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|  | **LABORATORY DEPARTMENT****POLICY AND PROCEDURES** | **Department: Serology** |
| **Number:** **1159.0.Sero.gu.reg11/2013** |

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| **ALERE DETERMINE HIV-1/2 Ag/Ab Combo** |

1. **Intended Use**

Alere Determine™ HIV–1/2 Ag/Ab Combo is an *in vitro*, visually read, qualitative immunoassay for the detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) to HIV Type1 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.

**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.**

1. **Test Principle**

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 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.

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 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 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.

1. **Specimen Collection/Treatment**

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| 1. Specimen
 | **Prior to specimen collection, provide test subjects with the Subject Information Notice.**Alere DetermineTM  HIV-1/2 Ag/Ab Combo can be used for testing fingerstick whole blood, venous whole blood, serum, or plasma specimens.  |
| 1. Specimen Transport
 | If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum, and plasma specimens should be shipped refrigerated with cold packs or wet ice. |
| 1. Specimen Storage
 | Serum and plasma specimens should be stored at 2-8o if the test is to be run within 7 days of collection. If testing is delayed for then 7 days, the specimen should be frozen (-20o C or colder).* 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 should be stored at 2-8o C if the test is run within 6 days of collection. **Do not freeze whole blood specimens.** If stored at 2-8oC, 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.
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| 1. Handling Precautions
 | 1. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection.
2. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples.
3. 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.
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1. **Reagents and Equipment**
2. **MATERIALS REQUIRED AND AVAILABLE AS AN ACCESSORY TO THE 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
1. **Reagents and 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 capillaries 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)

1. **Storage and Stability**

Alere 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.

1. **Quality Control**
2. **Internal Quality Control**

To ensure assay validity, a procedural control is incorporated in the device and is labeled “Control”. Any visible line (even very faint) in the control window should be interpreted as a valid result. If the control line does not turn pink/red by assay completion, the test result is invalid and the sample should be retested. If the problem persists, contact Alere™ Technical Support. Note: 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.

1. **External Quality Control**

*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 every 30 days whichever is more frequent. Controls should be tested in the same manner as serum or plasma samples in the following Test Procedure.*

Good Laboratory Practices (GLP) necessitate testing external control material along with the test samples to ensure proper performance of the test kit. Alere Determine™ Combo HIV-1, HIV-2, p24 Reactive and Nonreactive Controls are available separately for use with Alere Determine™ HIV-1/2 Ag/Ab Combo. The HIV Controls are used to verify proper functioning of the test and the operator’s ability to properly perform the test and to interpret the results.

The HIV-1 and HIV-2 Reactive Controls will produce a REACTIVE test result and have been manufactured to produce a visible Test “Ab” line. The HIV-1 p24 Antigen Control will produce a REACTIVE test result and has been manufactured to produce a visible Test “Ag” line. The Nonreactive Control will produce a NONREACTVE Test Result. Run the Controls as per the TEST PROCEDURE for serum/plasma samples (the use of Chase Buffer is not required) and interpret results as described in INTERPRETATION OF TEST RESULTS sections of the Product Insert. It is the responsibility of each facility using Alere Determine™ HIV–1/2 Ag/Ab Combo to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use.

*Please refer to the Alere Determine™ HIV-1/2 Ag/Ab Combo Package Insert for pictorial examples of REACTIVE, NONREACTIVE and INVALID Test Results.*

1. **Precautions**
2. **Safety Precautions**
3. Handle the samples, material contacting samples, and kit controls as if capable of transmitting infection.
4. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when handling patient samples.
5. 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.
6. 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 incinerate. 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.
7. 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.
8. 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.8
9. **Handling Precautions**
	1. If Desiccant Packet is missing, DO NOT USE. Discard Test Cards (all Test Units) and use a new Test Card.
	2. Do not use any Test Units from Test Cards if the pouch has been perforated.
	3. Each test device, lancet and disposable capillary tube for collection and transfer of fingerstick samples is for single use only.
	4. Do not use kit components beyond the expiration date printed on the label. Always check expiration date prior to testing.
	5. Adequate lighting is required to read a test result.
10. **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. Open the aluminum pouch containing the Alere 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 z iplock.

 *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. 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. Set a timer for 20 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. Set a timer for 20 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:**

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. Avoid squeezing 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.*

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.*

*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.*

1. Wait for one minute, then apply one drop of Chase Buffer to the Sample Pad.
2. Set a timer for 20 minutes after the addition of 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.*

1. **Interpretation of Test Results**

**ANTIBODY REACTIVE (Two Lines - Control 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 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 PRELIMINAR 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 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 Ag, Ab 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 HIV-1 p24 antigen.

**NONREACTIVE (One Line – Control Line)**

A pink/red line appears in the Control Area of the Test Unit, and no pink/red Ab or Ag lineappears 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 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.



**6. Limitations**

1. Alere 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. Alere Determine™ HIV–1/2 Ag/Ab Combo must be used in accordance with the instructions in this 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 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 is 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.
12. **Performance Characteristics**

 The clinical performance of Alere DetermineTM HIV-1/2 Ag/Ab Combo was established in a prospective clinical study conducted at 11 clinical trial sites located in the United States from 2010 – 2011. In this study, venous whole blood, serum and plasma specimens were evaluated from individuals either known to be infected with HIV-1 as confirmed by FDA-licensed confirmatory assays and/or FDA-approved NAT assays, at low risk for HIV infection, or at high risk for HIV infection. A subset of individuals also provided capillary fingerstick samples for evaluation. Refer to the Package Insert for detailed performance characteristics.

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| **Prepared by/Title/Date:**Michael Gilliam 10/16/2013**Approved by/Title/Date:**  | **Committee Approval/Date:**Policy & Procedure \_\_\_\_\_\_ | **Dates Reviewed/Revised:**  10/2013 |