either CDC or New York State Department of Health Axelrod Institute/Wadsworth laboratory
3. Reactive HIV-2 IA paired with HIV-2 detectable viral load performed by the University of Washington (state) laboratory.

The following laboratories can perform HIV-2 Immuno-Assay screening only:

1. LabCorp (certain locations; order Test Code #163550).
2. Quest Diagnostics (certain locations; order Test Code#34977)
3. Centers for Disease Control and Prevention (CDC)
4. Mayo Medical Laboratories (order Test Code HIV2L)

The following laboratories can perform HIV-2 qualitative RNA/DNA testing:

1. New York State Department of Health, Axelrod Institute, Wadsworth Laboratory. *Please note that the ordering facility must have a valid, current Memorandum of Understanding (MOU) with the laboratory in order to request the testing.*
2. Mayo Medical Laboratories (Order Test Code FHV2Q, or HIV-2 DNA/RNA PCR Qualitative)
3. Cambridge Biomedical Laboratory (CLIA# 22D0926993). The facility performs a non-FDA approved, but in-house CLIA validated qualitative HIV2 PCR using serum or plasma.

The following laboratory can perform HIV-2 Viral Load testing for monitoring infection status:

1. The University of Washington Medicine Laboratory. Order Test code HIV2VL, and follow instructions from this link from their online test catalog:

a. https://testguide.labmed.uw.edu/public/view/HIV2VL . Specimen can be blood or CSF collected as follows:
   i. Blood: Either 10ml EDTA lavender top or fill 5-8 ml in a Pearl top EDTA PPT tube
   ii. CSF: 3-4 ml of CSF in sterile collection tube.
b. Both sample types must be frozen at -70C within six (6) hours of collection.
c. Performing Lab: HMC-Retrovirus Lab at (206) 897-5210; performed twice weekly. LOINC = 69354-9; CPT code as of 6/8/2017 was 87539.