

General and Laboratory Considerations: Rapid HIV Tests Currently Available in the United States

Requirements for performing the tests, testing procedures, and interpretation of results

<http://www.cdc.gov/hiv/topics/testing/resources/factsheets/rt-lab.htm>

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Rapid HIV tests are simple to use and require little or no specialized equipment. They make it possible to provide test results at the time the test is done. Six rapid HIV tests approved by the U.S. Food and Drug Administration (FDA) are commercially available for use in the United States (listed in chronological order of their FDA approval dates):

1. OraQuick Rapid HIV-1/2 Antibody Test
2. Reveal G2 Rapid HIV-1 Antibody Test
3. Uni-Gold Recombigen HIV Test
4. Multispot HIV-1/HIV-2 Rapid Test
5. Clearview HIV 1/2 Stat Pak
6. Clearview Complete HIV 1/2

Test Kit Name	Manufacturer	Specimen Type	CLIA Category	Equipment Required
OraQuick Advance Rapid HIV-1/2 Antibody Test	Orasure Technologies, Inc http://www.orasure.com/	Whole Blood, Oral Fluid	Waived	Timer
		Plasma	Moderate Complexity	
Reveal G3 Rapid HIV-1 Antibody Test	MedMira, Inc. http://www.medmira.com/	Serum, Plasma	Moderate Complexity	Centrifuge, Refrigerator
Uni-Gold Recombigen HIV Test	Trinity BioTech http://www.unigoldhiv.com/	Whole Blood	Waived	Timer
		Serum, Plasma	Moderate Complexity	
Multispot HIV-1/HIV-2 RapidTest	Bio-Rad Laboratories http://www.bio-rad.com/	Serum, Plasma	Moderate Complexity	Centrifuge, Refrigerator, Lab Equipment
Clearview HIV 1/2 Stat Pak	Chembio Diagnostic Systems, Inc. http://www.chembio.com/	Whole Blood	Waived	Timer
		Serum, Plasma	Moderate Complexity	
Clearview Complete HIV 1/2	Chembio Diagnostic Systems, Inc. http://www.chembio.com/	Whole Blood, Serum, Plasma	Moderate Complexity*	Timer

*Manufacturer has applied for waiver for use with whole blood.

Requirements for Performing Rapid HIV Tests

Any organization that performs a rapid HIV test in order to provide results to patients is considered a laboratory under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). All laboratories must comply with the regulations of the CLIA program and with

applicable state requirements. (For more information on CLIA, which is administered by the Centers for Medicare & Medicaid Services (CMS), and a list of laboratory contact persons in each state, visit the CMS CLIA Web site [<http://www.cms.hhs.gov/CLIA/>].) An organization can either apply for its own CLIA certificate or, if authorized by CMS, make arrangements to be included with a CLIA-certified laboratory under a multiple-site exception.

The sale of rapid HIV tests is restricted to clinical laboratories that have an adequate quality assurance program and where persons who use the test will receive and use the instructional materials provided with the tests. The FDA also requires that persons tested with the rapid tests receive the "Subject Information" pamphlet provided with the test. Details about other restrictions that apply to the rapid HIV tests are outlined in the package inserts provided with the test kits.

Currently available rapid HIV tests are either "waived" or categorized as "moderate complexity" under the CLIA program. CLIA requirements for laboratories differ depending on the category of the test. The Clearview (Chembio) tests have only recently been approved by the FDA and are not yet commercially available.

Waived Testing

OraQuick is a waived test when it is used with whole blood or oral fluids. Uni-Gold and Clearview HIV 1/2 Stat Pak are waived tests when used with whole blood. The manufacturer of Clearview COMPLETE HIV 1/2 test has applied for a CLIA waiver.

