

DETERMINE™ HIV-1/2 Ag/Ab Combo TESTING PROCEDURE

PRINCIPLE:

The Determine™ HIV-1/2 Ag/Ag Combo assay is a qualitative immunoassay for the simultaneous detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) HIV Type 1 and Type 2 (HIV-1 and HIV-2) in capillary (fingerstick) whole blood. Although not used in our program, the test unit can also be used on human serum, plasma or venipuncture (venous) whole blood. It is intended for use as a point of care test to aid in the diagnosis of infection with HIV-1 and HIV-2, including an acute HIV-1 infection, and may distinguish acute HIV-1 infection from established HIV-1 infection when the specimen is positive for HIV-1 p24 antigen and negative for anti-HIV-1 and anti-HIV-2 antibodies.

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 specimen collection pipette provided in the test kit, and must be tested immediately.

The test units are stored until the expiration date on the box and pouch. They must be stored at a temperature between 2°-30° C (36°-86°F). If refrigerated, the pouch **must** be brought to room temperature before opening as the temperature range for testing is 15°-30° C (59°-86° F).

MATERIALS PROVIDED:

Each package contains the components to perform HIV tests:

- Aluminum ziplock pouch containing Alere Determine HIV1/2 Ag/Ag Combo Cards. Each card consists of 5 to 10 test units that can be separated from each other by tearing along the perforated lines. Each Test Unit has a cover that is to be removed for sample application and reading of results.
- Desiccant Package
- Chase Buffer
- Quick reference guide
- Package insert
- Information Notices: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.
- Customer Letter
- Disposable Capillary Tubes: For collection and transfer of fingerstick samples
- Disposable Pipettes (for External Controls and proficiency testing)
- Disposable Workstations: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.

MATERIALS REQUIRED, BUT NOT PROVIDED

- Clock, watch, or other timing device
- Disposable gloves
- Sterile gauze (for fingerstick whole blood specimens)
- Antiseptic wipes
- Biohazard disposal container

SPECIMEN STORAGE

The sample must be collected with the specimen collection pipette provided in the test kit, and must be tested *immediately*.

QUALITY CONTROL:

The controls must be stored at (2° - 8°C; 36° - 46° F) and are stable until the manufacturer's expiration date as stated on the box label. The date the controls are received, opened as well as the expiration date must be clearly marked on the box containing the controls. The Control Reagents are clear to straw-colored. Do not use if the Control Reagent appears visually cloudy or discolored.

EXTERNAL QUALITY CONTROL:

Determine™ HIV1/2 Ag/Ag Combo Controls contain:

- HIV-1 Reactive Control, 1 red capped vial (1.5 mL)
- HIV-2 Reactive Control, 1 green capped vial (1.5 mL)
- HIV-1 p24 Antigen Control, 1 lavender capped vial (1.5 mL)
- Nonreactive Control, 1 white capped vial (1.5 mL)
- Disposable pipettes for use in testing external controls and proficiency samples

When to Perform External 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 in the test environment** including such items as:
 - The temperature in the test storage area falling outside of 15°-30° C (59°-86°F).
 - A new location has been selected to perform testing.
4. After Invalid client test results on two consecutive attempts. Cease client testing until controls are run with valid results.

Reminder: If refrigerator storage temperature is out of range (2-8° C; 36° - 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. **DO NOT USE EXPIRED CONTROL SOLUTIONS.**
2. If you open new controls, write the date the controls are opened, and the new expiration date on the box containing the controls.
3. Remove the desired number of test units from the 5 or 10 test card units by bending and tearing at the perforation. The test(s) should be initiated within 2 hours after removing the protective foil cover from each test.
4. Remove the protective foil cover from each test and place it on a flat surface or in the workstation.
5. Open a Control Vial containing the Control Reagent.
6. Using the disposable pipette that comes with the external controls apply one hanging drop of control reagent to the Sample Pad (marked by the arrow symbol). **Use a new disposable pipette or pipette tip with each Control Reagent. Follow steps 5 and 6 until all control reagents have been pipetted.**
7. **DO NOT USE CHASE BUFFER** with control reagents! **BUFFER IS USED ONLY WITH BLOOD SPECIMENS.**
8. Start timing the test. Timing starts at 20 minutes but not more than 30 minutes.
9. Read the results between 20 and 30 minutes. Record the read time on the test log. If the test is not read within 30 minutes, discard the test materials and start again. The test may not be accurate and should be recorded as an operator error, (Code 3 on log sheet).
10. Dispose of the used Test Unit in a biohazard waste.
11. Reseal the Control Reagent Vials and store them in their original container at 2 to 8°C (36 to 46°F).
12. If controls do not give their expected results, you cannot do client testing. You must first figure out what's wrong. Check all temperature logs for out of range temperatures and then repeat the controls. If you do not get the expected results, discard the opened controls and if available open a new box. If you still do not get the expected results call the HIV support staff for instructions. You cannot test any patients until the controls perform correctly. Call our discordant line at (732) 236-7013 for further instructions.

INTERNAL QUALITY CONTROL:

In addition, the Determine™ HIV-1/2 Ag/Ab Combo Test has a built in quality control feature. A pink-purple line develops in the control window on the unit as a positive procedural control. If the line does not develop, the test result is invalid. Please refer to the Determine™ HIV-1/2 Ag/Ag Combo Package Insert for pictorial examples of Reactive, NonReactive and Invalid Test Results. The procedure for interpreting the internal quality control is included in the Interpretation of Test Results section below.

