DETERMINE™ HIV-1/2 Ag/Ab COMBO

Abbott

Detection of p24 may be inhibited by biotin in the sample, causing false negative results in acute infection. Therefore, do not test samples from patients who are taking biotin.

INTENDED USE

Determine™ HIV–1/2 Ag/Ab Combo is an *in vitro*, visually read, qualitative immunoassay for the simultaneous detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) to HIV Type 1 and Type 2 (HIV-1 and HIV-2) in human serum, plasma, capillary (fingerstick) whole blood 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. The test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV test are available, this test can be used in appropriate multi-test algorithms.

Determine HIV-1/2 Ag/Ab Combo is not intended for newborn screening or for use with cord blood specimens or specimens from individuals less than 12 years of age.

Determine HIV-1/2 Ag/Ab Combo is not intended for use in screening blood, plasma, cell, or tissue donors.

For Prescription Use Only.

CLIA Complexity: Waived
For Fingerstick Whole Blood

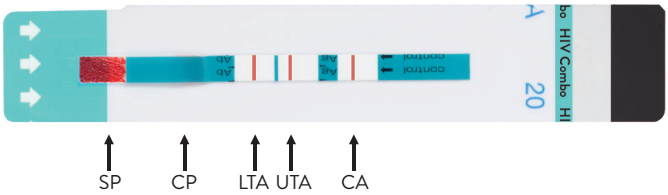
Any modification by the laboratory to the Determine HIV–1/2 Ag/Ab Combo test or the FDA approved Determine HIV–1/2 Ag/Ab Combo test instructions will result in the test no longer meeting the requirements for waived category.

A CLIA Certificate of Waiver is required to perform the test in a waived setting. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA or from your state health department.

CLIA Complexity: Moderate
For Venous Whole Blood, Serum and Plasma Samples

SUMMARY of DEVICE DESCRIPTION and BIOLOGICAL PRINCIPLE of the PROCEDURE

Determine HIV–1/2 Ag/Ab Combo is an immunochromatographic test for the simultaneous and separate qualitative detection of free HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2. The test device is a laminated strip that consists of a sample pad containing monoclonal biotinylated anti-HIV-1 p24 antibody, a conjugate pad containing monoclonal anti-HIV-1 p24 antibody-colloidal selenium and HIV-1 and HIV-2 recombinant antigen-colloidal selenium, and a nitrocellulose membrane with an immobilized mixture of recombinant and synthetic peptide HIV-1 and HIV-2 antigens in the Lower Test Area, immobilized streptavidin in the Upper Test Area, and an immobilized mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody in the Control Area.



SP = Sample Pad; CP = Conjugate Pad; LTA = Lower Test Area; UTA = Upper Test Area; CA = Control Area

A specimen (venipuncture or capillary whole blood, serum, or plasma) is applied to the sample pad (followed by chase buffer for venipuncture or fingerstick whole blood specimens) and migrates by capillary action through the conjugate pad and then through the nitrocellulose membrane.

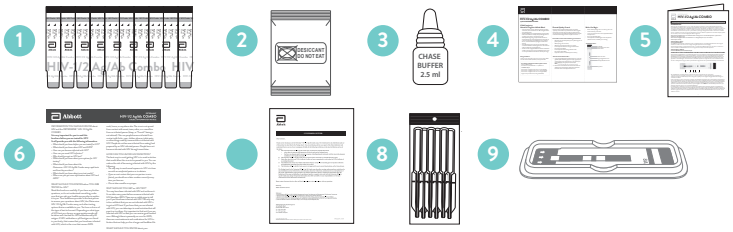
If HIV-1 p24 antigen is present in the specimen, it binds with the monoclonal biotinylated anti-HIV-1 p24 antibody from the sample pad and then with monoclonal anti-HIV-1 p24 antibody-colloidal selenium from the conjugate pad to form a complex (biotinylated antibody-antigen-colloidal selenium-antibody). This complex migrates through the solid phase by capillary action until it is captured by immobilized streptavidin at the Upper Test Area (labeled "Ag") where it forms a single pink/red "Ag" line. If HIV-1 p24 antigen is not present in the specimen or is below the limit of detection of the test, no pink/red Ag line is formed. ***NOTE**: The monoclonal biotinylated anti-HIV-1 p24 antibody used in this assay does not cross react with HIV-2 p26 antigen.*

If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to recombinant gp41 (HIV-1) and gp36 (HIV-2) antigen-colloidal selenium conjugates from the conjugate pad. The complex migrates through the solid phase by capillary action until it is captured by immobilized HIV-1 and HIV-2 synthetic peptide antigens and recombinant gp41 antigen at the Lower Test Area (labeled "Ab") and forms a single pink/red "Ab" line. If antibodies to HIV-1 and/or HIV-2 are absent or are below the detection limit of the test, no pink/red Ab line is formed.

To ensure assay validity, a procedural "Control" line containing a mixture of anti-HIV-1 antibody, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody is incorporated in the nitrocellulose membrane. For a test result to be valid there must be a visible pink/red Control line. During the testing procedure the colloidal selenium conjugates released from the conjugate pad will be captured by the antibodies and antigens immobilized in the Control Area and form a pink/red Control line for samples that are either positive or negative. ***NOTE**: A pink/red Control line may appear even when a test sample has not been applied to the test unit.*

COMPONENTS of DETERMINE™ HIV-1/2 Ag/Ab COMBO: MATERIALS PROVIDED

1. Aluminum ziplock pouch containing Determine HIV-1/2 Ag/Ab Combo Cards. Each card consists of 5 or 10 test units which 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 visualization of test results.
2. Desiccant package
3. Chase buffer: Containing sodium chloride, disodium hydrogen phosphate, and Nipasept as a preservative.
4. Quick reference card
5. Package insert
6. Subject information notices: 25
7. Customer letter
8. Disposable capillary tubes: 25. For collection and transfer of fingerstick samples.
9. Disposable workstations: 25



MATERIALS REQUIRED and AVAILABLE as an ACCESSORY to the KIT

Determine HIV–1/2 Ag/Ab Combo Controls (Catalog #7D2628). Each package contains:

- HIV-1 p24 antigen control: 1.5 mL, HIV-1 viral lysate in defibrinated pooled normal human plasma; negative for antibodies to HIV-1, HIV-2 and HCV; negative for HBsAg.
- HIV-1 reactive control: 1.5 mL, human plasma positive for anti-HIV-1 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-2 and HCV; negative for HBsAg.
- HIV-2 reactive control: 1.5 mL, human plasma positive for anti-HIV-2 antibodies, diluted in defibrinated pooled normal human plasma; negative for antibodies to HIV-1 and HCV; negative for HBsAg and HIV-1 p24.
- Nonreactive control: 1.5 mL, defibrinated normal human plasma; negative for antibodies to HIV-1, HIV-2, and HCV; negative for HBsAg and HIV-1 p24.
- 40 Disposable pipettes – for use testing the external controls only. The disposable pipettes are not to be used for testing patient samples.
- Package insert

MATERIALS AVAILABLE as an ACCESSORY to the KIT

Fingerstick Sample Collection Kit (Catalog #2604US199). Each collection kit contains:

- 100 Sterile safety lancets
- 100 Adhesive bandages
- 100 Ethanol swabs
- 100 Gauze pads

MATERIALS REQUIRED, but NOT PROVIDED

- Clock, watch, or other timing device
- Precision pipette capable of delivering 50 µL of sample with disposable tips, to be used in lieu of the disposable capillary tubes supplied with the kit (for other than fingerstick whole blood specimens)
- Disposable gloves
- Sterile gauze (for fingerstick whole blood specimens)
- Antiseptic wipes
- Biohazard disposal container
- Collection devices for specimens (other than fingerstick whole blood specimens)
- Sterile lancet capable of producing 50 µL of blood

STORAGE

Determine HIV–1/2 Ag/Ab Combo Test Cards and chase buffer must be stored at 2- 30°C (36- 86°F) until expiration date.

SPECIMEN STORAGE

Serum and plasma specimens may be stored at room temperature (15- 30°C) for up to two days before testing. If testing will not be performed within two days of sample collection, serum and plasma specimens should be stored at 2- 8°C if the test is to be run within 7 days of collection. If testing is delayed more than 7 days, the specimen should be frozen (-20°C or colder). Mix specimen well by gentle inversion of the tube immediately before testing.

