RAPID HIV

ORAQUICK® ADVANCE RAPID HIV – ½ ANTIBODY TEST

I. PRINCIPLE

Acquired Immune Deficiency Syndrome (AIDS), AIDS related complex (ARC) and pre-AIDS are thought to be caused by the Human Immunodeficiency Virus (HIV). HIV is known to be transmitted by sexual contact, by exposure to blood (including sharing contaminated needles and syringes) or by contaminated blood products, or it may be transmitted from an infected mother to her fetus during the prenatal period. Individuals infected with HIV produce antibodies against the HIV viral proteins. Testing for the presence of antibodies to HIV in bodily fluids (e.g., blood, oral fluid, and urine) is an accurate aid in the diagnosis of HIV infection. However, the implications of seropositivity must be considered in a clinical context. For example, in neonates, the presence of HIV is indicative of exposure to HIV, but not necessarily of HIV infection, due to the acquisition of maternal antibodies that may persist for up to 18 months. Conversely, absence of antibody to HIV cannot be taken as absolute proof that an individual is free of HIV infection or incapable of transmitting the virus. An antibody response to a recent exposure may take several months to reach detectable levels. HIV has been isolated from asymptomatic, seronegative individuals presumably before seroconversion following exposure.

The standard laboratory HIV testing algorithm used in the United States consists of screening with an enzyme immunoassay (EIA) and confirmation of repeatedly reactive EIAs using a Western blot test. Results are typically reported within 48 hours to two weeks, making these standard screening and supplemental tests inadequate to meet the need for rapid HIV diagnosis. The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is a point-of-care test to aid in the diagnosis of infection with HIV-1.

The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is a manually performed, visually red, 20-minute immunoassay for the qualitative detection of antibodies to HIV-1 in human blood obtained from a finger puncture or a venipuncture. The OraQuick rapid test is comprised of a single-use test device and a single-use vial containing a pre-measured amount of a buffered developer solution. Each component is sealed in separate compartments of a single pouch to form the test. The OraQuick® rapid test utilizes a proprietary lateral flow immunoassay procedure. The device plastic housing holds an assay test strip comprised of several materials that provide the matrix for the immunochromatography of the specimen and the platform for indication of the test results.

The assay test strip, which can be viewed through the test device result window, contains synthetic peptides representing the HIV envelope region and a goat anti-human IgG procedural control immobilized onto a nitrocellulose membrane in the Test (T) zone and the Control (C) zone respectively.

A blood specimen is collected and transferred into the vial of developer solution, followed by the insertion of the test device. The developer solution facilitates the flow of the specimen into the device and onto the test strip. As the diluted specimen flows through the device, 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, qualitatively indicating the presence of antibodies to HIV-1 in the specimen. The intensity of the line color is not directly proportional to the amount of antibody present in the specimen.

Further up the assay strip, the sample will encounter the C zone. This built-in procedural control serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device. A reddish-purple line will appear in the C zone during the performance of all valid tests, whether or not the sample is positive or negative for antibodies to HIV-1 and/or HIV-2 (refer to the Test Result and Interpretation of Test Result section below).

The test results are interpreted after 20 minutes, but not more than 40 minutes after the introduction of the test device into the developer solution containing the test specimen. No precision pipetting, pre-dilutions, or specialized instrumentation are required to perform the OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test.

II. CLINICAL SIGNIFICANCE

Unitypoint Health Pekin Laboratory personnel will utilize this procedure to perform and interpret Rapid HIV 1/2 antibody tests. The Rapid HIV 1/2 is performed only for:

1. Pregnant or postpartum women
2. Neonates of mothers who refuse testing
3. [U1]Sexual/body fluid assault exposures

III. SPECIMEN COLLECTION AND STORAGE:

A. Venipuncture Whole Blood:

1. Using standard venous phlebotomy procedures, collect a whole blood sample using a tube containing EDTA (lavender top).

B. Heel Stick:

1. Collect a whole blood sample using a tube containing ~~any of the following~~ anticoagulant~~s~~: EDTA (lavender top)~~., sodium heparin~~

~~(green top), sodium citrate (light blue top), or ACD Solution A (yellow top). Other anticoagulants have not been tested and may give an incorrect result.~~

C. If the specimens are not tested at the time of collection, the whole blood may be stored at 2ºC to 18º C (35ºF to 64º F) for up to 30 hours. Prior to testing, mix the blood tube gently by inversion several times to ensure a homogenous sample.[U2][U3]

IV. REAGENT

A. OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test Kit Controls: Package contains HIV-1 Positive Control (1 vial, black cap, 0.2 mL), HIV-2 Positive Control (1 vial, red cap, 0.2 mL) and Negative Control (1 vial, white cap, 0.2 mL), and a Package Insert.
B. Storage Instructions:
    1. Store unused OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Tests unopened at 2ºC to 27°C (35ºF to 80º F). Do not open the divided pouch until you are ready to perform a test. If stored refrigerated, ensure that the divided pouch is brought to ambient temperature (15ºC to 27°C; 59ºF to 80º F) before opening.

V. SUPPLIES/EQUIPMENT

A. 25 divided pouches, each containing: Test device (1), absorbent packet (1), developer solution vial (1) (each vial contains 1 ml of a phosphate buffered saline solution containing polymers and an antimicrobial agent)
B. 5 reusable test stands
C. 25 specimen collection loops
D. 25 subject information pamphlets
E. 1 package insert
F. Timer or watch capable of timing 20 to 40 minutes
G. Antiseptic wipe
H. Sterile lancet to obtain a heel stick whole blood specimen, or materials required to obtain a venipuncture blood specimen
I. Sterile gauze pads
J. Disposable gloves
K. Biohazard waste container

VI. QUALITY CONTROL

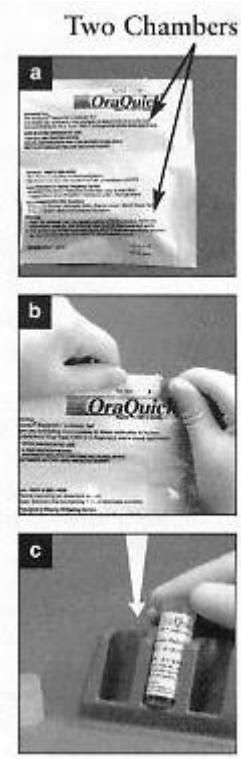
A. If controls do not react as expected, the test results are invalid and patient results cannot be turned out.

B. If the controls remain out of range, contact the pathologist as previous patient test results need to be reevaluated. Log all actions taken on Serology Action Log Sheet UPPK SER-0542.02.~~action log sheet.~~
C. Built In Control Features:
The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test has a built-in procedural control that demonstrates assay validity. A reddish-purple line in the Control (C) area of the result window indicates that a specimen was added and that the fluid migrated appropriately through the test device. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to Test Result and Interpretation of Test Result section below).
D. External Quality Control
OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test Kit Controls are available separately for use only with the OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test. The kit controls are used to verify your ability to properly perform the test and interpret the results. The positive control will produce a reactive test result and has been manufactured to produce a very faint Test (T) line. The negative control will produce a non-reactive test result. (Refer to Test Result and Interpretation of Test Result section below.)
E. Run the external controls under the following circumstances:
    1. When opening a new test kit lot,
    2. Whenever a new shipment of test kits is received,
    3. Each new operator prior to performing testing on patient specimens,
    4. If the temperature of the test storage area falls outside of 2ºC to 27º C (35ºF to 80º F),
    5. If the temperature of the testing area falls outside of 15ºC to 27º C (59°F to 80°F), and
    6. Every 30 days (minimum) lot is in use. Log on Serology HIV Monthly Log Sheet (UPPK SER-0542.03).

VII. PROCEDURE:

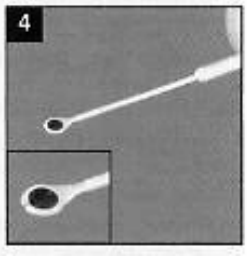
Prior to patient having the sample drawn, they receive information about the testing of HIV and must sign a consent form (UPPK SER-0542.01)

A. Step 1 - General Test Preparation:
    1. Open the two chambers of the OraQuick divided pouch ("Pouch") by tearing at the notches on the top of each side of the pouch (see pictures a and b). To prevent



contamination, leave the test device ("Device") in the pouch until you are ready to use it.

2. Remove the developer solution vial ("Vial") from the pouch. Hold the vial firmly in your hand. Carefully remove the cap from the vial by gently rocking the cap back and forth while pulling it off. Set the cap on your workspace.
3. Slide the vial into the top of one of the slots in the stand. DO NOT force the vial into the stand from the front of the slot as splashing may occur. Make sure the vial is pushed all the way to the bottom of the slot in the stand (see picture c).
4. Pick up an unused specimen collection loop ("Loop") by the thick "handle" end (see picture). Put the "rounded" end of the loop into the tube of blood. Make sure the loop is completely filled with blood (see picture 4).



Note: If the loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container. Get a new loop for the collection of the blood sample.

B. Step 2 – Mix:
   1. Immediately insert the blood-filled end of the loop all the way into the vial (see picture 5). Use the loop to stir the

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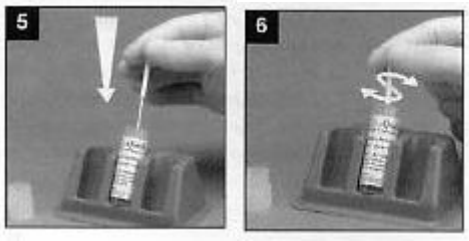
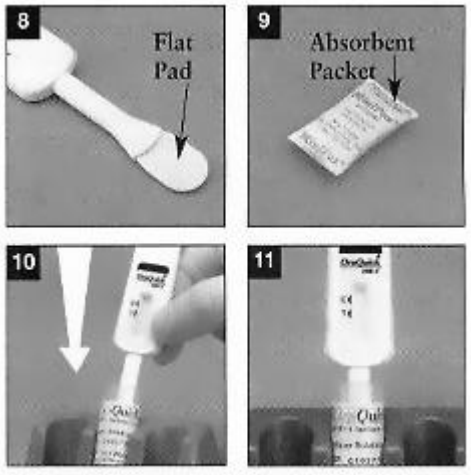
blood sample in the developer solution ("Solution") (see picture 6). Remove the used loop from the solution. Throw the used loop away in a biohazard waste container.



2. Check the solution to make sure that it appears pink. This means that the blood was correctly mixed into the solution. If the solution is not pink, discard all test materials in a biohazard waste container. Start the test over. Use a new pouch and a new blood sample.

C. Step 3 – Test:

1. Remove the device from the pouch. DO NOT touch the flat pad (see picture 8). Check to make sure that an absorbent packet is included with the device (see picture 9). If no absorbent packet is present, discard the device and obtain a new pouch for testing.
2. Insert the flat pad of the device all the way into the vial containing the sample (see picture 10). Make sure that the flat pad touches the bottom of the vial. The result window on the device should be facing



towards you (see picture 11).

Note: DO NOT cover the two holes in the back of the device with labels or other

materials. Doing so may cause an invalid result.

3. Start timing the test. DO NOT remove the device from the vial while the test is running. Pink fluid will appear and travel up the result window. The pink fluid will gradually disappear as the test develops (see picture 13). Read the results after 20 minutes but not more than 40 minutes using Ott-Lite® with attached magnification device.

VIII. INTERPRETATION AND RESULTS

A. Refer to the result window on the test device.
   1. Non-reactive (report as NEG):
      a. A reddish-purple line appears next to the triangle labeled "C," and **NO** line appears next to the triangle labeled "T."
      b. A Non-Reactive test result means that HIV-1 and HIV-2 antibodies were not detected in the specimen. The test result is interpreted as NEGATIVE for HIV-1 and HIV-2 antibodies.
   2. Reactive (report as POS – preliminary positive):
      a. A reddish-purple line appears next to the triangle labeled "C" AND a reddish-purple line appears next to the triangle labeled "T". One of these lines may be darker than the other.
      b. NOTE**:** The test is Reactive if any reddish-purple line appears next to the "T" triangle and next to the "C" triangle, no matter how faint these lines are.
      c. A Reactive test result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies. All positives will be sent to Methodist for a full serological evaluation. Order code HIVPAN. If full serological evaluation is positive Methodist will contact us to follow up with a confirmation via PCR testing at ARUP. Order code 3000867, Human Immunodeficiency Virus 1 (HIV-1) by Quantitative NAAT, Plasma.[U4] ~~Obtain a serum sample from patient for confirmation by~~

~~Western Blot (done through OSF system lab[U5]).~~ Notify patient's physician of PRELIMINARY POSITIVE result.

3. Invalid:
   a. NO reddish-purple line appears next to the triangle labeled "C"
   b. A red background in the result window makes it difficult to read the results after 20 minutes
   c. If any of the lines are NOT inside the "C" or "T" triangle areas
   d. An invalid test result means that there was a problem running the test, either related to the specimen or to the test device. An invalid result cannot be interpreted.
   e. Repeat the test with a new pouch and a new blood sample. Contact OraSure Technologies' Customer Service if you are unable to get a valid test result upon repeat testing.
4. Enter patients' and control results (positive or negative) on Serology Patient Worksheet (UPPK SER-0542.04) and result in computer.

~~e.~~

IX. EXPECTED VALUE:

A. Negative

X. METHOD LIMITATIONS

A. The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test must be used in accordance with the instructions in this package insert to obtain an accurate result.
B. Reading test results earlier than 20 minutes or later than 40 minutes may yield erroneous results.
C. This test is approved by FDA for use with oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens only. Use of other types of specimens, testing of venipuncture whole blood specimens collected using a tube containing an anticoagulant other than EDTA, sodium heparin, sodium citrate, or ACD Solution A, or testing of plasma specimens collected using a tube containing an anticoagulant other than EDTA may not yield accurate results.
D. Individuals infected with HIV-1 or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.
~~E.~~ Clinical data collected by the manufacturer validates the performance of this test in young adults and adults. A study done at Children's Hospital Chicago validates the use of this test in children.[U6] ~~Clinical data has not been collected to demonstrate the performance of the OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test in persons under 12 years of age[U7]~~
E.

F. A reactive result using the OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test suggests the presence of HIV-1 and/or HIV-2 antibodies in the specimen. The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
G. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
H. A non-reactive result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to recent exposure may take several months to reach detectable levels.
I. A person who has antibodies to HIV-1 or HIV-2 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 HIV infection is accurate.

XI. REFERENCES

A. OraSure Technologies, INC., Bethlehem, PA 18015, USA, OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test; 3001-0951, Rev3/13.

POLICY CREATION :	***Date***
Author: ***Sharrol Brisbin, MT (ASCP)***	***11/01/2004***
Medical Director: ***Kathryn Kramer, MD***	***11/01/2004***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date

~~Lead~~	~~Date~~	~~Coordinator/ Manager~~	~~Date~~	~~Medical Director~~	~~Date~~

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Reviewed by