

Chapter
10

Whole Blood HIV-1 Antibody by OraQuick

CHAPTER 10: QUALITATIVE HIV-1 SCREENING BY ORAQUICK

Procedure: Whole Blood anti-HIV Antibody by OraQuick

PRINCIPLE

The OraQuick Rapid HIV-1 Antibody Test is a single-use, qualitative immunoassay to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in fingerstick whole blood specimens. The OraQuick is a manually performed, visually read, 20-40 minute immunoassay system. It is comprised of a single-use test device and a single use vial containing a pre-measured amount of a buffered developer solution. The OraQuick test utilizes a lateral flow procedure. The assay test strip contains synthetic peptides representing the HIV envelope region in the Test zone, and a goat anti-human IgG procedural control in the Control zone, immobilized onto a nitrocellulose membrane.

A fingerstick whole blood specimen is collected and transferred into a vial of developer solution, followed by insertion of the test device into the developer vial. The developer solution facilitates the flow of the specimen into the device and onto the test strip. As the diluted specimen flows through the devices, it rehydrates the protein-A gold colorimetric reagent contained in the device. As the specimen continues to migrate up the strip, it encounters the T zone. If the specimen contains antibodies that react with the antigens immobilized on the nitrocellulose membrane, a reddish-purple line will appear, indicating the presence of antibodies to HIV-1 in the specimen. Further up the strip, the sample will encounter the C zone. A reddish-purple line at the C zone serves to demonstrate that a specimen containing antibodies was added to the vial and that the fluid has migrated adequately through the test device.

CLINICAL SIGNIFICANCE

Acquired Immune Deficiency Syndrome (AIDS), Aids related complex (ARC) and pre-AIDS are thought to be caused by the Human Immunodeficiency Virus (HIV). The first AIDS-related virus, HIV-1, has been isolated from patients with AIDS, and from healthy persons at high risk for AIDS. To date, eight HIV-1 subtypes have been identified worldwide, in addition to the highly divergent HIV-1 isolates from AIDS patients in Cameroon. A closely related but distinct second virus, designated HIV-2, has been isolated from West African patients with AIDS. Despite sharing a number of conserved genetic sequences, serological cross-reactivity between HIV-1 and HIV-2 is quite variable from sample to sample.

Using a rapid HIV test should increase the number of HIV-infected persons who may be diagnosed. The CDC estimates that nearly one third of the estimated 900,000 HIV-infected persons in the United States do not know their HIV status. As a result, they cannot benefit from early intervention with effective antiviral therapy. The OraQuick test provides a rapid preliminary result and is suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results.

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SPECIMEN REQUIREMENTS

No special patient preparation is needed. However, all test subjects should have received the "Subject Information" pamphlet prior to specimen collection.

A fingerstick blood sample should be collected using antiseptic technique. The sample must be collected immediately with the collection loop provided in the test kit, and must be tested immediately.

REAGENTS

OraQuick® Rapid HIV-1 Antibody Test Kit -- Catalog #: 1001-0052 (25 tests/kit), or
OraQuick® Rapid HIV-1 Antibody Test Kit -- Catalog #: 1001-0051 (100 tests/kit)

Vendor: OraSure Technologies, Inc., Bethlehem, PA, 800-672-7873

REAGENTS ARE TO BE OBTAINED BY CONTACTING RWJMS POCT PROGRAM (SEE CONTACT LIST)

EACH 25 (OR 100) TEST KIT CONTAINS

- 25 (or 100) Divided pouches, each containing a test device, developer solution vial and absorbent packet
- 5 (or 10) Reusable test stands
- 25 (or 100) Specimen collection loops
- 25 (or 100) Subject information pamphlets

MATERIALS REQUIRED BUT NOT PROVIDED:

- Timer or watch for timing 20-40 minutes

- Latex, vinyl or nitrile disposable gloves
- Biohazard waste container
- Clean, disposable, absorbent workspace cover

The test devices are **stable** until the expiration date on the box and pouch. They should be stored at room temperature, 35-80°F. If stored refrigerated, the pouch **must** be brought to room temperature before opening.