- Avoid repeated freeze/thaw cycles. Specimens that have been frozen and thawed more than 3 times cannot be used.
- All frozen specimens must be centrifuged at 10,000g for 5 min at room temperature. Carefully remove the 50 µL test sample from the supernatant. If a lipid layer is formed on the surface of the liquid, ensure that the sample is taken from the clear liquid below that layer.

Whole blood collected by venipuncture may be stored at room temperature (15- 30°C) for up to two days before testing. If testing will not be performed within two days of sample collection, whole blood collected by venipuncture should be stored at 2- 8°C if the test is to be run within 6 days of collection. **Do not freeze whole blood specimens.** If stored at 2- 8°C, bring specimen to room temperature before testing. Mix specimen well by gentle inversion of the tube immediately before testing.

Whole blood collected by fingerstick should be tested immediately.

TEST PROCEDURE and INTERPRETATION of RESULTS

A. TEST PROCEDURE

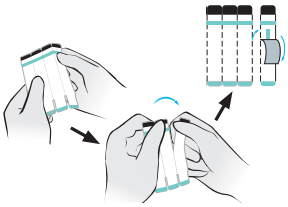
***NOTE**: Determine HIV-1/2 Ag/Ab Combo Controls should be tested prior to testing patient specimens when a new untrained operator performs testing, a new test kit lot is to be used, a new shipment of test kits is received, if the temperature of the test storage area falls outside of 2 to 30°C (36 to 86°F), if the temperature of the testing area falls outside of 15 to 30°C (59 to 86°F), and at periodic intervals indicated by the testing facility. Controls should be tested in the same manner as serum or plasma samples in the following Test Procedure.*

***NOTE:** The disposable pipettes provided with the Determine HIV-1/2 Ag/Ab Combo Control kit are for use with the external controls only and are NOT to be used for testing patient samples.*

Kit Component Preparation

1. Open the aluminum pouch containing the Determine HIV-1/2 Ag/Ab Combo Cards.
2. Remove the desired numbers of test units from the 5 or 10-test unit card by bending and tearing at the perforation.

   ***NOTE**: Removal of the test units should start from the right side of the card to preserve the lot number which appears on the left side of the card.*



3. Return the unused test units to the aluminum pouch and close the pouch with the ziplock.

   ***NOTE:** Store the unused cards and test units only in the aluminum pouch containing the desiccant package. Carefully close the ziplock, so that the cards are not exposed to ambient humidity during storage.*

4. Remove the protective foil cover from each test unit. Lay the test unit flat in the workstation. The test should be initiated within 2 hours after removing the protective foil cover from each test unit. Do NOT touch the sample pad with your fingers.

   ***NOTE:** Use of the workstation is optional. If the workstation is not used, place the test unit on a flat surface.*

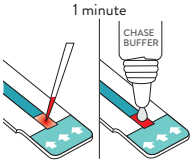
   NOTE: Determine HIV-1/2 Ag/Ab Combo must ONLY be used with capillary (fingerstick) or venous (venipuncture) whole blood, serum or EDTA plasma. Using other types of samples or testing of venipuncture whole blood and plasma samples collected using a tube containing an anticoagulant other than EDTA may not yield accurate results. For serum samples, collect blood without anticoagulant.

For **serum or plasma** samples:

1. Apply 50 µL of sample (precision pipette) by touching the tip of the pipette to the sample pad (marked by the arrow symbol). Do not add chase buffer when using serum or plasma specimens.
2. Read the test result between 20 and 30 minutes after the addition of the sample. Do not read test results after 30 minutes.

For **whole blood (venipuncture)** samples:

1. Using a precision pipette with a disposable tip, apply 50 µL of sample by touching the tip of the pipette to the sample pad (marked by the arrow symbol).
2. When all of the blood is transferred to the sample pad, wait one minute to ensure the chase buffer does not overflow the sample pad.
3. **Add one drop** of chase buffer to the sample pad.
4. Read the test result between 20 and 30 minutes after the addition of chase buffer. Do not read test results after 30 minutes.



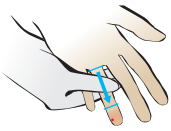
For **whole blood (fingerstick)** samples using the disposable capillary tube provided with the kit:

Caution: *The capillary tube must be used to collect the fingerstick sample.*

To optimize whole blood circulation:

- Warm the hand by washing in warm water (or holding it in a heating pad or hand warmer).
- Massage the finger with a downward motion several times before performing the fingerstick.

Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet capable of producing 50 µL of blood, puncture the skin just off the center of the finger pad and wipe away the first drop with sterile gauze.

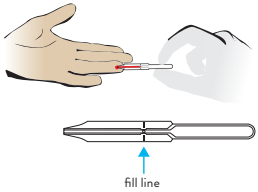


To collect an adequate sample volume:

- Quickly express blood down the fingertip by gently squeezing across the entire finger, to the last joint (not to the end of the fingertip).
- Do not squeeze or "milk" the fingertip to accelerate bleeding.

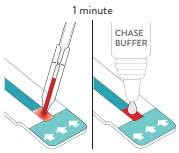
Collect the second drop of blood by holding the capillary tube **HORIZONTALLY**, and touch the tip of the capillary tube to the blood sample.

NOTE: *Filling of the capillary 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.*



To add sample to the test strip:

1. 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.*
2. When all of the blood is transferred to the sample pad, wait one minute to ensure the chase buffer does not overflow the sample pad.
3. **Add one drop** of chase buffer to the sample pad.
4. Read the test result between 20 and 30 minutes after the addition of the chase buffer. Do not read test results after 30 minutes.
   NOTE*: Discard the used pipette tips, capillary tube, test units and any other test materials into a biohazard waste container.*

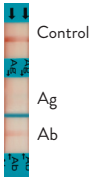


B. INTERPRETATION of TEST RESULTS

NOTE*: When testing whole blood samples, a faint pink background may be visible on the test membrane.*

ANTIBODY REACTIVE (Two Lines - Control and Ab Line)

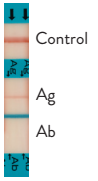
A PINK/RED Control line appears in the Control Area AND a PINK/RED Ab line **must** appear in the Lower Test Area of the test unit. The intensity of the Ab and Control lines may vary. Any visible PINK/RED line in both the Control and Lower Test Areas, regardless of intensity, is considered REACTIVE. 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 Line and Ag Line)

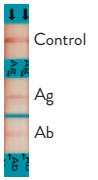
A PINK/RED Control line appears in the Control Area AND a PINK/RED Ag line **must** appear in the Upper Test Area of the test unit. The intensity of the Ag and Control lines may vary. Any visible PINK/RED line in both the Control and Upper Test Areas, regardless of intensity, is considered REACTIVE. 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.

NOTE: *A test result that is PRELIMINARY POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.*



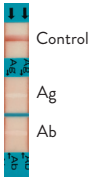
ANTIBODY REACTIVE and ANTIGEN (HIV-1 p24) REACTIVE (Three Lines - Control, Ab and Ag Lines)

A PINK/RED Control line appears in the Control Area AND a PINK/RED Ab line **must** appear 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 line in the Control Area, the Lower Test Area and the Upper Test Area, 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.



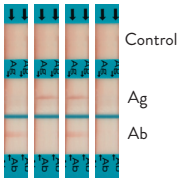
NONREACTIVE (One Line – Control Line)

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 NONREACTIVE 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 Test Area or the Upper Test Area of the test unit, the result is INVALID and the test should be repeated. If the problem persists, contact Abbott Technical Support.



RESTRICTIONS

- Sale of Determine HIV-1/2 Ag/Ab Combo is restricted to clinical laboratories that have an adequate quality assurance program, including planned and systematic activities that provide adequate confidence that requirements for quality will be met and where there is assurance that operators will receive and use the instructional material.
- Determine HIV-1/2 Ag/Ab Combo is approved for use only by an agent of a clinical laboratory.
- Non-clinical testing sites that offer waived rapid HIV tests must either have their own CLIA Certificate of waiver or have an agreement to work under the CLIA Certificate of an existing laboratory.
- Test subjects must receive the "Subject Information Notice" prior to specimen collection and appropriate information when the test results are provided.
- Determine HIV-1/2 Ag/Ab Combo is not approved for use to screen blood, plasma, cell or tissue donors.
- This assay has not been evaluated for newborn screening or for use with cord blood specimens or for use with specimens from individuals less than 12 years of age.

