I. PURPOSE

The OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test is a single-use qualitative immunoassay to detect antibodies to HIV-1 and HIV-2 in fingerstick whole blood, oral fluid, and venipuncture whole blood specimens. This procedure provides instructions on how to perform a rapid HIV test using the OraQuick® ADVANCE Rapid HIV-1/2 Antibody Test kit method.

II. ORDER

A physician's order, standard protocol, or order by another healthcare professional authorized to request laboratory tests is required for Point of Care qualitative HIV-1/2 Rapid Antibody tests.
III. MATERIALS

<table>
<thead>
<tr>
<th>Reagents/Controls</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test</td>
<td>Department of Health (City or State)</td>
</tr>
<tr>
<td>OraQuick® ADVANCE HIV-1/2 Kit Controls</td>
<td>Department of Health (City or State)</td>
</tr>
</tbody>
</table>

Additional Supplies

<table>
<thead>
<tr>
<th>Additional Supplies</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reusable Test Stands</td>
<td>Provided in OraQuick® HIV-1/2 Kit</td>
</tr>
<tr>
<td>Collection Loops</td>
<td>Provided in OraQuick® HIV-1/2 Kit</td>
</tr>
<tr>
<td>Disposable Gloves</td>
<td></td>
</tr>
<tr>
<td>Disposable Absorbent Workspace Cover</td>
<td></td>
</tr>
<tr>
<td>NIST-Certified Timing Device</td>
<td>POCT Office</td>
</tr>
<tr>
<td>JHMI Quality Control Labels (optional)</td>
<td>Standard Register Item #0209N</td>
</tr>
<tr>
<td>JHMI-approved Biohazard Waste Container</td>
<td></td>
</tr>
</tbody>
</table>

IV. STORAGE AND HANDLING REQUIREMENTS

<table>
<thead>
<tr>
<th>OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test Kits</th>
<th>Temperature</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerated (2-8°C)</td>
<td>Manufacturer's expiration date.</td>
<td></td>
</tr>
<tr>
<td>Room Temperature (15-27°C)</td>
<td>Manufacturer's expiration date.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OraQuick® ADVANCE HIV-1/2 Kit Controls</th>
<th>Refrigerated (2-8°C)</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once opened, 8 weeks or</td>
<td></td>
<td></td>
</tr>
<tr>
<td>manufacturer's expiration date,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whichever comes first.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. Once a box of Test Kits is opened, it is required that the box is labeled with the open date.
B. Once a box of Kit Controls is opened, it is required that the box is labeled with the open date and new expiration date.
1. Testing must be performed at temperatures in the range of 15-37°C.
C. If the Test Kits are stored refrigerated, supplies must be brought to room temperature prior to use.
D. Bring controls to room temperature (15-30 minutes) prior to use, and return to the refrigerator immediately after use.
E. Never use Divided Pouches or Controls past their expiration date.
F. The workspace area should be level and have adequate lighting in order to properly interpret the test results.

V. SPECIMEN TYPE

The OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test kit is approved by the FDA for the oral fluid, fingerstick whole blood, and venipuncture whole blood as a Waived Point of Care Test.

VI. SPECIMEN COLLECTION AND HANDLING

A. Prior to Specimen Collection:
1. Ensure patient has been provided informed consent and pre-test counseling, then prepare the order.
2. Properly identify the patient using at least two identifiers, neither of which may be the Room Number, prior to sample collection and testing.

3. Put on gloves.

B. Open the two chambers of the OraQuick® ADVANCE HIV-1/2 Divided Pouch by tearing the top notches on the top of each side of the pouch.

1. Leave the Test Device in the pouch until you are ready to use it.
2. Remove the Developer Solution Vial from the Pouch.
3. Label the Test Device and Vial with two patient identifiers.
   a. NOTE: A patient label may be used to meet this requirement, if available.
   b. NOTE: Do not cover the two holes on the back of the Test Device, as this may cause an Invalid test result.
4. Remove the cap carefully from the Vial by gently rocking the cap back and forth while pulling it off.
5. Slide the Vial into the top of one of the slots in the reusable stand, ensuring the Vial is pushed all the way to the bottom.

