SUMMARY OF SAFETY AND EFFECTIVENESS

Product: Alere Determine™ HIV-1/2 Ag/Ab Combo

I. General Information

Device Generic Name: Rapid HIV-1/2 Ag/Ab Test

Device Trade Name: Alere Determine™ HIV-1/2 Ag/Ab Combo

Applicant's Name and Address: Alere Scarborough, Inc.
10 Southgate Road
Scarborough, ME 04074
Tel: (207) 730-5737
Fax: (207) 730-5717

Manufacturer: Orgenics Ltd., (an Alere, Inc. Company)
North Industrial Area, 6 Dan St
Yavne, 70650
Israel

Premarket Approval Application (PMA) Number: BP 120037

Date of Panel Recommendation: Not Applicable

Office's Signatory Authority: Jay S. Epstein, M.D.
Director, OBRR/CBER

□ **I concur with the summary review.**
□ **I concur with the summary review and include a separate review to add further analysis.**
□ **I do not concur with the summary review and include a separate review.**

Office's Signatory Authority: Mary Malarkey
Director, OCBQ/CBER

□ **I concur with the summary review.**
□ **I concur with the summary review and include a separate review to add further analysis.**
□ **I do not concur with the summary review and include a separate review.**

Date of Notice of Approval to the Applicant:

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Discipline reviewed	Reviewer names
Clinical and Non-clinical/Analytical	Pawan K. Jain Krishnakumar Devadas
Product Design	Pawan K. Jain Krishna Devadas
CMC (Chemistry, Manufacturing, and Controls)	Krishnakumar Devadas Pawan K. Jain
Statistical	Paul Hshieh
Facility and GMP	Donald Ertel Lori Lawless
Bioresearch Monitoring	Dennis Cato
Labeling	Pawan Jain
Policy	Pradip Akolkar

II. Intended Use

Alere 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 but is negative for HIV-1 and HIV-2 antibodies. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test can be used in appropriate multi-test algorithms.

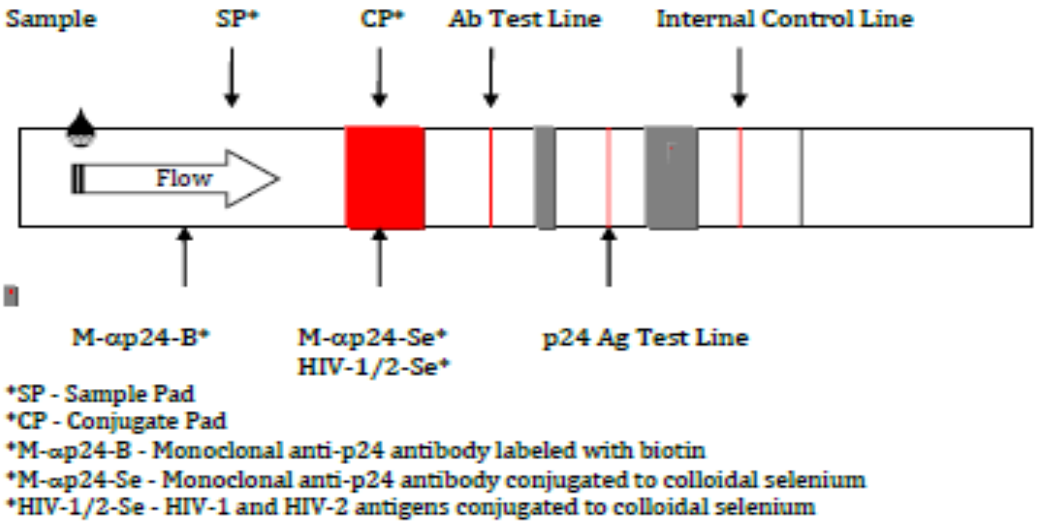
Alere Determine™ HIV–1/2 Ag/Ab Combo is not intended for newborn screening or for use with cord blood specimens or specimens from individuals less than 12 years of age.

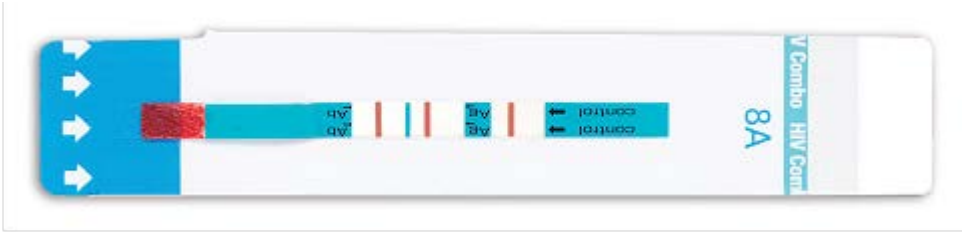
Alere Determine™ HIV–1/2 Ag/Ab Combo is not intended for use in screening blood, plasma, cell, or tissue donors.

