ANTI-HIV 1 / 2 ANTIBODY SCREENING by ORAQUICK ADVANCE

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

Fingerstick blood samples 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.

Oral samples should be collected by following specific technique. The sample must be tested immediately after collection.

The test devices are stable until the expiration date on the box and pouch. They must be stored at a temperature between (2°-27° C; 35°-80°F. If refrigerated, the pouch must be brought to room temperature before opening as the temperature range for testing is (15°-37° C; 59°-99° F).

QUALITY CONTROL:

EXTERNAL QUALITY CONTROL:

ORAQUICK® *ADVANCE* RAPID HIV-1/2 ANTIBODY CONTROL TEST KIT CONTAINS:

- HIV-1 Positive Control, 1 vial (Black cap, 0.2 mL)
- HIV-2 Positive Control, 1 vial (Red cap, 0.2 mL)
- Negative Control, 1 vial (White cap, 0.2 mL)

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

The controls must be stored at (2° - 8°C; 35°-46° F) and are stable until the expiration date stated on the box label. HOWEVER, ONCE OPENED THE CONTROLS EXPIRE IN 8 WEEKS. The date received, date opened and the expiration date must be clearly marked on the box containing the controls or on the individual vials.

When to perform quality control?

1. Whenever a new shipment of reagents is put into use, a set of controls shall be run on the first new box used from that shipment. Validation of each reagent lot will be performed prior to release through the RWJMS Rapid Testing Support Program. For this reason, each lot does not need to be re-validated on receipt at the testing site—just each shipment.
2. At the beginning of each week, one set of controls must be run, to make sure that the reagents have not deteriorated in storage.
3. Whenever there has been a change is the test environment including such items as:
   - The temperature in the test storage area falling outside of (2°– 27° C; 35°-80° F)
   - A new location has been selected to perform testing.

Reminder: If refrigerator storage temperature is out of range (2-8° C; 35° - 46° F), record the temperature and note any corrective action taken. This temperature problem may mean that the control solutions may give wrong results in the future. You need to know this in case you have a problem in the future, but a problem with refrigerator temperature does not mean that you need to run controls.

How to Perform Quality Control?

1. Check the expiration date of the external control solutions. Once opened, a control solution expires in 8 weeks not 2 months (You must count 8 weeks). DO NOT USE EXPIRED CONTROL SOLUTIONS.

2. If you open new controls, write the new expiration date on either the box or the vials.
3. Label the test vial and test device to indicate which control is being run on each device.
4. 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.
5. 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.
6. Reseal the Kit Control Reagent vials and store them in their original container at (2°-8° C or 35°-46° F).
7. 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.
8. Leave the Test Device in the Developer solution and start a timer and 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.
9. Dispose of the used Developer Solution Vial and the Test Device in a biohazard waste. If controls do not give their expected results, (Preliminary positive for the Black and Red caps, Negative for the White cap), the test is invalid and you cannot do client testing. You must first figure out what went wrong. Check all temperature logs for out of range temperatures and the repeat the controls. If you do not get the expected results, discard the controls and if available, open a new box of controls. If you still do not get expected results discontinue testing inform the HIV support team. You can not test any patients until the controls perform correctly. Instead of testing the client, offer to draw two white top tube and call our discordant line at (732) 236-7013 for pick up.

INTERNAL QUALITY CONTROL

In addition, the OraQuick® *ADVANCE* HIV-1/2 Antibody test has a built in quality control feature that demonstrates assay validity. A reddish-purple line develops next to the letter "C" on the test device indicating that a specimen was added and the fluid moved through the test device. If the "C" line does not develop, the test result is invalid and cannot be interpreted. If a red background color appears and interferes with the ability to read the test, the result is invalid. Before looking at the Test line "T" check for the reddish-purple line next to the "C". The Control line appears on all valid tests whether nonreactive or reactive.

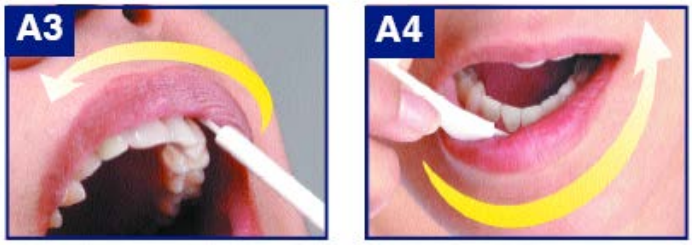
The procedure for interpreting the internal quality control is included in the Interpretation of Test Results section below.

TEST PROCEDURE:

1. Check to see whether client testing can be done by first following the Quality Control checklist (Section 2.6).
2. Check room temperature and record it on the test log. DO NOT continue if the temperature is outside the following range (15° -37° C) (59° – 99° F).
3. Make sure you are wearing disposable gloves.
4. 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.
5. Label both the Developer Solution Vial and the Test Device with the client's ID. Be careful not to block or cover the two holes on the back of the test device.
6. Carefully remove the cap from the vial and place the vial in the stand gently, without splashing the solution out of the vial.
7. Now, Follow Either: A. Using Oral Specimen or B. Using Fingerstick Specimen below:

A. Using Oral Specimen
   I. Before obtaining the specimen from the patient have the patient review "Oral Fluid Illustration" so that the patient understands the proper method of specimen collection. See illustration below.
   II. Wearing gloves, remove the test device from the pouch. Give the test device to the patient. Be careful not to touch the flat surface of the test device.