C. Specimen Collection:

1. Oral Fluid Collection.
   a. Confirm the presence of an Absorbent Packet in the pouch with the Test Device.
      i. NOTE: If none is present, discard the Test Device and obtain and open a new Divided Pouch for testing.
   b. Have the patient remove the labeled Test Device from its Pouch, ensuring the Flat Pad is not touched.
   c. Direct the patient to place the Flat Pad above the teeth against the outer gum and to gently swab one time completely around the outer gums, both upper and lower.
      i. NOTE: The Flat Pad must not come in contact with the roof of the mouth, the inside of the cheek, or the tongue.
      ii. NOTE: Both sides of the Flat Pad may be used during this collection procedure.
   d. Remove and discard the Absorbent Packet from the Pouch.
   e. Instruct the patient to re-insert the Test Device back into its Pouch.
      i. NOTE: The Test Device must be inserted into the Developer Solution Vial within 30 minutes of collection.
2. Refer to PHLEB024: Capillary Blood Collection (Heelstick and Fingerstick) for specific instructions on proper fingerstick technique.
   a. Fill an unused Specimen Collection Loop by placing the rounded end of the loop on the drop of blood, confirming the circle is completely filled.
      i. NOTE: If the loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container and recollect the patient specimen.
3. Venipuncture Whole Blood Collection.
   a. Using standard venous phlebotomy procedures, collect a whole blood specimen using a tube containing any of the following anticoagulants: EDTA, sodium heparin, lithium heparin, or sodium citrate. The blood collection specimen must be labeled with at least two patient identifiers.
      i. NOTE: Other anticoagulants have not been tested and may cause an inaccurate result.
      ii. NOTE: If the specimens are not tested at the time of collection, the whole blood may be stored at 2-8°C for up to 7 days or at 15-30°C for up to 3 days.
   b. Prior to testing, mix the blood tube gently by inversion several times to ensure a homogeneous specimen.
   c. Fill an unused Specimen Collection Loop by inserting the rounded end into the tube of blood, confirming the circle is completely filled.
i. NOTE: If the Loop is dropped or comes in contact with any other surface, discard it in a biohazard waste container and refill a new loop.

D. For Capillary Blood and Venipuncture Blood Collection, immediately insert the blood-filled end of the Specimen Collection Loop all the way into the Developer Vial.

E. Use the loop to stir the blood sample into the Developer Solution, then remove and discard in a biohazard waste container.

F. Verify that the Developer Solution is pink, indicating that the blood was thoroughly mixed into the Developer Solution.
   1. NOTE: If not pink, discard all test materials in a biohazard waste container and start the test over using a new Divided Pouch and a new blood sample.
   2. NOTE: To ensure accurate results, the Test Device must be inserted into the Developer Solution Vial within 60 minutes of introducing the blood specimen into the Developer Solution.

VII. SAFETY PRECAUTIONS

A. Follow ICPM IFC023 Infection Control and Prevention: Standard and Isolation Precautions.

B. All patient specimens, QC materials, and used Test Devices and Developer Solution Vials must be considered potentially infectious, handled with care, and disposed of in a JHMI-approved biohazard receptacle.

C. Refrigeration used for QC material and/or Test Kits is to be used only as designated.

D. Temperatures of refrigeration must be monitored, and documentation of that monitoring kept readily available for review.

VIII. PERFORMING QUALITY CONTROL (QC) TESTS

A. Internal Procedural Controls:
   1. The OraQuick® ADVANCE Rapid HIV-1/2 Antibody Test has a built-in procedural controls that demonstrate assay validity.
   2. Internal Positive Procedural Control:
      a. A reddish-purple line in the Control (“C”) area of the Result Window indicates application of sufficient specimen volume, proper wicking of the sample and reagent, and validity of the test procedure.
      b. The control line must appear on all tests, whether the sample is reactive or non-reactive, in order for the test result to be considered valid.

   3. Internal Negative Procedural Control:
      a. The background in the result window should be white.
      b. If a pink background in the result window makes result interpretation difficult, test results are Invalid.