CLIA Complexity: Moderate

III. Device Description

Alere 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 of 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 antibodies, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody in the Control Area.



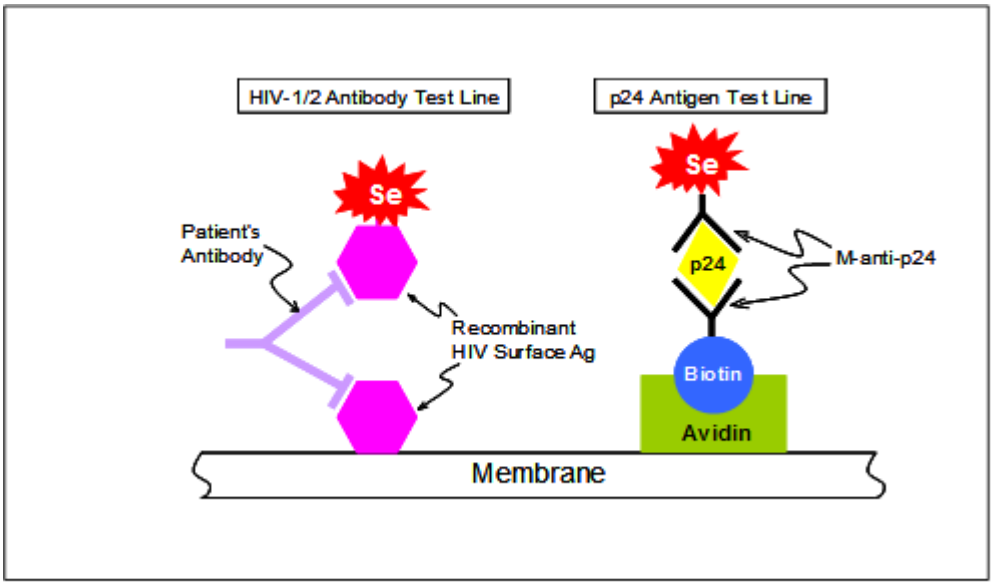


SP = Sample Pad; CP = Conjugate Pad; LTA = Lower Test Area; UTA = Upper Test Area; CA = Control Area

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 limit of detection 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 antibodies, 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 strip.

Components of Alere Determine™ HIV–1/2 Ag/Ab Combo are listed below:

Materials Provided

1. Alere 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 Guide
5. Package Insert
6. Subject Information Notices: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.
7. Customer Letter
8. Disposable Capillary Tubes: For collection and transfer of fingerstick samples.
9. Disposable Workstations: 25 in the 25 Test Units kit, and 100 in the 100 Test Units kit.

Materials Required and Available as Accessories to the Kit

- Fingerstick Sample Collection Kit
- Alere Determine™ HIV-1/2 Ag/Ab Combo Controls. 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.
  - Package Insert

Materials Required, but Not Provided

- Clock, watch, or other timing device
- Precision pipette capable of delivering 50µL 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)

IV. Quality Control of Manufacturing

Alere Determine™ HIV-1/2 Ag/Ab Combo kit components such as Test Units and Chase Buffer are manufactured at the Orgenics, Ltd. facility in Yavne, Israel. Reagents used in the manufacture of the kit components such as recombinant proteins, synthetic peptides, monoclonal antibodies, and conjugates are also manufactured by Orgenics, Ltd. or obtained from vendors certified by Alere Scarborough. Acceptance criteria and testing specifications have been established for all kit components and reagents by Alere Scarborough and approved by FDA. Every lot of the kit components and reagents must meet testing specifications to be acceptable for use in further manufacturing. Each lot of Test Units is subjected to a final performance test with a panel of samples containing low and high titers of HIV-1 p24 antigen, anti-HIV-1 antibodies, and anti-HIV-2 antibodies. Every lot of Alere Determine™ HIV-1/2 Ag/Ab Combo must meet the established performance specifications prior to release by Alere Scarborough.