III. Once the device is given to the patient, make sure the patient collecting the specimen swabs the upper and lower gums; do not allow the patient to swab the roof of the mouth, the tongue or inside of the cheek. Both sides of the pad can be used. See illustration below.



IV. Once swabbing is complete, collect the device from the patient and insert the device into the developer.
V. Return to TEST PROCEDURE #8 Below:

B. Using Fingerstick
I. Clean the finger of the person being testing with an antiseptic wipe, and allow area to dry.
II. Using a sterile lancet, puncture the skin off the center of the finger pad.
III. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid "squeezing" or "milking" the finger to obtain blood.
IV. Wipe away the first drop of blood with a sterile gauze pad. Allow a new drop to form.
V. Take a clean, unused Specimen Collection Loop, and fill the loop completely with blood.
VI. Immediately insert the blood-filled end of the loop into the solution in the vial and stir the blood sample into the solution (preferably in a back and forth motion).
VII. 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.

8. Insert the flat pad of the test device (labeled with the client's ID) into the developer solution. Make sure that the pad touches the bottom of the vial and that you can see the result window.
9. Start timing the test. Record the start time on the test log sheet.
10. Read the results after at least 20 minutes, but not more than 40 minutes have passed. Record the read time on the test log. If the test is not read within 40 minutes, discard the test materials and start again. The test is invalid.

TEST INTERPRETATION:

Preliminary Positive	A **complete reddish-purple** line in the zone next to the letter "T" (test) and a **complete reddish-purple** line next to the letter "C" (control) in the result window.
Negative	**No reddish-purple line** next to the letter "T", **and a complete reddish-purple** control line next to the letter "C" (control) in the result window.
No Result(Invalid)	If either: **No reddish-purple line** appears next to the letter "C" (control), or a partial line next to the letters 'T' or "C', **or a reddish background** makes it impossible to see a line in the "T" zone, the subject must be re-tested using a new test device. The reddish-purple result lines must appear inside the "C" or "T" triangle areas. IF THE LINES ARE NOT INSIDE THE TRIANGLES AREA, THE TEST IS INVALID, AND THE SUBJECT MUST BE RE-TESTED USING A NEW TEST DEVICE.

IF YOU WERE UNABLE TO GET A VALID RESULT AFTER TWO ATTEMPTS, RUN BOTH POSITIVE AND NEGATIVE CONTROLS, AND CALL THE NJ RAPID HIV TESTING SUPPORT OFFICE (732) 743-3624 FOR ASSISTANCE. OFFER TO DRAW TWO WHITE TOP TUBES AND CALL OUR DISCORDANT LINE AT (732) 236-7013 FOR PICK UP.

11. 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.
- Each site is responsible for maintaining inventory of the test kits. Every test kit is accounted for and recorded on the test log. Do not throw out expired test kits. They should be returned to RWJMS and noted on log with reason code 7. There is a reference sheet listing the reasons for non-patient test kit use with accompanying codes which should be noted on the test logs when a specific situation arises.
- 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.

Expected Values:

Negative if the person has not been exposed to the HIV virus.
This test is a screening test. If the result of this test is positive, please refer to the Preliminary Positive procedure for steps to follow.

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.
- This test is not approved for use in persons less than 12 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 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® *ADVANCE* Rapid HIV-1/2 Antibody Test for HIV variants for various geographic regions, 215 confirmed antibody positive specimens were tested 214 were reactive.

Individuals infected with HIV-1 or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.

Counseling should reflect these imperfections of testing.

References:

1. NCCLS, *Clinical Laboratory Technical Procedure Manual-Third Edition; Approved Guideline.* NCCLS document GP2-A3 (ISBN 1-56238). NCCLS Wayne, PA. 1996.
2. Product Insert, *OraQuick Rapid HIV-1 Antibody Test*, OraSure Technologies, 4/03.
3. Product Insert, *OraQuick ADVANCE Rapid HIV-1/2 Antibody Test*, OraSure Technologies, revised 10/07.
4. Product Insert, Oraquick ADVANCE Rapid HIV-1/2 Antibody Test, Orasure Technologies, revised 4/09.
5. Product Insert, Oraquick ADVANCE Rapid HIV-1/2 Antibody Test, Orasure Technologies, revised 4/12
6. Tietz, N.W., *Fundamentals of Clinical Chemistry,* 2nd ed., W.B.
7. Saunders. Philadelphia, PA. 1976.

Written by: Evan Cadoff, M.D	Date: 10/8/03
Approved by: Evan Cadoff, M.D.	Date: 10/8/03

Revised by: Patricia Ribeiro Date: _____
Reviewed by: Joanne Corbo MBA, MT (ASCP) Date: _____
Approved by: Evan Cadoff, M.D. Date: 8/6/2013