WARNINGS

For *In Vitro* Diagnostic Use

1. Read the package insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
2. Use of this test kit with specimen types other than those specifically approved for use with this device may produce inaccurate test results.
3. This test should be performed at 15 to 30°C (59 to 86°F). If stored refrigerated, ensure that the test units are brought to operating temperature before performing testing.
4. Do not open or remove the protective foil cover from the test unit until just prior to use (test units should be used within 2 hours of removing the protective foil).
5. Do not store unused cards or test units outside the provided ziplock aluminum pouch containing desiccant for long periods. Storage outside the original pouch could expose the test units to ambient humidity and affect the test performance.
6. Make sure the ziplock is well closed after returning the unused cards or test units to the aluminum pouch.
7. Do not use kit contents beyond labeled expiration date.
8. Ensure that the test subject's finger is completely dry before obtaining a fingerstick sample.
9. Ensure that a sample or control is applied to the sample pad. Failure to apply a sample may give a false negative test result.
10. Read test results in a well-lit area.
11. Reading test results for serum or plasma specimens earlier than 20 minutes or later than 30 minutes after addition of the serum or plasma specimen may yield erroneous results. Reading test results for capillary (fingerstick) or venous (venipuncture) whole blood specimens earlier than 20 minutes or later than 30 minutes after addition of the chase buffer may yield erroneous results.
12. Adding chase buffer before adding the sample will yield invalid test results.
13. A PINK/RED Control line does not indicate that a sample or control has been applied, but that liquid had been applied to the strip.
14. Specimens from individuals infected with HIV-1 and/or HIV-2 who are receiving antiretroviral (ART) therapy may produce false negative test results.
15. Specimens from individuals with Toxoplasma IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides (above 600 mg/dL), herpes simplex virus infection, hospitalized and cancer patients may give false positive test results.
16. Only the liquid in the chase buffer bottle should be used. Do not use water or other liquids.

PRECAUTIONS

1. Safety Precautions

a. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection.

b. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples.

c. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where samples and kit reagent materials are handled. Avoid any contact between hands, eyes, or mouth during sample collection and testing. Do not touch the sample pad.

d. Decontaminate and dispose of all specimens, reagents, disposable workstations, and other potentially contaminated materials in a biohazard waste container in accordance with local regulations. Lancets 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. The workstations are for single use only. The used workstation and test unit should be regarded as potentially infectious material. They should be disposed of together, without trying to remove the test unit from the workstation, in a biohazard waste container as indicated above.*

e. Clean and disinfect all spills of specimens or reagents using 10% bleach or other appropriate disinfectant. The bleach solution should be made fresh every day.

f. For additional information refer to: Centers for Disease Control and Prevention: Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendation for Post-exposure Prophylaxis.[7]

2. Handling Precautions

a. If desiccant package is missing, DO NOT USE. Discard test cards (all test units) and use a new test card.

b. Do not use any test units from test cards if the pouch has been perforated.

c. Each test unit, lancet and disposable capillary tube for collection and transfer of fingerstick samples is for single use only.

d. Do not use kit components beyond the expiration date printed on the label. Always check expiration date prior to testing.

e. Adequate lighting is required to read a test result.

LIMITATIONS of the TEST

1. Determine HIV-1/2 Ag/Ab Combo must ONLY be used with capillary (fingerstick) or venous (venipuncture) whole blood, serum or EDTA plasma. Using other types of samples or testing of venipuncture whole blood and plasma samples collected using a tube containing an anticoagulant other than EDTA may not yield accurate results. For serum samples, collect blood without anticoagulant.
2. Determine HIV–1/2 Ag/Ab Combo must be used in accordance with the instructions in the package insert to obtain accurate results.
3. This assay does not detect or has not been validated to detect HIV-2 antigen.
4. A Reactive result using Determine HIV-1/2 Ag/Ab Combo suggests the presence of HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2 in the sample. The Reactive result is interpreted as Preliminary Positive for HIV-1 p24 antigen and/or antibodies to HIV-1 and/or HIV-2. Determine HIV-1/2 Ag/Ab Combo is intended as aid in the diagnosis of infection with HIV-1/2. AIDS-related conditions are clinical syndromes, and their diagnosis can only be established clinically.
5. For a Reactive result, the intensity of the test line does not necessarily correlate with the titer of antigen or antibody in the sample.
6. Reactive test results should be confirmed by additional testing using other tests.
7. A Nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV.
8. A person who has HIV-1 p24 antigen or antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus. However, 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.
9. This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.
10. Specimens from individuals infected with HIV-1 and/or HIV-2 who are receiving antiretroviral (ART) therapy may produce false negative test results.
11. Specimens from individuals with Toxoplasma IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides (above 600 mg/dL), herpes simplex virus infection, hospitalized and cancer patients may give false positive test results.

SUMMARY of PRECLINICAL STUDIES

HIV-1 p24 Analytical Sensitivity

The analytical sensitivity of the Determine HIV-1/2 Ag/Ab Combo in serum was determined to be at least 25 pg/mL using the Etablissement Français du Sang HIV Ag panel by testing the greatest dilution provided. In addition, the sensitivity in serum was determined to be 12.5 pg/mL and 2 IU/mL by testing the maximum serial dilutions of the Applied Biosystems purified HIV-1 p24 antigen and the WHO HIV-1 p24 antigen standard, respectively, in normal human serum. The sensitivity in human whole blood was determined to be 25 pg/mL and 3 IU/mL by testing the maximum serial dilutions of the Applied Biosystems purified HIV-1 p24 antigen and the WHO HIV-1 p24 antigen standard, respectively, in normal human whole blood.

HIV-1 p24 Antigen Detection in Culture Supernatants

Forty-three (43) HIV-1 culture supernatants, representing HIV-1 group M subtypes A, C, D, CRF01_AE, F, G, CRF02_AG, G/H, H, J, K, and G/A, and HIV-1 group O and group N, were tested on Determine HIV-1/2 Ag/Ab Combo. Culture supernatants were diluted in HIV negative human serum before testing. Of the 43 culture supernatants, 40 tested positive for HIV-1 p24 antigen at a 1:27 dilution. One HIV-1 group O, one HIV-1 subtype H, and one HIV-1 subtype F specimen tested negative for HIV-1 p24 antigen (see Table 1). Results from this study demonstrated that Determine HIV-1/2 Ag/Ab Combo can detect HIV-1 p24 antigen from subtype B and from non-B subtypes, and from HIV-1 group O and group N.

Table 1: Detection of HIV-1 p24 Antigen from Group M Subtype Viral Isolate Culture Supernatants using Determine™ HIV-1/2 Ag/Ab Combo

Type and Subtype*	Positive /Specimens Tested
HIV-1 A	4/4
HIV-1 B	4/4
HIV-1 C	4/4
HIV-1 D	4/4
HIV-1 CRF01_AE	5/5
HIV-1 F	2/3
HIV-1 G	2/2
HIV-1 CRF02_AG	4/4
HIV-1 G/H	1/1
HIV-1 H	2/3
HIV-1 J	2/2
HIV-1 K	1/1
HIV-1 G/A	1/1
HIV-1 group O	3/4
HIV-1 group N	1/1
Total	40/43

** Culture supernatant from various isolates was spiked into negative human serum for testing.*

Seroconversion Panels

Twenty eight (28) commercially available seroconversion panels (serum/plasma) were tested on Determine HIV-1/2 Ag/Ab Combo, on an FDA-licensed anti-HIV-1/2 EIA and on an HIV-1 p24 antigen EIA with an estimated sensitivity of 15.7 pg/mL.

Detection of HIV-1 antibodies

As shown in Table 2, Determine HIV-1/2 Ag/Ab Combo detected HIV antibodies at the same bleed as an FDA-licensed anti-HIV-1/2 EIA in 14/28 panels (50%) and at a later bleed in 9/28 panels (32%). No antibodies were detected by either Determine HIV-1/2 Ag/Ab Combo or the FDA-licensed EIA in the remaining 5/28 panels (18%),

NOTE: *The five panels that were nonreactive for antibodies on both tests were reactive only for HIV-1 p24 antigen by Determine HIV 1/2 Ag/Ab Combo at the same bleed as the HIV-1 p24 Ag EIA assay.*

Detection of HIV-1 p24 antigen

Determine HIV-1/2 Ag/Ab Combo detected HIV-1 p24 antigen at the same bleed as the research use HIV-1 p24 antigen EIA in 18/28 panels (64%) and at a later bleed in 8/28 panels (29%). The HIV-1 p24 antigen EIA detected antigen but Determine HIV-1/2 Ag/Ab Combo did not detect antigen in multiple bleeds in 2/28 panels (7%). Determine HIV-1/2 Ag/Ab Combo detected HIV-1 p24 antigen less frequently (that is, in fewer bleeds) than the HIV-1 p24 antigen EIA in 18/28 panels (64%). Of these, in 12/28 panels (43%) Determine HIV-1/2 Ag/Ab Combo detected HIV-1 antibodies at the same bleed as HIV-1 p24 Ag EIA assay.