V. Facility Inspection

A pre-approval inspection was conducted by FDA at the manufacturing facility of Orgenics, Ltd., in Yavne, Israel, on January 27-31, 2013. During the inspection validation processes and the manufacturing controls were inspected, the inspection was classified as No Action Indicated (NAI), and a closeout memo was completed on May 24, 2013. Reviewers in the Office of Compliance and Biologics Quality (OCBQ)/Division of Manufacturing and Product Quality (DMPQ) recommended approval of this PMA.

VI. Stability

A real-time stability study of the kit was conducted according to the protocol specifications, and data were provided at 0, 6, 9, 12, 13, 14 -(b)(4)- months at temperatures 2-8^{0}C and 27-30^{0}C. Real-time stability data indicated that the test performed according to the specifications for (b)(4) months at both 2-8°C and 27-30°C. Based on this study, expiration dating for the Alere Determine™ HIV-1/2 Ag/Ab Combo was established and approved at 14 months when stored at 2-30^{0}C.

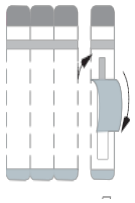
VII. Test Procedure and Interpretation of the Results

a. TEST PROCEDURE

NOTE: Alere Determine™ HIV-1/2 Ag/Ab Combo Controls should be tested prior to testing patient specimens when a new operator performs testing, a new test kit lot is to be used, a new shipment of test kits is received, 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.

Kit Component Preparation

1. 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.
2. Lay the Test Unit flat in the workstation and remove the protective foil cover from each Test Unit. The test should be initiated within 2 hours after removing the protective foil cover from each Test Unit.
   NOTE: Use of the workstation is optional. If the workstation is not used, place the Test Unit on a flat surface.

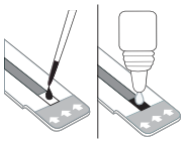


For serum or plasma samples:

1. Apply 50 µL of sample (precision 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 to the Sample Pad (marked by the arrow symbol).
2. Wait for one minute, then apply one drop of Chase Buffer to the Sample Pad.
3. Read the test result between 20 and 30 minutes after the addition of the Chase Buffer. Do not read test results after 30 minutes.

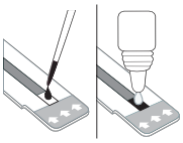


For whole blood (fingerstick) samples using the Disposable Capillary Tube:

1. Align the tip of the Capillary Tube containing the blood sample with 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.



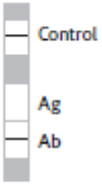
2. Wait for one minute, then apply one drop of Chase Buffer to the Sample Pad.
3. Read the test result between 20 and 30 minutes after the addition of the Chase Buffer. Do not read test results after 30 minutes.

NOTE: Discard the used pipette tips, Capillary Tubes, Test Units, and any other test materials into a biohazard waste container.

b. INTERPRETATION OF TEST RESULTS

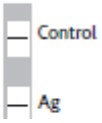
ANTIBODY REACTIVE (Two Lines – Control Line and Ab Line)

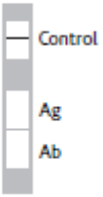
A pink/red Control line appears in the Control Area **AND** a pink/red Ab line appears in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary. Any visible pink/red color in both the Control and Lower Test Areas, regardless of intensity, is considered REACTIVE. A Reactive 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 Line and Ag Line)

A pink/red Control line appears 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.

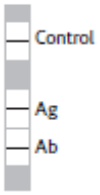




NOTE: A test result that is PRELIMINARY POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV 1 infection in the test subject. In this case the acute HIV-1 infection is distinguishe from an established HIV-1 infection in which antibodies to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

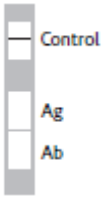
ANTIBODY REACTIVE AND ANTIGEN (HIV-1 p24)
REACTIVE (Three Lines - Control, Ab and Ag Lines)

A pink/red Control line appears in the Control Area **AND** a pink/red Ab line 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 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 for HIV-1 p24 antigen.



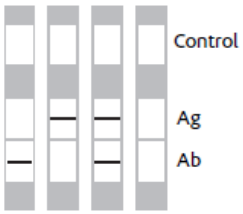
NONREACTIVE (One Line – Control Line)

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 Alere Technical Support.



VIII. Restrictions

- Sale of Alere 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.
- Alere Determine™ HIV–1/2 Ag/Ab Combo is approved for use only by an agent of a clinical laboratory.
- Test subjects must receive the "Subject Information Notice" prior to specimen collection and appropriate information when test results are provided.
- Alere 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.