   4. If either of these internal controls fails to react as expected, the test is reported as Invalid and must be repeated.

B. Three levels of External Quality Control - HIV-1 positive (black cap), HIV-2 positive (red cap), and negative (white cap) - will be performed:
   1. At least once per week on each test kit in use.
   2. When opening a new test kit, prior to use for patient testing.
   3. Whenever two consecutive "Invalid" results are obtained, either on the same client or two different clients.
   4. If there is a change in testing conditions, e.g. new location or temperature exceeding testing/storage temperature ranges.
   5. By each new operator as part of initial hands-on training prior to testing patient specimens.
   6. At least once a year by each operator to demonstrate competence for continued testing, as well as to meet regulatory requirements.

© Copyright 2023 by The Johns Hopkins Health System Corporation and/or The Johns Hopkins University
C. Procedure:
   1. Prepare materials for testing:
      a. Put on gloves.
      b. Verify that controls and Test Kits are dated and within both the manufacturer’s expiration date and open vial expiration date.
      c. Allow QC vials, and the Test Kit if stored refrigerated, to come to room temperature prior to use.
      d. Cover the workspace with a clean, disposable absorbent workspace cover, then place an OraQuick® ADVANCE HIV-1/2 Reusable Test Stand on the cover.
      e. Collection three Divided Pouches and open the chambers on them by tearing at the notches on the top of each side of the pouch.
         i. NOTE: Leave the Test Device in the pouch until ready for use. Label with the level of control to be tested.
      f. Remove the Developer Solution Vial from the pouch and label with the level of control to be tested.
      g. Set the timer to 20-40 minutes.
         i. NOTE: Use of a wall clock or wristwatch to time the test is insufficient; a NIST-certified timer must be used.
   2. Perform the test:
      a. Place the Developer Solution Vial into one of the slots in the reusable test stand, then remove the cap carefully by gently rocking the cap back and forth.
      b. Insert the round end of an unused Specimen Collection Loop into the appropriate vial of control solution, confirming the loop is completely filled.
         i. NOTE: If the control solution appears visually cloudy or discolored, do not use.
         ii. NOTE: Separate Specimen Collection Loops must be used for each test.
      c. Immediately immerse the filled Specimen Collection Loop in the Developer Solution, and stir to mix.
      d. Remove the Specimen Collection Loop from the Developer Solution Vial and discard in a JHMI-approved biohazard container.
      e. Without touching the flat pad, remove the Test Device from the Divided Pouch and insert into the Developer Solution Vial, making sure the flat pad touches the bottom of the vial and the Result Window faces forward.
         i. NOTE: If no absorbent packet is present in the Divided Pouch with the Test Device, a new device should be used for testing.
      f. Press Start on the timer.
      g. Read the results after 20 minutes but not more than 40 minutes in a fully lighted area, recording results and all applicable information on the HIV-1/2 Rapid Antibody QC Log Sheet (see Appendix A).
      h. Discard the used Developer Solution Vials, Test Devices, and other disposables into a JHMI-approved biohazard container.
      i. Recap the Kit Control vials and store them in their original container at 2-8°C.
      j. Both levels of QC must pass before patient testing can be performed.
D. Corrective Action:
   1. If any QC test fails to give the expected results (see Appendix B):
      a. Verify Divided Pouches and Kit Controls are within the expiration dates and that they have been properly stored.
      b. Ensure proper technique is being used, repeating testing.
      c. If QC fails a second time, open new vials of QC solution. Repeat test.
IX. PATIENT TEST PROCEDURE

A. Prepare for the test:
1. Confirm Divided Pouches are not expired and that QC has been performed successfully within the last week. If not, QC must be completed prior to patient testing. Refer to Performing Quality Control (QC) Tests.
2. Put on gloves.
3. Cover the workspace with a clean, disposable absorbent workspace cover, then place an OraQuick® ADVANCE HIV-1/2 Reusable Test Stand on the cover.
4. Open the Divided Pouch by tearing both of the notches at the top.
   a. NOTE: Leave the Test Device in the pouch until ready for use. Label with two patient identifiers, taking care not to cover the two holes on the back of the Test Device, as this may result in an Invalid test result.
5. Remove the Developer Solution Vial and label with at least two patient identifiers, not on the lid.
   a. NOTE: This can be accomplished by writing the identifiers, or by placing a patient label on the Developer Solution Vial.
6. Set timer to 20-40 minutes.
   a. NOTE: Use of a wall clock or wristwatch to time the test is insufficient; a NIST-certified timer must be used.