Detection of HIV infection by Determine HIV-1/2 Ag/Ab Combo compared to the FDA licensed EIA

The seroconversion panel studies showed that Determine HIV-1/2 Ag/Ab Combo detected HIV infection (as shown by the detection of HIV antigen and/or antibody) at an earlier bleed compared to HIV-1/2 antibodies by an FDA-licensed anti-HIV-1/2 EIA in 19/28 panels (68%) and at the same bleed in 9/28 panels (32%). The earlier detection of HIV infection by the Determine HIV-1/2 Ag/Ab Combo in all 19 panels was due to the detection of antigen in earlier bleeds compared to the detection of antibody by the FDA-licensed anti-HIV-1/2 EIA.

Table 2: Testing of Seroconversion Panels using Determine™ HIV-1/2 Ag/Ab Combo, an FDA-licensed anti-HIV-1/2 EIA, and an HIV-1 p24 Antigen EIA

Panel	Relative Day of Bleed	Determine™ HIV 1/2 Ag/Ab Combo		Anti-HIV 1/2 EIA	HIV 1 p24 Antigen EIA
		HIV 1 p24 Antigen	HIV 1/2 Antibody		
PRB-910 (J)	0	NR	NR	NR	NR
	14	R	NR	NR	RR
	26	NR	R	RR	NR
	28	NR	R	RR	NR
	32	NR	R	RR	NR
	35	NR	R	RR	NR
	40	NR	R	RR	NR
PRB-912 (L)	0	R	NR	RR	RR
	9	NR	R	RR	RR
	14	NR	R	RR	RR
	16	NR	R	RR	RR
	28	NR	R	RR	NR
	30	NR	R	RR	NR
PRB-925 (Y)	0	NR	NR	NR	NR
	10	NR	NR	NR	NR
	18	NR	NR	NR	NR
	22	NR	NR	NR	NR
	44	R	R	RR	RR
	49	NR	R	RR	RR
PRB-926 (Z)	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	7	R	NR	NR	RR
	9	R	NR	NR	RR
	27	NR	R	RR	RR
	32	NR	R	RR	RR
PRB-927 (AB)	0	NR	NR	NR	NR
	28	R	NR	RR	RR
	33	R	R	RR	RR
	35	NR	R	RR	RR
	40	NR	R	RR	RR
PRB-930 (AE)	0	R	NR	NR	RR
	3	R	NR	NR	RR
	7	R	R	RR	RR
	10	R	R	RR	RR
PRB-931 (AF)	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	7	NR	NR	NR	NR
	9	NR	NR	NR	NR
	15	R	NR	NR	RR
	28	R	R	RR	RR
	33	NR	R	RR	RR
	35	NR	R	RR	RR
	42	NR	R	RR	RR
PRB-934 (AI)	0	R	NR	NR	RR
	7	NR	R	RR	RR
	11	NR	R	RR	RR
PRB-938 (AM)	0	R	NR	NR	RR
	3	R	NR	NR	RR
	9	R	R	RR	RR
PRB-939 (AN)	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	7	NR	NR	NR	NR
	9	NR	NR	NR	NR
	14	NR	NR	NR	NR
	16	NR	NR	NR	RR
	21	R	NR	NR	RR
	23	R	NR	NR	RR
	103	NR	R	RR	RR

Panel	Relative Day of Bleed	Determine™ HIV 1/2 Ag/Ab Combo		Anti-HIV 1/2 EIA	HIV 1 p24 Antigen EIA
		HIV 1 p24 Antigen	HIV 1/2 Antibody		
PRB-940 (AP)	0	R	NR	NR	RR
	7	R	NR	NR	RR
	11	R	R	RR	RR
	15	NR	R	RR	NR
	18	NR	R	RR	NR
	22	NR	R	RR	NR
	25	NR	R	RR	NR
	29	NR	R	RR	NR
PRB-941 (AQ)	0	NR	NR	NR	NR
	4	NR	NR	NR	NR
	9	NR	NR	NR	RR
	18	R	NR	RR	RR
	21	NR	R	RR	NR
	25	NR	R	RR	NR
PRB-943 (AS)	0	NR	NR	NR	NR
	5	NR	NR	NR	NR
	7	NR	NR	NR	RR
	12	R	NR	NR	RR
	14	R	NR	RR	RR
	19	R	R	RR	RR
	21	R	R	RR	RR
PRB-945 (AU)	0	NR	NR	NR	NR
	3	NR	NR	NR	NR
	7	NR	NR	NR	RR
	13	R	NR	RR	RR
	15	R	R	RR	RR
	20	R	R	RR	RR
PRB-947 (AW)	0	NR	NR	NR	NR
	9	R	R	RR	RR
	11	NR	R	RR	RR
	20	NR	R	RR	NR
PRB-949 (AY)	0	NR	NR	NR	NR
	6	NR	NR	NR	NR
	9	NR	NR	NR	NR
	18	R	NR	NR	RR
	20	R	R	RR	RR
PRB-951 (BA)	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	8	NR	NR	NR	RR
	11	R	NR	NR	RR
	15	R	NR	NR	RR
	19	R	R	RR	RR
PRB-952 (BB)	0	NR	NR	NR	NR
	7	NR	NR	NR	NR
	10	R	NR	NR	RR
	14	R	NR	RR	RR
	17	NR	R	RR	RR
	21	NR	R	RR	NR
PRB-955 (BE)	0	NR	NR	NR	NR
	3	NR	NR	NR	RR
	7	R	NR	NR	RR
	12	R	NR	RR	RR
	14	NR	R	RR	RR
PRB-956 (BF)	0	NR	NR	NR	NR
	40	NR	NR	NR	NR
	42	NR	NR	NR	NR
	47	R	NR	NR	RR
	50	R	NR	NR	RR

Panel	Relative Day of Bleed	Determine™ HIV 1/2 Ag/Ab Combo		Anti-HIV 1/2 EIA	HIV 1 p24 Antigen EIA
		HIV 1 p24 Antigen	HIV 1/2 Antibody		
PRB-957 (BG)	0	NR	NR	NR	NR
	7	NR	NR	NR	NR
	9	NR	NR	NR	NR
	14	NR	NR	NR	NR
	16	NR	NR	NR	RR
	23	R	NR	RR	RR
	28	R	R	RR	RR
PRB-959 (BI)	0	NR	NR	NR	RR
	7	R	NR	NR	RR
	9	R	NR	RR	RR
	14	NR	R	RR	RR
	19	NR	R	RR	RR
	21	NR	R	RR	RR
	26	NR	R	RR	RR
PRB-960	0	NR	NR	NR	NR
	4	NR	NR	NR	NR
	7	NR	NR	NR	NR
	11	NR	NR	NR	NR
	14	NR	NR	NR	NR
	18	NR	NR	NR	NR
	21	NR	NR	NR	NR
	28	R	NR	NR	RR
	30	R	NR	NR	RR
PRB-961	0	NR	NR	NR	NR
	5	NR	NR	NR	NR
	7	NR	NR	NR	NR
	12	NR	NR	NR	NR
	14	NR	NR	NR	NR
	19	NR	NR	NR	NR
	21	NR	NR	NR	NR
	27	R	NR	NR	RR
	29	R	NR	NR	RR
PRB-962	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	7	NR	NR	NR	NR
	9	NR	NR	NR	NR
	14	R	NR	NR	RR
	17	R	NR	NR	RR
PRB-963	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	7	NR	NR	NR	NR
	9	NR	NR	NR	NR
	14	NR	NR	NR	NR
	17	R	NR	NR	RR
	21	R	NR	NR	RR
PRB-965	0	NR	NR	NR	NR
	5	NR	NR	NR	RR
	7	NR	NR	NR	RR
	12	NR	R	RR	RR
	14	NR	R	RR	RR
	21	NR	R	RR	RR

Panel	Relative Day of Bleed	Determine™ HIV 1/2 Ag/Ab Combo		Anti-HIV 1/2 EIA	HIV 1 p24 Antigen EIA
		HIV 1 p24 Antigen	HIV 1/2 Antibody		
PRB-966	0	NR	NR	NR	NR
	2	NR	NR	NR	NR
	20	NR	NR	NR	NR
	22	NR	NR	NR	NR
	30	NR	NR	NR	NR
	35	NR	NR	NR	NR
	37	NR	NR	NR	NR
	44	NR	NR	NR	RR
	48	NR	R	RR	RR
	51	NR	R	RR	RR

R = Reactive, NR = Nonreactive, RR = Repeatedly Reactive

Reactivity with HIV-1 Low Titer Panels

Two commercially available low titer panels of serum and plasma specimens (30 specimens total) were used to evaluate the Determine HIV-1/2 Ag/Ab Combo (two lots), and the results were compared to those from three FDA-licensed HIV antibody EIAs and two HIV-1 p24 antigen EIAs (with limits of detection of 12.5 pg/mL and 25 pg/mL).