QUALITY CONTROL

EXTERNAL QUALITY CONTROL:

OraQuick® Rapid HIV-1 Antibody Test Kit Controls – Catalog #:1001-0049

Vendor: OraSure Technologies, Inc., Bethlehem, PA, 800-672-7873

EACH KIT CONTAINS

- Positive Control, 1 vial (Black Cap, 0.2 mL)
- Negative Control, 1 vial (White cap, 0.2 mL)

The controls are human, plasma-based reagents. The positive control contains antibodies that will show a REACTIVE result and the Negative Control will show a Non-Reactive result when run with the Orasure Rapid HIV-1 Antibody Test. The controls are liquid, ready to use and require no reconstitution or dilution. Both controls contain .2 mL of photochemically inactivated human plasma which is either positive or negative for antibodies to HIV-1 diluted by a defibrinated pool of normal human plasma. Both specimens are negative for Hepatitis B surface antigen and Hepatitis C antibody.

The controls should be stored at (2 - 8°C) and are stable until the expiration date stated on the the box label. **HOWEVER, ONCE OPENED THE CONTROLS EXPIRE IN 21 DAYS.** This expiration date should be clearly marked on the box containing the controls or on the individual vials.

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External quality control is to be performed:

- **Whenever a new shipment of reagents is put into use**, both positive and negative controls shall be run on the first new box used from that shipment.
- At the beginning of **each week**, at least one control must be run, to make sure that the reagents have not deteriorated in storage.
- **If a patient is to be test anonymously, controls must be run, unless they were already run, that day.**
- **Each month**, each person doing testing must run at least one control as a means of demonstrating continued competency.
- The quality control requirements listed above can overlap:
 1. If more than one person will be doing testing, they may divide up the quality control, with one person running the negative control, and one person running the positive control.
 2. If a new box of reagent is about to be opened, the controls for the week can be run on the new box.
- Validation of each reagent lot will be performed prior to release through the RWJMS POCT program. For this reason, **each lot does not need to be re-validated on receipt at the testing site—just each shipment.**
- **Whenever there has been a change is the test environment** including such items as:
 1. The temperature in the test storage area falling outside of 2° – 27° C (35°-80° F)
 2. The temperature of the testing area falling outside of 15° -27° C (59° – 80° F)
 3. A new location has been selected to perform testing
 4. Room lighting has been significantly altered

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TEST PROCEDURE: EXTERNAL QUALITY CONTROL SOLUTIONS

1. Check the expiration date of the external control solutions. Once opened, a control solution expires in 21 days. **DO NOT USE EXPIRED CONTROL SOLUTIONS.**

2. Insert the round end of an unused Specimen Collection Loop into the vial of the control reagent. Visually inspect the loop to make sure that it is completely filled with the control reagent. Use separate unused Specimen Collections Loops for each control reagent. NOTE: The Kit Control reagents are clear to straw-colored. Do not use if the reagent appears visually cloudy or discolored.
3. Immediately immerse the control-reagent-filled Specimen Collection Loop in the developer solution inside the Developer Solution Vial. Use the Specimen Collection Loop to stir the specimen in the developer solution. Remove the Specimen Collection Loop from the Developer Solution Vial and discard in a biohazardous waste container.
4. Remove the Test Device from the Divided Pouch without touching the flat pad. Insert the Test Device, flat pad first, into the Developer solution Vial containing the specimen. Be sure that the result window faces forward and the flat pad touches the bottom of the Developer Solution Vial.
5. Leave the Test Device in the Developer solution and start a timer or record the start time. Do not remove the Test Device from the vial until you have read the results. Read the results after 20 minutes but not more than 40 minutes in a fully lighted area. Read the results as described in the Interpretation of Test Results section below.
6. Dispose of the used Developer Solution Vial and the Test Device in a biohazard waste container.
7. Reseal the Kit Control Reagent vials and store them in their original container at 2-8° C (35-46° F).

INTERNAL QUALITY CONTROL

In addition, the OraQuick HIV-1 Antibody test has a built in quality control feature. A reddish-purple line develops next to the letter "C" on the cassette as a positive procedural control. If the line does not develop, the test result is invalid. Absence of interfering background is a negative control. If a red background color appears and interferes with the ability to read the test, the result is invalid.

The procedure for interpreting the internal quality control is included in the test procedure section.