IX. 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 the 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 use kit contents beyond labeled expiration date.
6. Ensure that the test subject's finger is completely dry before obtaining a fingerstick sample.
7. Ensure that a sample or Control is applied to the Sample Pad. Failure to apply a sample may give a false negative test result.
8. Read test results in a well-lit area.
9. 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.
10. A pink/red Control line does not indicate that a sample or Control had been applied, but that liquid had been applied to the strip.
11. Specimens from individuals infected with HIV-1 and/or HIV-2 who are receiving "Highly Active Antiretroviral Therapy" (HAART) may produce false negative test results.
12. Specimens from individuals with *Toxoplasma* IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides, herpes simplex virus infection, and hospitalized and cancer patients may give false positive test results.

X. Precautions

1. Safety Precautions

    a. Handle the samples, materials 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.
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^{o}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 Recommendations for Post-exposure Prophylaxis.*[7]

2. Handling Precautions

a. If Desiccant Package is missing or damaged, 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.

XI. Limitations of the Test

1. Alere Determine^{TM} 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. Alere Determine^{TM} 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 Alere 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. Alere Determine™ HIV-1/2 Ag/Ab Combo is intended as an 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 "Highly Active Antiretroviral Therapy" (HAART) may produce false negative test results.
11. Specimens from individuals with *Toxoplasma* IgG, human anti-mouse antibodies, rheumatoid factor, elevated triglycerides, herpes simplex virus infection, and hospitalized and cancer patients may give false positive test results.

XII. Alternative Practices and Procedures

Detection of antibodies against HIV epitopes and HIV-1 p24 antigen can be done in a variety of ways, including enzyme or chemiluminescent immunoassays (detection of antibodies and antigen), Western blot (detection of antibodies), and immunochromatographic assays (detection of antibodies and antigen). Immunochromatographic assays may be either lateral or transverse flow. Tests may be intended for laboratory use by laboratory professionals, at the point-of-care by healthcare professionals, or for home use by lay persons.

Most rapid HIV assays for detection of HIV antibodies are based on lateral flow technology. The signal mechanism for immunochromatographic tests may utilize enzymatic or chemiluminescent reactions or entail visualization of a metal colloid (primarily gold, although selenium has also been used). Capture antigens may be peptides, proteins, fusion peptides or viral lysate. Samples for these tests are serum, plasma, whole blood, and oral fluid. Usually a qualitative or semi-quantitative result is reported. Most tests are utilized as screening assays and require confirmation. Rapid tests may also be used as part of a diagnostic testing algorithm HIV-1 p24

antigen in clinical specimens is typically detected using laboratory-based assays that capture HIV-1 p24 antigen on an anti-HIV-1 p24 antibody-coated solid support.

XIII. Marketing History

A previous version of this device has been marketed exclusively outside of the United States.

XIV. Potential Adverse Effects of the Device on Health

Potential adverse effects of Alere Determine™ HIV-1/2 Ag/Ab Combo relate to the risk of false positive and false negative results. While performance studies indicate that this risk is likely to be very small, the potential for inaccurate results exists. The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert.

XV. Summary of Preclinical Studies:

HIV-1 p24 Analytical Sensitivity

The analytical sensitivity of the Alere Determine™ HIV-1/2 Ag/Ab Combo in serum was determined to be at least as low as 25 pg/mL using the Establissement Français du Sang HIV Ag panel by testing the greatest dilution provided. In addition, the sensitivity in serum was determined to be as low as 12.5pg/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 as low as 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 Alere 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 Alere 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 in human serum using Alere Determine™ HIV-1/2 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 Alere Determine™ HIV-1/2 Ag/Ab Combo, on the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA, and on a research use HIV-1 p24 antigen EIA with an estimated sensitivity of 15.7 pg/mL.

Detection of HIV-1 antibodies

As shown in Table 2, Alere Determine™ HIV-1/2 Ag/Ab Combo detected HIV antibodies at the same bleed as Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA in 14/28 panels (50%) and at a later bleed in 9/28 panels (32%). No antibodies were detected by either Alere Determine™ HIV-1/2 Ag/Ab Combo or Abbott HIVAB HIV-1/HIV-2 (rDNA) 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 Alere Determine HIV 1/2 Ag/Ab Combo at the same bleed as HIV-1 p24 Ag EIA assay.

Detection of HIV-1 p24 antigen

Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo did not detect antigen in multiple bleeds in 2/28 panels (7%). Alere 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%).