As shown in Table 3, Determine HIV-1/2 Ag/Ab Combo detected HIV-1 infection (that is, detected HIV-1 antigen and/or antibody) in all specimens for which at least one HIV antibody EIA or one HIV-1 p24 antigen EIA was repeatedly reactive. The concordance rates of antibody reactivity for the Determine HIV-1/2 Ag/Ab Combo with HIV antibody EIA Test 1 and Test 2 were 27/30 and 26/30, respectively. A lower concordance rate, 21/30, of the Determine HIV-1/2 Ag/Ab Combo with HIV antibody EIA Test 3 correlated with reduced sensitivity of the EIA Test 3 compared with EIA Test 2. The concordance rates of antigen reactivity for the Determine HIV-1/2 Ag/Ab Combo with research use HIV-1 p24 antigen EIA Test 1 and Test 2 were 25/30 and 27/30, respectively.

Table 3: Low Titer HIV Panels – Comparison of Determine™ HIV-1/2 Ag/Ab Combo to HIV Antibody and HIV-1 p24 Antigen Reference Tests

Specimen Information		Determine™ HIV 1/2 Ag/Ab Combo			HIV Antibody EIA			HIV 1 p24 Antigen EIA	
Panel	Member	HIV 1 p24 Antigen	HIV 1/2 Antibody	Overall (Antigen or Antibody)	Test 1	Test 2	Test 3	Test 1	Test 2
PRB108(M)	1	NR	R	R	RR	RR	RR	NR	NR
	2	NR	NR	NR	NR	NR	NR	NR	NR
	4	NR	R	R	RR	RR	RR	NR	RR
	5	NR	R	R	RR	RR	RR	NR	NR
	7	NR	R	R	RR	RR	RR	NR	NR
	8	NR	R	R	RR	RR	RR	NR	NR
	9	NR	R	R	RR	RR	NR	RR	NR
	10	NR	R	R	RR	RR	NR	RR	RR
	12	R	NR	R	RR	RR	NR	RR	RR
	15	R	R	R	RR	RR	RR	RR	RR
PRB109	1	R	R	R	RR	RR	NR	RR	RR
	2	R	R	R	NR	RR	NR	RR	RR
	3	R	NR	R	NR	RR	NR	RR	RR
	4	R	R	R	RR	RR	NR	RR	RR
	5	NR	R	R	RR	RR	RR	RR	RR
	6	R	NR	R	RR	RR	RR	RR	RR
	7	R	NR	R	NR	RR	NR	RR	RR
	8	NR	NR	NR	NR	NR	NR	NR	NR
	9	R	R	R	RR	RR	NR	RR	RR
	10	R	R	R	RR	RR	RR	RR	RR
	11	R	R	R	RR	RR	RR	RR	RR
	12	R	R	R	RR	RR	RR	RR	RR
	13	R	NR	R	NR	NR	NR	RR	RR
	14	R	R	R	RR	RR	RR	RR	RR

Specimen Information		Determine™ HIV 1/2 Ag/Ab Combo			HIV Antibody EIA			HIV 1 p24 Antigen EIA	
Panel	Member	HIV 1 p24 Antigen	HIV 1/2 Antibody	Overall (Antigen or Antibody)	Test 1	Test 2	Test 3	Test 1	Test 2
PRB109	15	R	R	R	RR	RR	RR	RR	RR
	16	R	R	R	RR	RR	NR	RR	RR
	17	R	R	R	RR	RR	NR	RR	RR
	18	R	R	R	RR	RR	RR	RR	RR
	19	NR	R	R	RR	RR	RR	RR	NR
	20	*R/NR	R	R	RR	RR	RR	RR	NR

R = Reactive, NR = Nonreactive, RR = Repeatedly Reactive

**Sample PRB109-20 generated an HIV-1 p24 antigen Reactive result on one lot and a Nonreactive result on the other.*

Reactivity with Worldwide HIV-1 Specimens

Sensitivity of Determine HIV-1/2 Ag/Ab Combo for HIV-1 specimens from various worldwide geographic regions (Ghana, South Africa, Uganda, Spain, Argentina, Ivory Coast, Zimbabwe, Thailand, Columbia, France, UK and Belgium), was assessed by testing 223 HIV-1-positive specimens. Specimens (serum/plasma) tested included group M subtypes A (12), B (47), C (12), D (12), F (10), G (13), H (8) J (8), K (4), HIV-1 group O specimens (15) and different recombinant forms (82). All 223 specimens tested Reactive by Determine HIV-1/2 Ag/Ab Combo.

Table 4: Testing HIV-1 Specimens from Various Geographic Regions using the Determine™ HIV-1/2 Ag/Ab Combo

HIV Subtype	Number of Specimens	Determine™ HIV 1/2 Ag/Ab Combo Reactive
A	12	12
B	47	47
C	12	12
D	12	12
F	10	10
G	13	13
H	8	8
J	8	8
K	4	4
Group O	15	15
CRF01_AE	8	8
CRF02_AG	3	3
CRF06_cpx	3	3
CRF01	2	2
CRF02	3	3
CRF06	2	2
CRF09	2	2
CRF11	1	1
AE	3	3
AG	9	9
A/AE	4	4
A/D	2	2
A/G	1	1
AG/A	2	2
AG/B	1	1
AG/F	1	1
AG/G	1	1
AG/K	3	3
B/D	2	2
C/B	2	2
D/A	3	3
D/B	2	2
D/C	2	2
D/F	1	1
F/B	2	2
G/AG	2	2
G/B	3	3
H/C	1	1
J/G	1	1
K/A	2	2

HIV Subtype	Number of Specimens	Determine™ HIV 1/2 Ag/Ab Combo Reactive
K/AE	1	1
K/C	6	6
K/F	1	1
Total	223	223

Effect of Unrelated Medical Conditions and Potentially Interfering Substances on Specificity and Analytical Sensitivity

To assess the impact of unrelated medical conditions and potentially interfering substances on the specificity of Determine HIV-1/2 Ag/Ab Combo, a total of 1205 specimens from a variety of medical conditions unrelated to HIV infection or containing potential interfering substances were tested. The list of medical conditions and potentially interfering substances and the test results are shown in Table 5. Of the 1205 specimens, 1184 gave Nonreactive results. The following 21 samples gave false Reactive results: 1/55 herpes simplex virus (HSV) (1.8%), 1/55 Toxoplasma IgG (1.8%), 2/55 cancer (3.6%), 8/560 hospitalized patients (1.4%), 2/54 individuals with human anti-mouse antibodies (HAMA) (3.7%), 4/150 rheumatoid factor (RF) (2.7%), and 3/55 with elevated triglycerides (5.6%).

To assess the impact of unrelated medical conditions and potentially interfering substances on the analytical sensitivity of Determine HIV-1/2 Ag/Ab Combo, a total of 310 specimens from a variety of medical conditions unrelated to HIV infection or containing potentially interfering substances were tested spiked with an HIV-1-positive specimen to a low level of antibody reactivity. In addition, 300 specimens were spiked to a low level of HIV-1 p24 antigen reactivity. All spiked samples generated Reactive results (see Table 5), indicating that none of the unrelated medical conditions or potentially interfering substances affected detection of HIV-1 antibodies or p24 antigen by Determine HIV-1/2 Ag/Ab Combo.