B. Perform the patient test:
1. Place the Developer Solution Vial into one of the slots in the reusable test stand, then remove the cap carefully by rocking the cap back and forth.
2. Collect the patient specimen, as outlined in Specimen Collection and Handling.
   a. For capillary and venous blood collection specimens, continue with the following steps:
      i. Confirm the round end of the Specimen Collection Loop is completely filled with blood.
      ii. Immediately immerse the filled Specimen Collection Loop in the Developer Solution, and stir to mix.
      iii. Remove the Specimen Collection Loop from the Developer Solution Vial and discard in a JHMI-approved biohazard container.
3. Regardless of specimen type, remove the Test Device from the Divided Pouch without touching the flat pad and insert into the Developer Solution Vial, making sure the flat pad touches the bottom of the vial and the Result Window faces forward.
   a. NOTE: Test Devices that have been used to collect Oral Fluid specimens must be introduced to the Developer Solution Vial within 30 minutes of collection.
4. Press Start on the timer.
   a. NOTE: Do not remove the Test Device from the Developer Solution Vial during this time period.
5. Read the results after 20 minutes but not more than 40 minutes in a fully lighted area, recording results and all applicable information on the HIV-1/2 Rapid Antibody Patient Result Log (see Appendix C). Immediately result the test in the patient's electronic medical record, as applicable.
6. Discard the used Developer Solution Vials, Test Devices, and other disposables into a JHMI-approved biohazard container.

X. EXPECTED RESULTS

Expected results on the OraQuick® ADVANCE HIV-1/2 Rapid Antibody test are Non-Reactive, meaning HIV-1 and/or HIV-2 antibodies are not detected in the specimen and the patient is presumed not to be infected with HIV. For additional information about Expected Results for the Intended Use Population, refer to the OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test package insert.

XI. RESULTS INTERPRETATION

A. Reactive: Presence of pink lines in both the "C" zone and in the "T" zone (see Figure 1) indicates that HIV-1 and/or HIV-2 antibodies have been detected in the specimen and the patient is presumed to be infected with HIV. The lines may vary in intensity, but test is reactive regardless of how faint these lines appear.

B. Non-Reactive: Presence of a pink line in the "C" zone, but no line in the "T" zone (see Figure 2) indicates that HIV-1 and/or HIV-2 antibodies were not detected in the specimen and the patient is presumed not to be infected with HIV.

C. Invalid: No line appears in the "C" zone, a pink background interferes with the test interpretation, or any partial line appears on one side of the "C" or "T" zones (see Figure 3). This is considered failed Internal Procedural quality control. No patient/external QC results can be reported until the failed Internal Procedural quality control is corrected.

Note: Individuals with a reactive result must undergo appropriate clinical follow-up and confirmatory testing, according to CDC recommendations for supplemental testing, and unit-specific procedures.

Figure 1 (Reactive)

Figure 2 (Nonreactive)
XII. RESULTS REPORTING

All patient test results must be manually documented on the HIV-1/2 Rapid Antibody Patient Result Log (see Appendix C). The results on the Internal Procedural Control for each patient's test must be documented, and the log subsequently used to ensure results are properly documented in Epic using manual result entry.

A. Each of the fields must be filled out when completing the HIV-1/2 Rapid Antibody Patient Result Log Sheet.
   1. Record patient's results as "NR" for Nonreactive/Negative and "R" for Reactive/Positive.
      a. NOTE: Do not use (+) or (-) for recording test results.

B. All Reactive results must be sent for confirmatory testing; complete the relevant sections of the Patient Result Log Sheet.