Detection of HIV infection by Alere Determine™ HIV-1/2 Ag/Ab Combo compared to the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA

The seroconversion panel studies showed that Alere 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 Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA in 19/28 panels (68%) and at the same bleed in 9/28 panels (32%). The earlier detection of HIV infection by the Alere Determine™ HIV-1/2 Ag/Ab Combo in all 19 panels was due to the earlier detection of antigen compared to the detection of antibody by Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA.

Table 2: Testing of Seroconversion Panels using Alere Determine™ HIV-1/2 Ag/Ab Combo, Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA, and an HIV-1 p24 Antigen EIA

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) 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	0	R	NR	RR	RR
	9	NR	R	RR	RR

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1 p24 Antigen EIA
		HIV-1 p24 Antigen	HIV-1/2 Antibody		
(L)	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

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1 p24 Antigen EIA
		HIV-1 p24 Antigen	HIV-1/2 Antibody		
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
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

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1 p24 Antigen EIA
		HIV-1 p24 Antigen	HIV-1/2 Antibody		
	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

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1 p24 Antigen EIA
		HIV-1 p24 Antigen	HIV-1/2 Antibody		
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
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

Panel	Relative Day of Bleed	Determine™ HIV-1/2 Ag/Ab Combo		Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1 p24 Antigen EIA
		HIV-1 p24 Antigen	HIV-1/2 Antibody		
	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
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 Alere Determine™ HIV-1/2 Ag/Ab Combo (two lots), and the results were compared to those from three FDA-licensed HIV antibody EIAs (Test 1 was Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA, Test 2 was Bio-Rad Genetic Systems HIV-1/HIV-2 *PLUS O* EIA, and Test 3 was Organon Teknika Vironostika HIV-1 Microelisa System), and two research use HIV-1 p24 antigen EIAs (with limits of detection of 12.5 pg/mL and 25 pg/mL).

As shown in Table 3, Alere Determine™ HIV-1/2 Ag/Ab Combo detected HIV-1 antibodies and HIV-1 p24 antigen overall (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 Alere 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 Alere 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 Alere 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 Alere 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

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	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
	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, NA = Not Available

*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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo.

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

HIV Subtype	Number of Specimens	Alere 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
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 Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo.

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

Specimen Description	Alere 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	0/20	10/10	10/10

Specimen Description	Alere 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
(CMV)			
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: 5mg/mL; Bilirubin: 0.25mg/mL; Protein: 0.05g/mL).

Reproducibility

The reproducibility of Alere Determine™ HIV-1/2 Ag/Ab Combo was evaluated at three independent sites using three lots of Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo was calculated to be 482/485 = 99.4% (95% confidence interval 98.2 to 99.9%).

XVI. Summary of Clinical Studies:

HIV-1 Sensitivity

SERUM

The sensitivity of Alere Determine™ HIV-1/2 Ag/Ab Combo to detect infection with HIV in serum specimens was evaluated by testing 903 specimens from individuals known to be infected with HIV-1. All 903 specimens were repeatedly reactive on an FDA-licensed EIA and positive on a licensed HIV-1 Western blot (WB) or licensed immunofluorescence assay (IFA). Of these, 902 specimens tested Reactive using Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo, 30 specimens tested Reactive, including all 23 WB positives, and 625 tested Nonreactive.

The sensitivity of Alere 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 6). Of these, 925 tested Reactive using Alere Determine™ HIV-1/2 Ag/Ab Combo (902 known positive and 23 high risk). Alere Determine™ HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Alere 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 6: 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	Alere 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 Alere 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 Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo, 28 specimens tested Reactive, including all 23 WB positives, and 627 tested Nonreactive.

The sensitivity of Alere 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 7). Of these, 924 tested Reactive using Alere Determine™ HIV-1/2 Ag/Ab Combo (901 known positive and 23 high risk). Alere Determine™ HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Alere 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 7: 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	Alere 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 Alere 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 Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo, 28 specimens tested Reactive, including all 23 WB positives, and 626 tested Nonreactive.

The sensitivity of Alere 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 8). Of these, 924 tested Reactive using Alere Determine™ HIV-1/2 Ag/Ab Combo (901 known positive and 23 high risk). Alere Determine™ HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Alere 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 8: 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	Alere 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 Alere 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 Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo, 24 specimens tested Reactive, including all 22 WB positives, and 630 tested Nonreactive.