TEST PROCEDURE

1. Open both portions of the test pouch. Make sure that an absorbent packet was packed with the test device. If not, discard the device and open a new pouch.
2. **Label both the Developer Solution Vial and the test Device with the subject's name or ID.** Be careful not to block or cover the two holes on the back of the test device.
3. Carefully remove the cap from the vial and place the vial in the stand gently, without splashing the solution out of the vial.
4. Make sure that you are wearing disposable gloves.
5. Clean the finger of the person being testing with an antiseptic wipe, and allow to dry.
6. Using a sterile lancet, puncture the skin off the center of the finger pad. You may apply gentle pressure beside the puncture, but do not squeeze or 'milk' the finger to obtain blood.
7. Take a clean, unused Specimen Collection Loop, and fill the loop completely with blood.
 - For a control, fill the loop with control solution. Note that the developer in the vial will not turn pink.
8. Immediately insert the blood-filled end of the loop into the solution in the vial and stir the blood sample into the solution. Make sure that the solution appears pink. If blood did not fully fill the loop, or if the solution is not pink, discard the test materials and start again with a new pouch, new lancet, and fresh fingerstick.
9. Insert the flat pad of the test device (labeled with the test subject's ID) into the vial with the blood in the developer solution. Make sure that the pad touches the bottom of the vial, and that you can see the result window.
10. Start timing the test. Record the start time on the test log sheet.
11. Read the results after at least 20 minutes, but not more than 40 minutes have passed. If the test is not read within 40 minutes, discard the test materials and start again. The test is invalid.
12. **Interpretation of test results:**

Preliminary Positive	A reddish-purple line in the zone next to the letter "T" (test) and a reddish-purple control line next to the letter "C" (control) in the result window.
Negative	No reddish-purple line next to the letter "T", and a reddish-purple control line next to the letter "C" (control) in the result window.
No Result	If either:

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No reddish-purple line appears next to the letter “C” (control), **or a reddish background** makes it impossible to see a line in the “T” zone, the subject must be re-tested using a new cassette.

The reddish-purple result lines should appear next to the triangles next to the letters T and C. **IF THE LINES ARE NOT NEXT TO THE TRIANGLES, THE TEST IS INVALID, AND THE SUBJECT MUST BE RE-TESTED USING A NEW CASSETTE.**

If there is no valid result after two attempts on one subject, run both positive and negative controls, and call for assistance.

13. Record test results:

- Record results on the testing log and report the result to the counselor for recording in the counseling records.
- Control and patient results should be recorded on the same log. Enter the control lot number in the Patient/Control ID column.
- Start a new page with each new box of reagents. The box should end at the end of a page.
- Completed log pages (or copies of them) must be returned to the POCT office, and must be kept for at least two years. If there is a problem with the test materials or methods, we need to track back and see which test subjects were tested when, and with which lot of reagents.

PROFICIENCY TESTING

The RWJMS Point of Care Testing program subscribes to the College of American Pathologists Survey Program. Depending on the survey, between two and three challenges are sent per year, with multiple samples per challenge. In addition, the POCT Quality Assurance program requires participation in additional **competency assessments periodically**. These samples will be distributed at the time of an on-site visit by the laboratory director or an individual designated to provide technical oversight by the director.

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- These samples must be run as much as possible like patient samples.
- Proficiency Test results will be graded by the CAP and the NJ DHSS and the evaluation sent to us about one month after we send in our test results. Successful performance is necessary to maintain our license.
- Quality Assurance or Competency Assessment specimens will be evaluated by the Director or his/her designee.
- The results may not be discussed with any other lab employees, at your or any other lab, until after the due date of the test results.

EXPECTED VALUES:

Negative if the person has not been exposed to the HIV-1 virus.

This test is a screening test. If the result of this test is positive, the follow-up testing protocol and appropriate counseling of the subject tested must be initiated.

PROCEDURAL NOTES

Reading the result earlier than 20 minutes or later than 40 minutes may yield erroneous results. If the result is not read within 40 minutes, the test **is invalid and** must be repeated.

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This test is not approved for use in persons under 13 years of age. It cannot be used to test newborns or infants for infections that may have been acquired from their mothers.

For positive (reactive) results, the intensity of the line does not necessarily correlate with the amount of antibody in the specimen, or with the stage of disease.

LIMITATIONS OF THE PROCEDURE

A negative (non-reactive) result does not preclude the possibility of exposure to, or infection with, HIV. It can take several months for antibodies to appear after exposure to the HIV-1 virus.

No test is 100% accurate:

- ~~In one study of 521 specimens that were repeatedly reactive using a licensed EIA, and positive by Western blot, 519 gave a reactive (positive) result with OraQuick.~~
- In another study of 625 high-risk subjects, 20 were repeatedly reactive by EIA. Of those 20, 17 were positive by OraQuick, and were also positive by Western Blot. Of the remaining three (which were negative by OraQuick), two were negative and one indeterminate by Western Blot.
- To assess the sensitivity of the OraQuick Rapid HIV-1 Antibody Test for HIV-1 variants for various geographic regions, 215 confirmed antibody positive specimens were tested. 214 were reactive. Counseling should reflect these imperfections of testing.

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REFERENCES

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