For waived tests, there are no federal requirements for personnel, quality assessment, or proficiency testing. Waived tests can be done in traditional laboratories or clinical settings and also in settings such as doctors' offices, HIV counseling and testing sites, mobile vans, and health fairs. To perform waived tests, an organization must obtain a certificate of waiver from the CLIA program (or, if authorized by CMS, be included with a CLIA-certified laboratory under a multiple-site exception) and follow the manufacturer's instructions for the test procedure. For laboratories that plan to perform waived testing, more information on CLIA requirements is outlined in the CLIA Certificate of Waiver Fact Sheet (<http://www.cdc.gov/hiv/topics/testing/resources/factsheets/roftCLIA.htm>) and at the CDC CLIA Web site (<http://www.phppo.cdc.gov/clia/regs/toc.aspx>). CDC also has developed guidelines on quality assurance practices for laboratories planning to use waived HIV rapid tests (http://www.cdc.gov/hiv/topics/testing/resources/guidelines/ga_guide.htm).

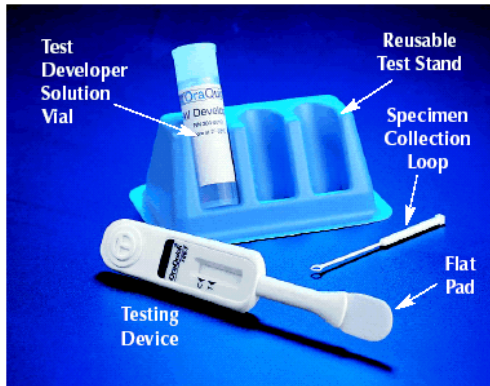
Moderate Complexity Testing

Reveal and Multispot are categorized as moderate complexity tests. OraQuick is categorized as moderate complexity when used with plasma. Uni-Gold, Clearview HIV Complete HIV 1/2, and Clearview HIV 1/2 Stat Pak are categorized as moderate complexity when used with serum or plasma.

A laboratory that performs moderate complexity tests must register with the CLIA program and meet specific CLIA standards for personnel, quality assessment, proficiency testing, and inspections. For organizations that plan to perform moderate complexity testing, more information on CLIA requirements and the steps necessary to obtain a certificate for moderate complexity testing are outlined in the moderate complexity overview section of the CDC CLIA Web site (<http://www.phppo.cdc.gov/clia/moderate.aspx>).

Summary of the Testing Procedures

OraQuick Advance Rapid HIV 1/2 Antibody Test



The OraQuick test is approved for use with whole blood specimens obtained by fingerstick or by venipuncture, with oral fluid specimens, and with plasma. It is intended for use at point of care, in medical and nonmedical settings. However, it can also be performed in a laboratory after the specimen has been obtained.

To conduct the test, place a vial of developer solution in the plastic stand. The reusable stand holds the test device at the correct angle to ensure accurate test results. When testing a fingerstick specimen, clean the fingertip with alcohol and prick the fingertip with a lancet

(needle) to get a small drop of blood. The blood is collected with a specimen loop and transferred to a small plastic vial containing a premeasured volume of developing solution, into which the sample is mixed. The testing process is the same for whole blood or plasma obtained by venipuncture. For whole blood, insert the specimen loop into the tube of blood after the tube has been inverted to ensure that the blood is thoroughly mixed. For plasma, first centrifuge the blood to separate the blood cells from plasma, and insert the specimen loop into the plasma. Then insert the specimen loop into the test vial and mix. Collect oral fluid specimens by using the absorbent pad on the end of the test device to swab the outer surface of the upper and lower gums. Then insert the test device into the test vial. Test results must be read no sooner than 20 minutes, but no later than 40 minutes, after the OraQuick device is added to the developer solution.

The test result is read directly from the OraQuick device.



If 1 reddish band appears at the control (C) location the test result is negative for HIV antibodies.

If 2 reddish bands appear, one at the control (C) location and one at the test (T) location, the test is “reactive” (that is to say, the test result is preliminary positive for HIV-1 or HIV-2 antibodies).

If no band appears at the C location, if any bands appear outside the C or T locations, or if a pink-red background appears in the device window, the test is invalid and must be repeated.

The OraQuick test includes an internal control that verifies that specimen has been added and that the test has been run correctly. Positive and negative external controls must be run by each new operator before performing testing on patient specimens, whenever a new lot of test kits is used, if the conditions of testing or storage (e.g., temperature) fall outside the range recommended by the manufacturer, and at periodic intervals specified in the laboratory's quality

assurance program. External controls are not included with the test kits and must be ordered separately from the manufacturer.