Table 5: Determine™ HIV-1/2 Ag/Ab Combo Reactivity with Specimens from Individuals with Unrelated Medical Conditions and Specimens with Potentially Interfering Substances

Specimen Description	Determine™ HIV 1/2 Ag/Ab Combo (# Reactive/Total Tested)		
	Specificity Testing: Unspiked Samples	Sensitivity Testing: HIV 1 Samples (Weak Reactive)	Sensitivity Testing: p24 Antigen Samples
Human T-cell Lymphotropic Virus (HTLV)	0/10	10/10	10/10
Epstein Barr Virus (EBV)	0/20	10/10	NT
Cytomegalovirus (CMV)	0/20	10/10	10/10
Hepatitis C Virus (HCV)	0/30	10/10	10/10
HBsAg	0/8	NT	NT
Herpes Simplex Virus (HSV)	1/55	20/20	20/20
Syphilis	0/20	10/10	10/10
Toxo IgG	1/55	20/20	20/20
Cancer	2/55	20/20	20/20
Alcoholic Cirrhosis	0/10	10/10	10/10
Flu Vaccine	0/10	10/10	9/9
Anti-HBc	0/10	NT	NT
Multiparous Females	0/10	NT	NT
Drugs	0/10	NT	NT
Hospitalized patients	8/560	55/55	56/56
HAMA	2/54	20/20	20/20
RF	4/150	21/21	21/21
Triglycerides*	3/55	21/21	21/21
Hemoglobin**	0/21	21/21	21/21
Bilirubin**	0/21	21/21	21/21
High Serum Protein**	0/21	21/21	21/21

**Naturally occurring specimens containing more than 500 mg/dL*

***Specimens artificially created by adding the potentially interfering substance to normal human serum (Lyophilized hemoglobin: 5 mg/mL; Bilirubin: 0.25 mg/mL; Protein: 0.05 g/mL).*

To assess the impact of biotin on the performance of Determine HIV-1/2 Ag/Ab Combo, whole blood and serum samples were spiked with HIV-1, HIV-2, or p24 antigen to a low level of reactivity. These samples and presumed non-reactive samples were spiked with biotin concentrations up to 3600ng/mL and tested on the Determine HIV-1/2 Ag/Ab Combo test. No assay interference was observed with biotin spiked HIV-1 or HIV-2 samples or with presumed non-reactive serum and whole blood samples. Two of 20, or 10% of samples, had false negative results for the p24 antigen in whole blood spiked with biotin at 150 ng/mL. Thirteen of 20, or 65% of samples, had false negative results for the p24 antigen in serum spiked with biotin at 200 ng/mL.

Table 6: Determine™ HIV-1/2 Ag/Ab Combo – % p24 Ag Inhibition

Sample Type	Level of Biotin (ng/mL)	Determine™ HIV 1/2 Ag/Ab Combo # Detected	% False Negative Results
Serum	3600	0/20	100%
	200	7/20	65%
	150	20/20	0%
	100	20/20	0%
	75	20/20	0%
	50	20/20	0%
	0	20/20	0%
Whole Blood	3600	0/20	100%
	200	1/20	95%
	150	18/20	10%
	100	20/20	0%
	75	20/20	0%
	50	20/20	0%
	0	20/20	0%

Reproducibility

The reproducibility of Determine HIV-1/2 Ag/Ab Combo was evaluated at three independent sites using three lots of Determine HIV-1/2 Ag/Ab Combo. A blind-coded panel that consisted of six contrived blood specimens (one HIV-1 antibody/antigen-negative, one high positive for HIV-1 antibody, one low positive for HIV-1 antibody, one positive for HIV-2 antibody, one high positive for HIV-1 p24 antigen (25 pg/mL), and one low positive for HIV-1 p24 antigen (12.5 pg/mL), was tested according to the Package Insert on three days by three operators at each of three sites. A total of 485 tests were performed. The overall reproducibility of Determine HIV-1/2 Ag/Ab Combo was calculated to be 482/485 = 99.4% (95% confidence interval 98.2 to 99.9%).

SUMMARY of CLINICAL PERFORMANCE

HIV-1 SENSITIVITY

Serum:

In addition, 655 specimens from individuals at high risk for infection with HIV-1 were tested. Of these, 23 specimens tested repeatedly reactive using an FDA-licensed EIA and positive on WB or IFA. On testing these 655 specimens using Determine HIV-1/2 Ag/Ab Combo, 30 specimens tested Reactive, including all 23 WB positives, and 625 tested Nonreactive.

The sensitivity of Determine HIV-1/2 Ag/Ab Combo for serum specimens was estimated using 926 true HIV-1 positives (903 known positives and 23 true positives identified from the high risk population) (see Table 7). Of these, 925 tested Reactive using Determine HIV-1/2 Ag/Ab Combo (902 known positive and 23 high risk). Determine HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Determine HIV-1/2 Ag/Ab Combo for serum specimens in these studies was 925/926 = 99.9% (95% confidence interval 99.4 to 100.0%).

Table 7: Detection of HIV-1 Antibodies and/or p24 Antigen in Serum Specimens from Individuals Known to be Infected with HIV-1 and at High Risk for Infection with HIV-1

True Status	Determine™ HIV 1/2 Ag/Ab Combo		
	Reactive	Nonreactive	Total
Positive[1]	925	1	926
Negative	7[2]	625	632
Total	932	626	1558

[1] True status based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).

[2] Seven specimens were false Reactive on Determine HIV-1/2 Ag/Ab Combo based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA) or an HIV-1 PCR assay.

Plasma:

The sensitivity of Determine HIV-1/2 Ag/Ab Combo to detect infection with HIV in plasma specimens was evaluated by testing 902 specimens from individuals known to be infected with HIV-1. All 902 specimens were repeatedly reactive on an FDA-licensed EIA and positive on a licensed HIV-1 WB or licensed IFA. Of these, 901 specimens tested Reactive using Determine HIV-1/2 Ag/Ab Combo.

In addition, 655 specimens from individuals at high risk for infection with HIV-1 were tested. Of these, 23 specimens tested repeatedly reactive using an FDA-licensed EIA and positive on WB or IFA. On testing these 655 specimens using Determine HIV-1/2 Ag/Ab Combo, 28 specimens tested Reactive, including all 23 WB positives, and 627 tested Nonreactive.

The sensitivity of Determine HIV-1/2 Ag/Ab Combo for plasma specimens was estimated using 925 true HIV-1 positives (902 known positives and 23 true positives identified from the high risk population) (see Table 8). Of these, 924 tested Reactive using Determine HIV-1/2 Ag/Ab Combo (901 known positive and 23 high risk). Determine HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Determine HIV-1/2 Ag/Ab Combo for plasma specimens in these studies was 924/925 = 99.9% (95% confidence interval 99.4 to 100.0%).

Table 8: Detection of HIV-1 Antibodies and/or p24 Antigen in Plasma Specimens from Individuals Known to be Infected with HIV-1 and at High Risk for Infection with HIV-1

True Status	Determine™ HIV 1/2 Ag/Ab Combo		
	Reactive	Nonreactive	Total
Positive[1]	924	1	925
Negative	5[2]	627	632
Total	929	628	1557

[1] True status based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).
[2] Five specimens were false Reactive on Determine HIV-1/2 Ag/Ab Combo based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA) or an HIV-1 PCR assay.

Venous Whole Blood

The sensitivity of Determine HIV-1/2 Ag/Ab Combo to detect infection with HIV in venous (venipuncture) whole blood specimens was evaluated by testing 902 specimens from individuals known to be infected with HIV-1. All 902 specimens were repeatedly reactive on an FDA-licensed EIA and positive on a licensed HIV-1 WB or licensed IFA. Of these, 901 specimens tested Reactive using Determine HIV-1/2 Ag/Ab Combo.

In addition, 654 specimens from individuals at high risk for infection with HIV-1 were tested. Of these, 23 specimens tested repeatedly reactive using an FDA-licensed EIA and positive on WB or IFA. On testing these 654 specimens using Determine HIV-1/2 Ag/Ab Combo, 28 specimens tested Reactive, including all 23 WB positives, and 626 tested Nonreactive.