The sensitivity of Alere 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 9). Of these, 929 tested Reactive using Alere Determine™ HIV-1/2 Ag/Ab Combo (907 known positive and 22 high risk). Alere Determine™ HIV-1/2 Ag/Ab Combo gave false Nonreactive results for one specimen (known positive). The estimated sensitivity of Alere 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 9: 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	Alere 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 Alere 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.

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HIV-2 Sensitivity

The sensitivity of Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo. The sensitivity of the Alere 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 % C.I. 98.5 – 100).

A total of 552 specimens from an area endemic for infection with HIV-2 were obtained from repository sources and were tested using the Bio-Rad Multispot HIV-1/HIV-2 Rapid Test and the Alere Determine™ HIV-1/2 Ag/Ab Combo. One specimen that tested invalid using the Alere Determine™ HIV-1/2 Ag/Ab Combo is not included. The Bio-Rad Multispot 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 Bio-Rad GS HIV-1/HIV-2 *Plus O* EIA and/or WB, and 246 were nonreactive. All 291 positive specimens tested Reactive using the Alere Determine™ HIV-1/2 Ag/Ab Combo (see Table 10).

Table 10: Detection of Antibodies to HIV-2 in Specimens from HIV-2 Endemic Populations*

	Bio-Rad Multispot HIV-1/HIV-2 Rapid Test		
		Reactive*	Nonreactive
Alere Determine™ HIV-1/2 Ag/Ab Combo	Reactive	291[a]	12[C]
	Nonreactive	14[b]	234

* One specimen tested invalid using the Alere Determine™ HIV-1/2 Ag/Ab Combo and 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 Bio-Rad GS HIV-1/HIV-2 *Plus O* EIA and/or WB.

[c] Seven out of these 12 specimens tested reactive for antibodies using Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 11).

Based on these studies, the specificity of Alere 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 Alere 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 11: Performance of Alere 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]	Alere 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 Alere Determine ™ HIV-1/2 Ag/Ab Combo and is not included.

PLASMA

The specificity of Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 12).

Based on these studies, the specificity of Alere 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 Alere 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 12: Performance of Alere Determine™ HIV-1/2 Ag/Ab Combo on Plasma Specimens from Individuals Presumed to be Negative for HIV Infection

Study Population	Total Samples Tested	True Negative[1]	Alere 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 Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 13).

Based on these studies, the specificity of Alere 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 Alere 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 13: Performance of Alere 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]	Alere 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 Alere 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 Alere Determine™ HIV-1/2 Ag/Ab Combo but tested negative using additional testing (see Table 14).

Based on these studies, the specificity of Alere 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 Alere 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 14: Performance of Alere 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]	Alere 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 Alere 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.

XVII. Conclusions Drawn from the Studies

Risk/Benefit Analysis

Alere Determine™ HIV-1/2 Ag/Ab Combo provides useful information to the patient and healthcare provider on the HIV status of an individual in the point-of-care setting, and can serve as an aid in the diagnosis of infection with HIV-1 or HIV-2. This test is also able to distinguish an acute HIV-1 infection from an established HIV-1 infection when the specimen is positive for HIV-1 p24 antigen but is negative for HIV-1 and HIV-2 antibodies. It has the potential, as a rapid test, to lead to diagnosis

in a short turnaround time for the test result, counseling and treatment. These features of the test are helpful to patients and public health surveillance initiatives in preventing the transmission of HIV infection.

Risks associated with a point-of-care HIV assay relate primarily to its rate of false negative and false positive results. Performance studies have demonstrated that Alere Determine™ HIV-1/2 Ag/Ab Combo has a high level of sensitivity and specificity; i.e., the rate of false Reactive or false Nonreactive results with Alere Determine™ HIV-1/2 Ag/Ab Combo is very small.

Overall, the information provided by the sponsor indicates that the benefits of Alere Determine™ HIV-1/2 Ag/Ab Combo outweigh the risks associated with its use.

Safety and Effectiveness

Trained operators conducted testing in accordance with the Package Insert of Alere Determine™ HIV-1/2 Ag/Ab Combo. Performance studies showed that the rate of false positive and false negative results is very small. The sensitivity and specificity of Alere Determine™ HIV-1/2 Ag/Ab Combo Assay for all specimen types studied (fingerstick whole blood, venous whole blood, serum, and plasma) are greater than or equal to 99% with the lower boundary of the 95% confidence interval greater than or equal to 98% for all sample types. It is expected that operator proficiency will be assured under CLIA regulations for performance of a moderate complexity test.