Shelf Life of Kits: 6 months from data of manufacture if stored at room temperature

Shelf Life of Controls: 1 year unopened or 8 weeks after opening if refrigerated

For more information on the OraQuick test, see the test kit package insert (<http://www.orasure.com/uploaded/398.pdf>) or visit the manufacturer's Web site (<http://www.orasure.com/>).



Reveal G3 Rapid HIV-1 Antibody Test

The Reveal G3 test is intended for use as a point-of-care test, but it requires some laboratory equipment. Positive and negative external controls (supplied with the test) must be reconstituted with buffer solution. These control reagents, each sufficient for 5 tests, can be stored refrigerated for up to 7 days after they are reconstituted.

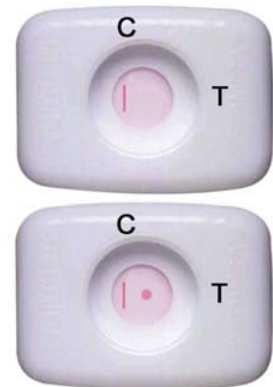
To do the test, draw a blood specimen from a vein and centrifuge the specimen to separate the blood cells from the serum or plasma. Place a buffer solution

in the test cartridge, and allow it to be absorbed. Add the serum or plasma specimen to the test cartridge and allow it to be absorbed. Then place the InstaGold cap in the cartridge and add 12 drops of buffer. Then remove the cap and read the test result directly from the cartridge. An additional 3 drops of buffer may be added to clarify the test results

A red band on the test cartridge indicates the test result is “non-reactive,” that is, negative for HIV-1 antibodies.

A red dot with a red band on the test cartridge indicates the test result is “reactive,” that is, preliminary positive for HIV-1 antibodies.

Absence of the red band on the test cartridge after the test has been run, or a pinkish-red background throughout the window of the cartridge indicates that the test is invalid and must be repeated.



Unlike the Reveal test initially approved by the FDA, the Reveal G3 test contains an internal procedural control that verifies that the specimen has been added and the test has been run correctly. External controls (known HIV-positive and -negative specimens supplied with the test kit) must be run by each new operator before performing testing on patient specimens, whenever a new lot of test kits is used, if the conditions of testing or storage (e.g., temperature) fall outside the range recommended by the manufacturer, and at periodic intervals specified in the laboratory's quality assurance program.

Shelf Life: Information pending

For more information on the Reveal G3 test, see the test kit package insert (http://www.reveal-hiv.com/pdf/g3_insert.pdf) or visit the manufacturer's Web site (<http://www.reveal-hiv.com/>).

Uni-Gold Recombigen HIV

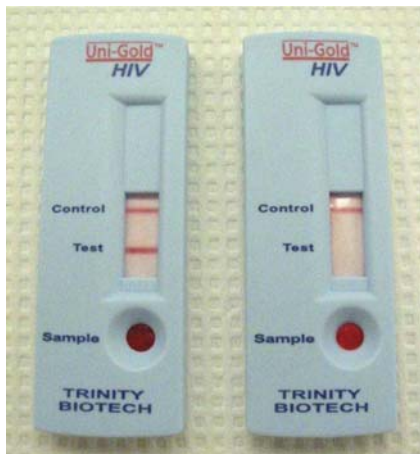
The Uni-Gold test is a single-use rapid test for the detection of HIV-1 antibodies in plasma, serum, or anticoagulated whole blood obtained by fingerstick or venipuncture. It is intended for use as a point-of-care test.

To conduct the test,

- draw an adequate specimen (serum, plasma, or whole blood) to the first gradation on the pipette supplied with the kit, or obtain a fingerstick blood specimen with the pipette supplied for this purpose.
- Hold the pipette vertically over the sample port and add 1 drop of specimen.
- Add 4 drops of the wash solution from the dropper bottle to the sample port.
- Set timer for 10 minutes.



The test result is read directly from the device 10 to 12 minutes after the specimen is added.



A reddish line at the “control” region with no line at the “test” region indicates that the test is negative for HIV-1 antibodies.

A reddish line of any intensity at both the “test” and “control” regions indicates the test is “reactive”, that is, preliminary positive for HIV-1 antibodies.