The sensitivity of Determine HIV-1/2 Ag/Ab Combo for venous whole blood specimens was estimated using 925 true HIV-1 positives (902 known positives and 23 true positives identified from the high risk population) (see Table 9). Of these, 924 tested Reactive using Determine HIV-1/2 Ag/Ab Combo (901 known positive and 23 high risk). Determine HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Determine HIV-1/2 Ag/Ab Combo for venous whole blood specimens in these studies was 924/925 = 99.9% (95% confidence interval 99.4 to 100.0%).

Table 9: Detection of HIV-1 Antibodies and/or p24 Antigen in Venous Whole Blood Specimens from Individuals Known to be Infected with HIV-1 and at High Risk for Infection with HIV-1

True Status	Determine™ HIV 1/2 Ag/Ab Combo		
	Reactive	Nonreactive	Total
Positive[1]	924	1	925
Negative	5[2]	626	631
Total	929	627	1556

[1] True status based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA),
[2] Five specimens were false Reactive on Determine HIV-1/2 Ag/Ab Combo based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA) or an HIV-1 PCR assay.

Capillary (Fingerstick) Whole Blood

The sensitivity of Determine HIV-1/2 Ag/Ab Combo to detect infection with HIV in capillary (fingerstick) whole blood specimens was evaluated by testing 908 specimens from individuals known to be infected with HIV-1. All 908 specimens were repeatedly reactive on an FDA-licensed EIA and positive on a licensed HIV-1 WB or licensed IFA. Of these, 907 specimens tested Reactive using Determine HIV-1/2 Ag/Ab Combo.

In addition, 654 specimens from individuals at high risk for infection with HIV-1 were tested. Of these, 22 specimens tested repeatedly reactive using an FDA-licensed EIA and positive on WB or IFA. On testing these 654 specimens using Determine HIV-1/2 Ag/Ab Combo, 24 specimens tested Reactive, including all 22 WB positives, and 630 tested Nonreactive.

The sensitivity of Determine HIV-1/2 Ag/Ab Combo for fingerstick whole blood specimens was estimated using 930 true HIV-1 positives (908 known positives and 22 true positives identified from the high risk population) (see Table 10). Of these, 929 tested Reactive using Determine HIV-1/2 Ag/Ab Combo (907 known positive and 22 high risk). Determine HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Determine HIV-1/2 Ag/Ab Combo for fingerstick whole blood specimens in these studies was 929/930 = 99.9% (95% confidence interval 99.4 to 100.0%).

Table 10: Detection of HIV-1 Antibodies and/or p24 Antigen in Fingerstick Whole Blood Specimens from Individuals Known to be Infected with HIV-1 and at High Risk for Infection with HIV-1

True Status	Determine™ HIV 1/2 Ag/Ab Combo		
	Reactive	Nonreactive	Total
Positive[1]	929	1	930
Negative	2[2]	630	632
Total	931	631	1562

[1] True status based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA),

[2] Two specimens were false Reactive on Determine HIV-1/2 Ag/Ab Combo based on testing by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA) or an HIV-1 PCR assay.

HIV-2 SENSITIVITY

The sensitivity of Determine HIV-1/2 Ag/Ab Combo to detect HIV-2 antibodies was assessed by testing 250 specimens that were confirmed positive for HIV-2 antibodies only. These specimens were obtained from repository sources. All 250 specimens tested Reactive using Determine HIV-1/2 Ag/Ab Combo. The sensitivity of the Determine HIV-1/2 Ag/Ab Combo for detection of antibodies to HIV-2 in these studies was estimated to be 250/250 = 100% (95% confidence interval 98.5 to 100.0%).

A total of 552 specimens from an area endemic for infection with HIV-2 were obtained from repository sources and were tested using an FDA-approved anti-HIV-1/2 test that can distinguish between infection with HIV-1 and HIV-2, and the Determine HIV-1/2 Ag/Ab Combo. One specimen that tested invalid using the Determine HIV-1/2 Ag/Ab Combo is not included. The FDA-approved anti-HIV-1/2 test results for the remaining 551 specimens were as follows: 246 tested positive for antibodies to HIV-2 only, 31 were positive for antibodies to HIV-1 only, 13 were positive for antibodies to both HIV-1 and HIV-2, one was not differentiated for antibodies to HIV-1 or HIV-2, 14 were Reactive but were negative on additional testing using an FDA licensed anti-HIV-1/2 EIA that detects antibody against HIV-1 group O, and/or WB, and 246 were nonreactive. All 291 positive specimens tested Reactive using the Determine HIV-1/2 Ag/Ab Combo (see Table 11).

Table 11: Detection of Antibodies to HIV-2 in Specimens from HIV-2 Endemic Populations*

Determine™ HIV 1/2 Ag/Ab Combo	FDA-approved Anti-HIV 1/2 Test		
		Reactive*	Nonreactive
	Reactive	291[a]	12[c]
	Nonreactive	14[b]	234

** One specimen tested invalid using the Determine HIV-1/2 Ag/Ab Combo is not included.*

[a] Out of these 291 reactive specimens, 246 tested positive for antibodies to HIV-2 only, 31 tested positive for antibodies to HIV-1 only, 13 tested positive for antibodies to HIV-1 and HIV-2, and one was not differentiated for antibodies to HIV-1 or HIV-2.

[b] These 14 reactive specimens tested negative for infection with HIV-1 or HIV-2 on additional testing using an FDA-licensed anti-HIV-1/2 EIA that detects antibody against HIV-1 group O, and/or WB.

[c] Seven out of these 12 specimens tested reactive for antibodies using Determine HIV-1/2 Ag/Ab Combo. Five out of these seven specimens tested negative for infection with HIV-1 or HIV-2 by additional testing, one tested indeterminate, and one was not further tested because of small specimen volume. In addition, five of the remaining 12 tested reactive for HIV-1 p24 antigen but tested negative for infection with HIV-1 using a PCR assay.

Specificity

Serum:

The specificity of Determine HIV-1/2 Ag/Ab Combo was evaluated by testing serum specimens from 1062 individuals at low risk of infection (i.e., from a population with less than 1% prevalence of HIV infection) and 655 individuals at high risk of infection (i.e., clinics with more than 1% prevalence of HIV infection) at five clinical trial sites for each population. Specimens from seven individuals at low risk for infection with HIV and specimens from 23 individuals at high risk for infection with HIV tested repeatedly reactive on a licensed EIA and positive on HIV-1 WB or IFA and were excluded from the analysis. Of the remaining 1687 specimens, seven specimens from individuals at high risk for infection with HIV tested Reactive using Determine HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 12).

Based on these studies, the specificity of Determine HIV-1/2 Ag/Ab Combo using serum specimens was estimated to be 1055/1055 = 100% (95% confidence interval 99.6 to 100%) for individuals at low risk for HIV infection, and 625/632 = 98.9% (95% confidence interval 97.7 to 99.5%) for individuals at high risk for HIV infection. The overall specificity of Determine HIV-1/2 Ag/Ab Combo using serum specimens in these studies was estimated to be 1680/1687 = 99.6% (95% confidence interval 99.2 to 99.8%).

Table 12: Performance of Determine™ HIV-1/2 Ag/Ab Combo on Serum Specimens from Individuals Presumed to be Negative for HIV Infection

Study Population	Total Samples Tested	True Negative[1]	Determine™ HIV 1/2 Ag/Ab Combo Nonreactive
Low Risk	1062	1055	1055[2]
High Risk	655	632	625
Total	1717	1687	1680

[1] For the high risk population, antibody result confirmation was performed by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).
[2] One additional specimen tested false Nonreactive using Determine HIV-1/2 Ag/Ab Combo and is not included.

Plasma:

The specificity of Determine HIV-1/2 Ag/Ab Combo was evaluated by testing plasma specimens from 1059 individuals at low risk of infection (i.e., from a population with less than 1% prevalence of HIV infection) and 655 individuals at high risk of infection (i.e., clinics with more than 1% prevalence of HIV infection) at five clinical trial sites for each population. Specimens from eight individuals at low risk for infection with HIV and specimens from 23 individuals at high risk for infection with HIV tested repeatedly reactive on a licensed EIA and positive on HIV-1 WB or IFA and were excluded from the analysis. Of the remaining 1683 specimens, five specimens from individuals at high risk for infection with HIV tested Reactive using Determine HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 13).

Based on these studies, the specificity of Determine HIV-1/2 Ag/Ab Combo using plasma specimens was estimated to be 1051/1051 = 100% (95% confidence interval 99.6 to 100%) for individuals at low risk for HIV infection, and 627/632 = 99.2% (95% confidence interval 98.2 to 99.7%) for individuals at high risk for HIV infection. The overall specificity of Determine HIV-1/2 Ag/Ab Combo using plasma specimens in these studies was estimated to be 1678/1683 = 99.7% (95% confidence interval 99.2 to 99.8%).

Table 13: Performance of Determine™ HIV-1/2 Ag/Ab Combo on Serum Specimens from Individuals Presumed to be Negative for HIV Infection

Study Population	Total Samples Tested	True Negative[1]	Determine™ HIV 1/2 Ag/Ab Combo Nonreactive
Low Risk	1059[2]	1051	1051
High Risk	655	632	627
Total	1714	1683	1678

[1] For the high risk population, antibody result confirmation was performed by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).
[2] One specimen tested Reactive for HIV-1 p24 antigen on the Determine HIV-1/2 Ag/Ab Combo and was positive by an HIV-1 PCR, and is not included.

Venous (Venipuncture) Whole Blood

The specificity of Determine HIV-1/2 Ag/Ab Combo was evaluated by testing venous whole blood specimens from 1062 individuals at low risk of infection (i.e., from a population with less than 1% prevalence of HIV infection) and 654 individuals at high risk of infection (i.e., clinics with more than 1% prevalence of HIV infection) at five clinical trial sites for each population. Specimens from seven individuals at low risk for infection with HIV and specimens from 23 individuals at high risk for infection with HIV tested repeatedly reactive on a licensed EIA and positive on HIV-1 WB or IFA and were excluded from the analysis. Of the remaining 1686 specimens, five specimens from individuals at high risk for infection with HIV tested Reactive using Determine HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 14).

Based on these studies, the specificity of Determine HIV-1/2 Ag/Ab Combo using venous whole blood specimens was estimated to be 1055/1055 = 100% (95% confidence interval 99.6 to 100%) for individuals at low risk for HIV infection, and 626/631 = 99.2% (95% confidence interval 98.2 to 99.7%) for individuals at high risk for HIV infection. The overall specificity of Determine HIV-1/2 Ag/Ab Combo using venous whole blood specimens in these studies was estimated to be 1681/1686 = 99.7% (95% confidence interval 99.3 to 99.9%).

Table 14: Performance of Determine™ HIV-1/2 Ag/Ab Combo on Venous Whole Blood Specimens from Individuals Presumed to be Negative for HIV Infection

Study Population	Total Samples Tested	True Negative[1]	Determine™ HIV 1/2 Ag/Ab Combo Nonreactive
Low Risk	1062	1055	1055
High Risk	654	631	626
Total	1716	1686	1681

[1] For the high risk population, antibody result confirmation was performed by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).

Capillary (Fingerstick) Whole Blood

The specificity of Determine HIV-1/2 Ag/Ab Combo was evaluated by testing fingerstick whole blood specimens from 707 individuals at low risk of infection (i.e., from a population with less than 1% prevalence of HIV infection) and 654 individuals at high risk of infection (i.e., clinics with more than 1% prevalence of HIV infection) at five clinical trial sites for each population. Specimens from two individuals at low risk for infection with HIV and specimens from 22 individuals at high risk for infection with HIV tested repeatedly reactive on a licensed EIA and positive on HIV-1 WB or IFA and were excluded from the analysis. Of the remaining 1337 specimens, two specimens from individuals at high risk for infection with HIV tested Reactive using Determine HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 15).

Based on these studies, the specificity of Determine HIV-1/2 Ag/Ab Combo using fingerstick whole blood specimens was estimated to be 705/705 = 100% (95% confidence interval 99.5 to 100%) for individuals at low risk for HIV infection, and 630/632 = 99.7% (95% confidence interval 98.9 to 100%) for individuals at high risk for HIV infection. The overall specificity of Determine HIV-1/2 Ag/Ab Combo using fingerstick whole blood specimens in these studies was estimated to be 1335/1337 = 99.8 (95% confidence interval 99.5 to 99.9%).

Table 15: Performance of Determine™ HIV-1/2 Ag/Ab Combo on Fingerstick Whole Blood Specimens from Individuals Presumed to be Negative for HIV Infection

Study Population	Total Samples Tested	True Negative[1]	Determine™ HIV 1/2 Ag/Ab Combo Nonreactive
Low Risk	707	705	705[2]
High Risk	654	632	630[3]
Total	1361	1337	1335

[1] For the high risk population, antibody result confirmation was performed by EIA and either licensed HIV-1 Western blot or licensed immunofluorescence assay (IFA).

[2] One additional specimen tested false Nonreactive using Determine HIV-1/2 Ag/Ab Combo and is not included.

[3] One additional specimen tested false negative using the licensed EIA and is not included.

CLIA WAIVER STUDY

The performance of Determine HIV-1/2 Ag/Ab Combo was evaluated in a prospective study conducted at 9 geographically diverse sites located in the United States. At each site, Determine HIV-1/2 Ag/Ab Combo was conducted by operators who had no laboratory experience and were representative of users at CLIA waived testing sites (intended use). The 53 test operators (intended users) who participated in the study were not given any training on the use of the test. There were 1730 subjects with unknown HIV status and 745 subjects known to be HIV positive. The subjects with unknown HIV status were tested with Determine HIV-1/2 Ag/Ab Combo and the comparator method. The subjects known to be HIV positive were tested with Determine HIV-1/2 Ag/Ab Combo and their HIV status was not known to the operators. Fingerstick blood from each subject with unknown HIV status was tested with Determine HIV-1/2 Ag/Ab Combo by the operators at each site. HIV status for each subject with unknown HIV status was determined by a composite reference method (comparator method) which consists of an FDA approved EIA with supplemental Western blot and PCR assays as required. The result of Determine HIV-1/2 Ag/Ab Combo was compared to the HIV status of the subject. The invalid rate was 0.49% (14/2869) (95%CI: 0.29%, 0.82%).

The positive percent agreement and negative percent agreement between Determine HIV-1/2 Ag/Ab Combo and the HIV status for the study specimens are presented in Table 16 below.

Table 16: Positive Percent Agreement and Negative Percent Agreement between Determine™ HIV-1/2 Ag/Ab Combo and the HIV Status of Individuals with Known and Unknown HIV Status

Study Population	Number of Subjects	Positive Percent Agreement	95% two-sided Confidence Interval	Negative Percent Agreement	95% two-sided Confidence Interval
HIV Status Unknown	1730	93.8% (30/32)	79.9% - 98.3%	99.6% (1692/1698)	99.2% - 99.8%
Known HIV-1 Positive	745	99.9% (744/745)*	99.2% - 100%	N/A	N/A
Total	2475	99.6% (774/777)	98.9% - 99.9%	99.6% (1692/1698)	99.2% - 99.8%

**1 Subject was well controlled on antiretroviral (ART) therapy.*

Additionally, a study was conducted to evaluate the ability of untrained operators to detect HIV antibodies and p24 antigen in weakly reactive samples. The samples were prepared in fresh whole blood spiked with patient plasma samples positive for HIV-1 and HIV-2 antibodies. The weakly reactive p24 Ag sample was prepared using a commercially available analytical standard spiked into the whole blood. The concentrations determined to be near the assay cutoff for each of the analytes were based on a series of dilutions; for each analyte, the dilution that resulted in 95% detection when tested repeatedly was selected for the study. Randomly coded panels consisting of 3 contrived weakly reactive whole blood samples were tested with Determine HIV-1/2 Ag/Ab Combo at 3 intended use sites by 9 untrained operators (60 measurements in total per sample). The testing was done over 5 consecutive days with samples integrated into the daily workflow at each site.

Table 17 below shows performance of the test with samples near the cutoff of the assay in the hands of intended users (across all sites).

Table 17: Performance of the Determine™ HIV-1/2 Ag/Ab Combo Run by Intended Users with Weakly Reactive Samples

Sample	Dilution	Percent Reactive	95% Confidence Interval
HIV-1 Weakly Reactive	1:1500	97% (58/60)	88.6% - 99.1%
HIV-2 Weakly Reactive	1:2000	95% (57/60)	86.3% - 98.3%
True Negative	N/A	3% (2/62)	0.9% - 11.0%

Using risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated the test is insensitive to stresses of environmental conditions and potential user errors.

SYMBOLS

℞ Only	Prescription Only
IVD	*In Vitro* Diagnostics

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