PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Determine HIV 1&2 Ag/Ab Combo Procedure

Department of Microbiology

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1.0 Clinical Significance

The Determine™ HIV 1/2 Ag/Ab Combo test is a rapid assay to be used solely at Sacred Heart for the purpose of screening antepartum patients or for testing healthcare workers and source patients following incidents of healthcare worker exposure.

2.0 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.

The assay can utilize venipuncture or capillary whole blood, serum, or plasma. Specimen is applied to the sample pad, followed by chase buffer for 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. 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.

3.0 Scope

This procedure is classified under CLIA as moderately complex. It should be carried out by technical personnel familiarized and trained to perform and interpret the assay.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. Information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure,

the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens.
- Laboratory Coat – must be worn when handling specimens and reagents.
- Biological Safety Cabinet – must be used when performing testing.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

Alere Determine™ HIV-1/2 Ag/Ab Combo must ONLY be used with whole blood in EDTA (lavender top), serum, or EDTA plasma. Using other types of samples, or testing of whole blood and plasma samples collected using a tube containing an anticoagulant other than EDTA, may not yield accurate results.

Serum and plasma specimens should be stored at 2-8°C if the testing will be performed within 7 d of collection. If testing is delayed more than 7 d, the specimen should be frozen (-20°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 10,000 x *g* 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 2-8°C if the test is to be run within 6 d 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.

6.0 Materials

6.1 Equipment

- Pipette capable of delivering 50 µL
- Timer

6.2 Consumables

- Pipette tips

6.3 Reagents

Alere Determine™ HIV-1/2 Ag/Ab Combo (Catalog Number 7D2648, 25 tests). Store kit at 2-30°C until expiration date.

1. Aluminum ziplock pouch containing Alere Determine™ HIV-1/2 Ag/Ab Combo cards. Each card consists of multiple 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. Chase Buffer: Containing sodium chloride, disodium hydrogen phosphate, and Nipasept as a preservative.
3. Quick Reference Guide
4. Disposable workstations

6.4 Control Materials

Alere Determine™ HIV–1/2 Ag/Ab Combo Controls.

1. 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.
2. 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.
3. 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.
4. 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.

7.0 Interfering Substances

Studies for potential interfering substances were performed by the manufacturer.

1. To assess the impact of unrelated medical conditions and potentially interfering substances on the specificity, 1205 specimens from a variety of medical conditions unrelated to HIV infection or containing potential interfering substances were tested. 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%).
2. To assess the impact of unrelated medical conditions and potentially interfering substances on the analytical sensitivity, all spiked samples generated reactive results, 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.

8.0 Warnings and Precautions

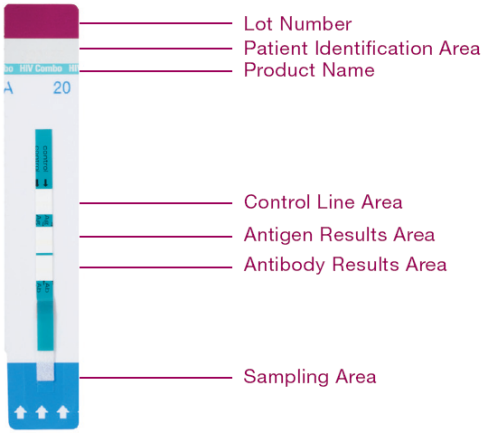
1. Do not open or remove the protective foil cover from the test unit until just prior to use (test units should be used within 2 h of removing the protective foil).
2. Do not store unused cards or test units outside the provided ziplock aluminum pouch containing desiccant for long periods. Storage outside the original pouch could expose the test units to ambient humidity and affect the test performance.
3. Make sure the ziplock is well closed after returning the unused cards or test units to the aluminum pouch.
4. Do not use kit contents beyond labeled expiration date.
5. Reading test results for serum or plasma specimens earlier than 20 min or later than 30 min after addition of the serum or plasma specimen may yield erroneous results. Reading test results for whole blood specimens earlier than 20 min or later than 30 min after addition of the Chase Buffer may yield erroneous results.

9.0 Procedure

9.1 Preparation of Kit Components

1. Open the aluminum pouch containing the Alere Determine™ HIV-1/2 Ag/Ab Combo Cards.
2. Remove the desired number of test units from the 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. 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 h after removing the protective foil cover from each test unit.