No line at the “control” region (irrespective of a line forming at the “test” region) or lines not adjacent to the respective regions indicate the test is invalid and must be repeated

The Uni-Gold test includes an internal control that indicates whether the test is functioning correctly. However, the formation of the control line on the Uni-Gold test does not validate that the specimen has been added to the test.

Consequently, a test with no specimen added may appear the same as a test with a negative result (that is, a band in the control region and no band in the test region). When testing whole blood, you must observe the red color at the specimen sample port to validate that specimen was added. Positive and negative external controls should be run by each new operator before performing testing on patient specimens, whenever a new lot of test kits is used, if the conditions of testing or storage (e.g., temperature) fall outside the range recommended by the manufacturer, and at periodic intervals specified in the laboratory's quality assurance program. External controls are not included with the test kits and must be ordered separately from the manufacturer. The controls require refrigeration and can be stored for 21 days after they are opened.

Shelf Life of Kits: 1 year from date of manufacture if stored at room temperature

For more information on the Uni-Gold test, see the test kit package insert (http://www.unigoldhiv.com/package_insert.pdf) or visit the manufacturer's Web site (<http://www.trinitybiotech.com/>).

Multispot HIV-1/HIV-2 Rapid Test

The Multispot test is a single-use rapid test that detects and differentiates circulating antibodies to HIV types 1 and 2 in fresh or frozen human serum and plasma. It has been approved by FDA to differentiate HIV-1 from HIV-2 antibodies. Multispot requires a refrigerator for the reagents and some laboratory equipment for processing and diluting the specimen.



To conduct the test,

- Dilute the specimen in specimen diluent and then add it to the test cartridge through a prefilter.
- After the diluted specimen has been completely absorbed, remove the prefilter. If antibodies against HIV-1 and/or HIV-2 are present in the specimen, they bind to the antigens on the microparticles in the specific spots on the cartridge membrane.
- Add the conjugate to the cartridge. The conjugate binds to the human antibody-antigen complexes that are immobilized in the spots on the cartridge membrane.
- Perform a wash step to remove the unbound conjugate
- Add development reagent and a stop solution to the cartridge.
- Examine the membrane visually for the presence of the color purple on the procedural control spot and on the test spots.



The test result is read directly from the device at any time after the test is completed.

The purple color on the test spots is proportionate to the amount of antibodies against HIV-1 and/or HIV-2 that have been bound to the antigen-coated microparticles and detected by the conjugate.

The procedural control spot will turn purple when the test has been performed correctly. If no color appears at the control spot, the test is invalid and

must be repeated with a new cartridge.

Shelf Life of Kits: 1 year refrigerated, 3 months at room temperature

For more information on the Multispot test, see the test kit package insert (<http://www.fda.gov/cber/pmalabel/p040046LB.pdf>) or visit the manufacturer's Web site (<http://www.bio-rad.com/>).

ClearView HIV 1/2 Stat Pak

The Clearview HIV 1/2 Stat Pak is a single-use rapid test that detects antibodies to HIV-1 and HIV-2 in fingerstick whole blood, venous whole blood, serum, or plasma specimens. The Clearview HIV 1/2 Stat Pak assay is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 or HIV-2



To conduct the test,

- Place the Clearview HIV 1/2 Stat Pak cartridge on a flat surface.
- Label the test device with patient name or identification number.
- Touch the 5 μ L sample loop (provided in kit) to the specimen, allowing the specimen to fill the opening of the loop.
- Holding the sample loop vertically, touch it to the sample pad in the center of the sample (S) well of the device to dispense about 5 μ L of specimen onto the sample pad.
- Invert the bottle of running buffer bottle and hold it vertically over the sample well. Add 3 drops (about 105 μ L) of buffer slowly, drop by drop, into the sample (S) well.
- Wait 15 minutes.

The test result is read directly from the device.