C. Refer to unit-specific protocols for documentation of results in Epic.

XIII. INTERFERENCES

The OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test was evaluated against common interfering substances in order to assess their potential effect on assay performance. None of the interfering substances or concurrent disease states had any impact on the assay performance at the concentrations evaluated. For a full list of interfering substances and the concentrations tested, refer to the OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test package insert.

XIV. LIMITATIONS

A. Reading test results earlier than 20 minutes or later than 40 minutes may yield inaccurate test results.

B. This test is approved for use with fingerstick or venipuncture whole blood, or oral fluid specimens only as a waived point of care test. Use of other types of specimens, or venipuncture whole blood specimens collected using a tube containing anticoagulants other than EDTA, lithium heparin, sodium heparin, or sodium citrate may yield inaccurate results.

C. Clinical data has not been collected to demonstrate the performance of the OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test in persons under 12 years of age.

D. A reactive result using the OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test suggests the presence of HIV-1 and/or HIV-2 antibodies in the specimen, and the intensity of the test line does not necessarily correlate with the HIV antibody titer in the specimen. The OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test is intended as an aid in the diagnosis of HIV infection AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.

E. A non-reactive result does not exclude the possibility of exposure to HIV or infection with HIV. An antibody response to recent exposure may take several months to reach detectable levels.

F. Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.
G. Participation in an HIV vaccine study may result in an individual developing antibodies to the vaccine, while their HIV infection status being unknown. Clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing to device whether a diagnosis of HIV infection is accurate.

XV. PROCEDURE NOTES
A. If Oral Fluid is to be collected, confirm the patient has not had anything to eat, drink, or has not chewed gum for at least 15 minutes prior to collection.
   1. NOTE: If the patient has used any oral care products, wait at least 30 minutes.
B. Do not interchange Test Devices and Developer Solution Vials from kits with different lot numbers.
C. All single use test devices, ie Divided Pouch contents, must be labeled with identifiers (two for patients, one for QC), as outlined in the Performing Quality Control (QC) Tests and Patient Test Procedure sections of this procedure.
D. To ensure accurate results, the Test Device must be inserted into the Developer Solution Vial within a set time period:
   1. For Oral Fluid specimens, the prepared Test Device must be inserted within 30 minutes of collection.
   2. For Capillary and Venipuncture Whole Blood specimens, the Test Device must be inserted within 60 minutes after introducing the specimen into the Developer Solution.
E. Avoid microbial contamination and exercise care in handling kit components.

XVI. OPERATOR TRAINING AND COMPETENCY
A. All staff members acting as Counselors must have attended PHPA sponsored or approved Level I HIV Counselor training, been assigned a unique counselor number, and completed a PHPA sponsored (or approved) training on HIV rapid testing techniques.
B. In addition, testing at JHH may only be performed by currently certified staff members who have been trained by a Point of Care Coordinator, Nurse Educator, or designated unit trainer. Training records must be kept in the employee's personnel file, and a copy sent to the Point of Care Testing Office.
C. Initial training will include:
   1. Review the policy online.
   2. Completion of the Initial Training and Competency Assessment Checklist (see Appendix D), to be kept in the employee's personnel file.
   3. Successful performance and documentation of all three levels of external quality control.
   4. Passing score on the quiz following the MyLearning module.

In order to maintain competency, all testing personnel must successfully complete and document all three levels of external quality control and the MyLearning module quiz at least once a year. The competency calendar follows the fiscal year: July 1 - June 30.

XVII. REFERENCES
B. OraQuick® ADVANCE HIV-1/2 Rapid Antibody Test Kit Controls Package Insert. OraSure Technologies, Inc. Item #3001-1202, rev. 02/08.
HIV Testing Using the OraQuick ADVANCE HIV-1/2 Rapid Antibody Test Procedure

XVIII. SIGNATURES

<table>
<thead>
<tr>
<th>Electronic Signature(s)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>William Clarke</td>
<td>11/09/2023</td>
</tr>
<tr>
<td>Medical Director of Point of Care Testing</td>
<td></td>
</tr>
</tbody>
</table>