PROFICIENCY TESTING:

Proficiency Test (PT) specimens, as required by the Proficiency Testing Program policy, are tested in the same manner as External Quality Control samples. Reporting of PT results varies; follow specific instructions with each PT challenge.

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° -30° C) (59° – 86° F).
3. Before testing make sure the testing area has adequate lighting.
4. Make sure you are wearing disposable gloves
5. Open 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. Note on the test log that the absorbent packet was not enclosed. This makes the packet invalid.
6. **Remove the test unit from the perforation. Label the Test Unit with the client's ID number (not much space on unit; may use last 3 digits of client ID).**
7. **Using Fingerstick Whole Blood**
 - Clean the finger of the person being tested with an antiseptic wipe, and allow to it dry.
 - Using a sterile lancet, puncture the skin on the side of the finger pad (toward the thumb).
 - Hold the finger downward. Apply gentle pressure beside the point of the puncture.
 - Wipe away the first drop of blood with a sterile gauze pad. Allow a new drop to form.
 - Avoid squeezing the fingertip to accelerate the bleeding. Collect the second drop of blood by holding the capillary tube horizontally, and touch the tip of the capillary tube to the blood sample.
Note: Filling of the capillary tube is automatic-do not squeeze the bulb while sampling. Maintain this position until the flow of the sample has reached the fill line and stopped.
 - Touch the tip of the Capillary Tube containing the blood sample to the Sample Pad (marked by the arrow symbol) and gently squeeze the bulb. Avoid air bubbles. Wait until all the blood is transferred from the Capillary Tube to the Sample Pad.

Caution: Do not lift the Capillary Tube from the Sample Pad before all the blood has been transferred – a bubble may form which will prevent the complete transfer of sample. If a sample won't expel, cover the small opening at the mark on the capillary with a gloved finger. Then squeeze the bulb until the sample is fully dispensed onto the Sample Pad.
8. Wait one minute, then apply one drop of Chase Buffer to the Sample Pad.
9. Start timing the test. Record the start time on the test log sheet.
10. Read the results between 20 and 30 minutes. Record the read time on the test log. If the test is not read within 30 minutes, discard the test materials and start again. If the test is not read within 20 to 30 minutes it may not be accurate. Record it as an operator error, (Code 3).
11. After recording the result immediately discard the test unit into a biohazard waste container.

TEST INTERPRETATION:

Antibody Reactive	A pink-red line in the Control Area and a pink/red Ab line appears in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary. Any visible pink/red color in both the Control and Lower Test Areas, regardless of intensity, is considered Reactive. 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.
Antigen (HIV-1 p24) Reactive (Two Lines – Control and Ag Line)	A pink-red line in the Control Area and a pink/red Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ag and Control lines may vary. Any visible pink/red color in both the Control and Upper Test Areas, regardless of intensity, is considered Reactive. A Reactive test result means that HIV-1 p24 antigen has been detected in the specimen. The test result is interpreted as Preliminary Positive for HIV-1 p24 antigen.
Antibody Reactive and Antigen (HIV-1 p24) Reactive (Three Lines – Control, Ab and Ag)	A pink-red line in the Control Area and a pink/red Ab line appears in the Lower Test Area AND a pink/red Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ab, Ag and Control lines may vary. Any visible pink/red color in

Lines)	the Control Area, the Lower and Upper Test Areas, regardless of intensity, is considered Reactive. The test result is interpreted as Preliminary Positive for HIV-1 and/or HIV-2 antibodies and HIV-1 p24 antigen.
Non-Reactive (One Control Line)	A pink-red Control line appears in the Control Area of the Test Unit, and no pink/red Ab or Ag line appears in the Lower Test Area and the Upper Test Area of the Test Unit, respectively. A Non-Reactive test result means that HIV-1 or HIV-2 antibodies and HIV-1 p24 antigen were not detected in the specimen.
Invalid (No Control Line)	If there is no pink-red Control line in the Control Area of the Test Unit, even if a pink/red line appears in the Lower or Upper Test Area of the Test Unit, the result is INVALID and the test should be repeated. If the problem persists, contact the Rapid HIV Support Program for assistance at 732-743-3624 or call our Discordant Hotline of 732-236-7013.

IF YOU WERE UNABLE TO GET A VALID RESULT AFTER TWO ATTEMPTS, RUN CONTROLS, AND CALL THE NJ RAPID HIV TESTING SUPPORT OFFICE (732) 743-3624 FOR ASSISTANCE OR CALL OUR DISCORDANT LINE AT (732) 236-7013.

Record Test Results:

- Record results on the testing log and if indicated report the result to the appropriate person for documentation.
- Control and patient results should be recorded on the same log. Enter the patient identification number in the Patient/Control ID column.
- Utilize NJHIV RAPID HIV TEST LOGS only. Every test kit utilized must be logged including controls run, wasted test units and so on.
- As log pages are completed they must be faxed to the NJ Rapid HIV Testing office (utilizing the fax number on the test log), and they 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.
- 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.
- The log sheet is a communication tool between the test site and the NJ Rapid HIV Testing Support team.

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 preliminary positive, please refer to the Rapid-2-Rapid procedure for steps to follow.

Procedural Notes:

- Reading the result earlier than 20 minutes or later than 30 minutes may yield erroneous results. If the result is not read within 20 minutes, the test is invalid and must be repeated.
- This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.
- 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:**

This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.

Use only materials that are supplied and/or required in accordance with the Alere Determine HIV-1/2 Ag/Ab Combo test kit.

For more information regarding Limitations of the Procedure please refer to your package insert.

Counseling should reflect these imperfections of testing.

References: SEE INSIDE PACKAGE INSERT

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