- Read the test results after 15 to 20 minutes. Reactive test results may be observed and read earlier than 15 minutes. To verify a nonreactive test result, wait the entire 15 minutes. Do not read results after 20 minutes.
- A reactive test will show two pink or purple lines—1 in the test area and 1 in the control area. The line in the test area may look different from the line in the control area. Intensities of the test and control lines may vary, but a test result with visible lines in both the test and control areas, regardless of intensity, is considered reactive, which means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as preliminarily positive for HIV-1 and/or HIV-2 antibodies.
- A nonreactive test will have 1 pink/purple line in the control area, but no line in the test area. A nonreactive test result means that neither HIV-1 nor HIV-2 antibodies were detected in the specimen. The test result is interpreted as negative for both antibodies.
- The test is invalid if there is no pink-purple line in the control area. Similarly, the test is invalid if any lines appear outside the control area or test area. An invalid test cannot be interpreted. An invalid test must be repeated with a new device.

Shelf Life of Kits: 24 months from the date of manufacture if stored at room temperature

For more information on the Clearview HIV 1/2 Stat Pak, see the test kit package insert (<http://www.chembio.com/pdfs/6240%20HIV102%20Stat%20Pak%20Prod%20Insert%20Rev%201.pdf>) or visit the manufacturer's Web site (<http://www.chembio.com/>).

ClearView Complete HIV 1/2

The Clearview Complete HIV 1/2 (*formerly known as the Sure Check HIV 1/2*) is a single-use rapid test that detects antibodies to HIV-1 and HIV-2 in fingerstick whole blood, venous whole blood, and serum or plasma specimens. It is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV-1 or HIV-2. The shape of the device resembles a syringe with a narrow sampler tip and the test strip enclosed in the barrel. The single-use vial of buffer is stored in the base of the barrel.



To conduct the test,

- For fingerstick whole blood: touch the blood drop with the test sampler tip until the blood flows into and fills the tip.
- For venous whole blood, serum, or plasma: invert sampler and pipette 2.5 μ L of specimen into sampler tip.
- Firmly press the sampler tip into the buffer vial through the foil cover until sampler and buffer vial snap together.
- Wait 15 minutes, keeping the sampler tip/buffer vial mechanism upright in the cardboard rack supplied with the kits.

The test result is read directly from the device.



- Read the test results after 15 to 20 minutes. Reactive test results may be observed and read earlier than 15 minutes. To verify a nonreactive test result, wait the entire 15 minutes. Do not read results after 20 minutes.
- A reactive test will show two pink/purple lines— 1 in the test area and 1 in the control area. A test result with visible lines in both the test and control areas, regardless of intensity, is considered reactive, which means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as preliminarily positive for HIV-1 and/or HIV-2 antibodies.
- A nonreactive test will have 1 pink/purple line in the control area, but no line in the test area. A nonreactive test result means that neither HIV-1 nor HIV-2 antibodies were detected in the specimen and the test result is interpreted as negative for both antibodies.
- The test is invalid if there is no pink-purple line in the control area. Similarly, a test is invalid if any lines appear outside the control area or the test area. An invalid test cannot be interpreted. An invalid test must be repeated with a new device.

Shelf Life of Kits: 24 months from the date of manufacture if stored at room temperature

For more information on the Clearview Complete HIV 1/2, see the test kit package insert (<http://www.chembio.com/pdfs/6260%20HIV202%20Sure%20Check%20Prod%20Ins%20Rev%201.pdf>) or visit the manufacturer's Web site (<http://www.chembio.com/>).

Interpretation of Rapid HIV Test Results

The results of rapid HIV tests are interpreted the same way as the results of other HIV screening tests.

- A nonreactive result from a single test is considered negative. However, if the person whose test result is negative may have been exposed to HIV within the past 3 months, it may be too early for the test to detect HIV antibodies. A repeat test at a later time is recommended.
- A reactive result from a rapid test is considered a preliminary positive result. The test does not have to be repeated before the result is reported as "preliminary positive." It must be followed up with another type of test a Western blot, an immunofluorescence assay, or an RNA test to confirm the result. The person is considered HIV-positive only if the confirmatory test result is positive. A small proportion of specimens produce indeterminate results in the confirmatory test. If this happens, the test should be repeated after 1 month.

Special Note: *Specimens from HIV-infected persons receiving highly active antiretroviral therapy may produce false-negative results on rapid tests.*

For more information see CDC's Revised Counseling, Testing, and Referral Guidelines: <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm>