Policy Statement	Core Laboratory personnel are responsible for ensuring the specimen submitted for testing is acceptable and the procedure for performing the test is adhered to.
Purpose	This procedure provides technical instruction for the performance of HIV using the HIV OraQuick Kit.
Scope	This procedure applies to testing personnel authorized to perform testing on the HIV OraQuick Kit. This group includes, but is not limited to Medical Laboratory Technicians/Technologists, as well as leads and supervisory personnel.
Responsibility	All above personnel are responsible for following the OraQuick procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
Related Documents	CORE 6950 R Infectious Disease Reportable Tests CORE 6952 F QraQuick HIV Lot Comparison CORE 6952 Fa HIV External QC Record

Specimen Collection and Handling:
Specimen Whole Blood (EDTA, sodium heparin, or sodium citrate).

Specimens may be stored at 2-30 C for up to five days. Prior to testing, mix the blood tube gently by inversion several times to ensure a homogeneous sample.

Handling Conditions:

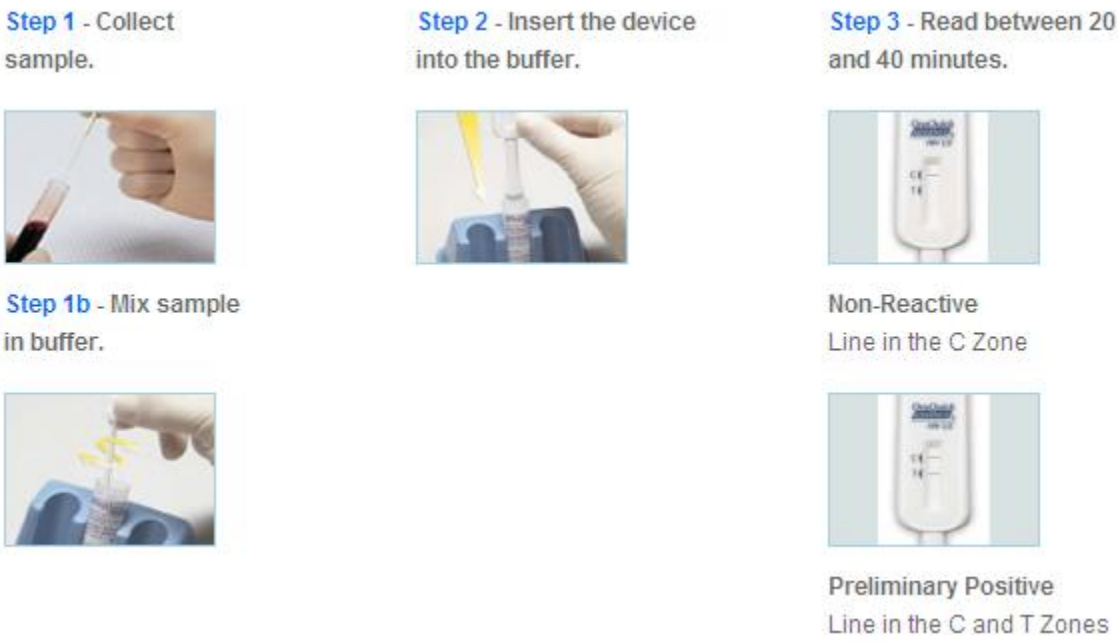
1. Use all Specimen Collection Loops, Test Devices, and Developer Solution Vials only once and dispose of properly. Do not reuse any of these test components.
2. Do not use the test beyond the expiration date printed on the Divided Pouch. Always check expiration date prior to testing.
3. Do not interchange Test Devices and Developer Solution Vials from kits with different lot numbers.
4. Avoid microbial contamination and exercise care in handling the kit components.
5. To ensure accurate results, the Test Device must be inserted into the Developer Solution Vial within 60 minutes after introducing the whole blood sample.
6. Adequate lighting is required to read a test result.

Procedure:

Prior to testing, allow the test kit to come to ambient temperature (15°-27°C) before use. Refer to the *External Quality Control* section in this procedure to determine if the Kit Controls should be run.

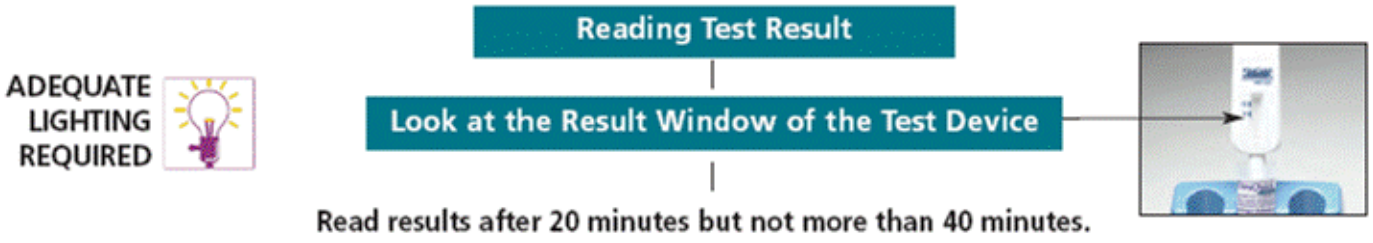
1. Open the two chambers of the OraQuick® *ADVANCE* Divided Pouch ("pouch") by tearing at the notches on the top of each side of the pouch. To prevent contamination; leave the Test Device ("device") in the pouch until you are ready to use it.
2. Remove the Developer Solution Vial ("vial") from the pouch. Hold the vial firmly in your hand.
3. Label the vial and/or device with the patient name or the specimen accession number. Carefully remove the cap from the vial by gently rocking the cap back and forth while pulling it off.
4. Slide the vial into the top of one of the slots in the provided 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.
5. Pick up an unused Specimen Collection Loop ("loop") by the thick handle end. Put the rounded end of the loop into the tube of blood. Make sure that the loop is completely filled with blood.
6. Immediately insert the blood-filled end of the loop all the way into the vial. Use the loop to stir the blood sample in the Developer Solution ("solution"). Remove the used loop from the solution. Throw the used loop away in a biohazard waste container.
7. 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. Repeat the test using a new pouch of supplies.
8. Remove the device from the pouch. DO NOT touch the flat pad. Check to make sure that an absorbent packet is included with the device. If no absorbent packet is present, discard the device and obtain a new pouch for testing. Insert the flat pad of the device all the way into the vial containing the blood sample. Make sure that the flat pad is completely inserted in the vial. The result window on the device should be facing towards you. DO NOT cover the two holes in the back of the device with labels or other materials. Doing so may cause an Invalid result.
9. 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.
10. Read the results after 20 minutes but not more than 40 minutes in a fully light area.
11. After reading the results and inputting results into Meditech, the vial and device should be disposed in the biohazardous waste container.

Visual Depiction:



Test Interpretation:

After the test has been developing for at least 20 minutes, review the Result Window on the Test Device.



Non-Reactive: Line in C Zone

Line in C Zone.
No HIV antibodies detected.

CDC recommendations for reporting HIV negative results:[1]

- HIV-negative test results may be conveyed without direct personal contact between the patient and the health-care provider.
- Persons known to be at high risk for HIV infection also should be advised of the need for periodic retesting and should be offered prevention counseling or referred for prevention counseling.

Reactive: Lines in C and T Zones.

Examples of preliminary positive results. Line in C Zone in each test.
Line in T Zone. Line in T Zone. Faint Line in T Zone.

Invalid: Repeat Test

No Line in C Zone | Red background obscures results | Any partial line at C or T Zones | Lines are outside of C or T Zones

Reactive results are confirmed by Western Blot. Testing is performed by a reference laboratory. Orders for Western Blot must be entered by the medical technologist performing the assay. The test mnemonic WBL should be entered under a new requisition using the specimen collection date and time.

An Invalid test result means that there was a problem running the test, either related to the specimen or to the device. An Invalid result cannot be interpreted. Specimens should be repeated with a new pouch of supplies. If the error continues, notify the Lead Technologist.

Result Reports:

Non-Reactive Results Report: Negative – "Negative for HIV1 and HIV2 antibodies" comment is appended to each result.

Reactive Results Report: Preliminary Positive – "Preliminary Positive for HIV1 and /or HIV2, See Western Blot for Confirmation" comment is appended to each result.

Procedure for Reporting Results:
Each positive result is called to the patient care area. Documentation of the call is entered into Meditech as a result comment.

In the case of an Employee Exposure Incident, all results are called. Results should be called to the patient location area designated on the requisition. This includes both positive and negative results. Documentation of the call is entered into Meditech as a result comment.

Equipment and Materials Required to Perform Test:

- OraQuick *ADVANCE*® Rapid HIV1/2 Antibody Test – Divided Pouch containing:
  - Test Device
  - Developer Solution Vial
- Reusable Test Stand
- Specimen Collection Loop
- Timer
- Biohazardous Waste Container

Reagent Storage Requirements:
Store unused OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Tests unopened at 2º-27ºC.

Quality Control:

Built-in Control
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 Interpretation* section.) Documentation of the built-in procedural control is required with each patient. Presence of the built-in control is document in Meditech under the test requisition.

External Quality Control:
OraQuick® *ADVANCE* Rapid HIV – 1/2 Antibody Test Kit Controls are the external quality controls utilized with this test. The external controls should be performed under the following circumstances:

- Weekly,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2º-27ºC
- If the temperature of the testing area falls outside of 15º-37ºC

Refer to the OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test Kit Controls package insert for instructions on the use of these reagents. Contact the Lead Technologist and OraSure Technologies' Customer Service if the Kit Control reagents do not produce the expected results.

Lot Comparisons/Parallel Testing
Before a new reagent lot and/or shipment is put into use, the performance is checked with external controls to ensure that equivalent results are obtained with the new lot. The controls should be tested in the same manner as patient samples. (See *Procedure* section) Results should be retained in the designated binder.

Principle:
The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is a single-use, qualitative immunoassay to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in oral fluid, fingerstick whole blood, venipuncture whole blood and plasma specimens. The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is

intended for use as an aid in the diagnosis of infection with HIV-1 and HIV-2. The OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test is a manually performed, visually read, 20 minute immunoassay for the qualitative detection of antibodies to HIV-1 and HIV-2 in human oral fluid, whole blood obtained from a finger puncture or a venipuncture, and plasma. The OraQuick® *ADVANCE* 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® *ADVANCE* 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.

An oral fluid specimen is collected using the flat pad on the test device, followed by the insertion of the test device Into the vial of developer solution. A fingerstick whole blood, venipuncture whole blood or plasma 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 and/or HIV-2 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 pipeting, predilutions, or specialized instrumentation are required to perform the OraOuick *ADVANCE* Rapid HIV-1/2 Antibody Test.

Warnings

For *in vitro* Diagnostic Use

1. Read the package insert completely before using the product. Follow the instructions carefully. Not doing so may result in inaccurate test results.
2. Before performing testing, all operators MUST read and become familiar with Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health-Care Settings.
3. FDA has approved this kit for use with oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens only. Use of this test kit with specimen types other than those specifically approved for use with this device may result in inaccurate test results.
4. This test should be performed at temperatures in the range of (15°- 37°C, 59°- 99°F). If stored refrigerated, ensure that the Divided Pouch is brought to operating temperature (15°- 37°C, 59°- 99°F) before performing testing.
5. If the test kit is stored at temperatures outside of ambient temperature (2°- 27°C, 35°- 80°F), or used outside of the operating temperature (15°- 37°C, 59°- 99°F), use the Kit Controls to ensure performance of the test.
6. Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.

Precautions

Safety Precautions

1. Handle blood specimens and materials contacting blood specimens as if capable of transmitting infectious agents.
2. Do not drink, eat, or smoke in areas where specimens are being handled or testing is being performed.
3. Wear disposable gloves while handling blood specimens and performing testing of blood specimens. Change gloves and wash hands thoroughly after performing each test. Dispose of used gloves in a biohazard waste container.
4. Oral fluid is not considered potentially infectious unless it contains blood. Use of gloves for oral fluid testing is optional. Test administrators with breaks in the skin (cuts, abrasions, or dermatitis) should wear gloves when performing oral fluid testing. Wash hands thoroughly after performing each oral fluid test and after contact with oral fluid.
5. Dispose of all test specimens and materials used in the test procedure in a biohazard waste container. Lancets and venipuncture materials should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may

be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions that contain bleach.

6. Wipe all spills thoroughly with a solution of 10% bleach or other appropriate disinfectant Bleach solutions should be made fresh each day.
7. For additional information on biosafety, refer to "Universal Precautions far Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other Blood-borne Pathogens in Health-Care Settings"[59] and "Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations far Postexposure Prophylaxis".

Handling Precautions

1. Use all Specimen Collection Loops, Test Devices, and Developer Solution Vials only once and dispose of properly (see Safety Precautions), Do not reuse any of these test components.
2. Do not use the test beyond the expiration date printed on the Divided Pouch. Always check expiration date prior to testing.
3. Do not interchange Test Devices and Developer Solution Vials from kits with different lot numbers. 4. Avoid microbial contamination and exercise care In handling the kit components.
5. To ensure accurate results, the Test Device must be inserted into the Developer Solution Vial within 60 minutes after introducing the fingerstick whole blood, venipuncture whole blood or plasma sample.
6. When collecting oral fluid specimens the Test Device must be inserted into the Developer Solution Vial within 30 minutes of collection. A Test Device containing an oral fluid specimen that is not inserted into the Developer Solution Vial within 10 minutes of collection should be either stored on a flat surface or returned to the Divided Pouch after the desiccant has been removed from the Divided Pouch. Far a 10-30 minute delay In insertion, return the Test Device containing the oral fluid specimen to the Divided Pouch after the desiccant has been removed from the Divided Pouch. Ensure that the Divided Pouch containing the Test Device is kept in a horizontal position until the Test Device is inserted into the Developer Solution Vial.
7. Adequate lighting is required to read a test result.

References:

1. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. *Science* 1984; 224:500-3.
2. Curran JW, Morgan WM, Hardy AM, et al. The epidemiology of AIDS: current status and future prospects. *Science* 1985; 229:1352-7.
3. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. *Science* 1986; 233:343-6.
4. Sehulster LM, Hollinger FB, Dreesman GR, and Melnick JL. Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. *Appl Env Microbiol* 1981; 42:762-7.
5. CDC. Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR* 1988; 37(24): 377-388.
6. CDC. Approval of a New Rapid Test for HIV Antibody. *MMWR* 2002; 51(46): 1051-1052 and Erratum 51(47): 1075.
7. CDC: Revised Guidelines for HIV Counseling, Testing, and Referral and Revised Recommendations for HIV Screening of Pregnant Women. *MMWR* 2001; 50(19): 32-35.
8. CDC: Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Post exposure Prophylaxis. *MMWR* 2001; 50(RR-11): 1-42.
9. Package insert OraQuick® *ADVANCE* Rapid HIV-1/2 Antibody Test