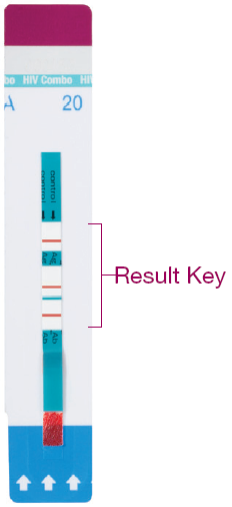
9.2 Serum or Plasma Samples

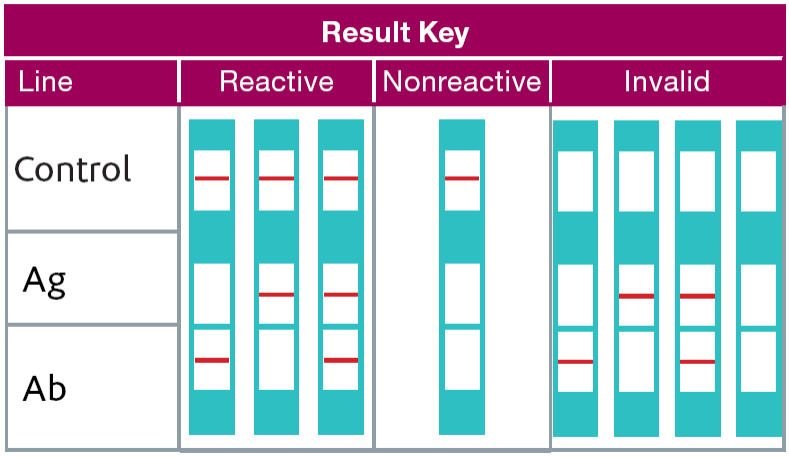
1. Using a pipette, apply 50 µL of sample to the sample pad (marked by the arrow symbol). Do not add chase buffer when using serum or plasma specimens.
2. Read the test result between 20 and 30 min after the addition of the sample. Do not read test results after 30 min.

9.3 Whole Blood Samples

1. Using a pipette, apply 50 µL of sample to the sample pad (marked by the arrow symbol).
2. Wait for 1 min, then apply one drop of chase buffer to the sample pad.
3. Read the test result between 20 and 30 min after the addition of chase buffer. Do not read test results after 30 min.

10.0 Interpretation, Reporting, & Reflexive Testing





10.1 Antibody Reactive

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 a preliminary positive for HIV-1 and/or HIV-2 antibodies. Document result on the Immunology Test log and in LIS.

Report: **Reactive for either HIV1 or HIV2 antibodies by rapid screening. Test results will be confirmed by additional testing. [R12AB]**

Notify the ordering clinician and epidemiology. A positive test result must be confirmed by the Human Immunodeficiency Virus (HIV) Combo Antigen/Antibody (HIV-1/O/2) by ELISA, with Reflex to HIV-1/HIV-2 Antibody Differentiation by Multispot test at ARUP (test code 2007980).

10.2 Antigen Reactive

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. A test result that is 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 patient. The test result is interpreted as preliminary positive for HIV-1 p24 antigen. Document result on the Immunology Test log and in LIS.

Report: **Reactive for HIV1 antigen by rapid screening. Test results will be confirmed by additional testing. [R1AG]**

Notify the ordering clinician and epidemiology. A positive test result must be confirmed by the Human Immunodeficiency Virus (HIV) Combo Antigen/Antibody (HIV-1/O/2) by ELISA, with Reflex to HIV-1/HIV-2 Antibody Differentiation by Multispot test at ARUP (test code 2007980).

10.3 Antibody & Antigen Reactive

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 HIV-1 p24 antigen. Document result on the Immunology Test log and in LIS.

Report: **Reactive for HIV1 antigen and either HIV1 or HIV2 antibodies by rapid screening. Test results will be confirmed by additional testing. [R1AG12]**

Notify the ordering clinician and epidemiology. A positive test result must be confirmed by the Human Immunodeficiency Virus (HIV) Combo Antigen/Antibody (HIV-1/O/2) by ELISA, with Reflex to HIV-1/HIV-2 Antibody Differentiation by Multispot test at ARUP (test code 2007980).

10.4 Nonreactive

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. Document result on the Immunology Test log and in LIS.

Report: **Nonreactive for HIV1 and HIV2 antibodies and HIV1 antigen by rapid screening. [NR12AB]**

10.5 Invalid

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, notify the supervisor and/or technical specialist.

If repeated testing is invalid, the specimen should be referred to PAML for testing (test code 12HIVR).

11.0 Quality Control & Quality Assurance

11.1 Internal Control

Procedural controls are included in the test. A pink/red colored line appearing in the control area is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the results area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result. If the control line does not appear, or if the background is not clear, do not report results obtained from that device. Repeat testing using a new device. If problem persists, notify supervisor and/or technical specialist. Document the internal control result on the Immunology Test Log.

11.2 External Quality Control

Positive and Negative controls must be run with each new test kit lot number or shipment and every 30 d while in use. Controls should also be run by a new operator prior to performing tests on patient specimens. The Alere Determine™ HIV-1/2 Ag/Ab Combo Controls are available separately for use with the Alere Determine™ HIV-1/2 Ag/Ab Combo assay. The HIV controls are used to verify the operator's ability to properly perform the test and to interpret the results and check the performance of each kit for reagent failure. Control material should be stored at 2-8°C and used up to the expiration date. New lots and/or shipments of kits should be checked using the same batch of control materials that were used to check the old lot. Quality control testing using external controls should be performed by following the procedure for testing patient samples. 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 nonreactive test result. Document external control results in LIS.

11.3 Quality Assurance

All test results should be documented on the Immunology Test Log and resulted in LIS. Results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Document review on the Immunology Test Log.

12.0 Limitations

1. Alere Determine™ HIV-1/2 Ag/Ab Combo must only be used with 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. This assay does not detect or has not been validated to detect HIV-2 antigen.
3. 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.
4. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antigen or antibody in the sample.
5. Reactive test results should be confirmed by additional testing using other tests.
6. A nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV.
7. 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.
8. This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 12 years of age.

9. 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.
10. 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.

13.0 Validation Information

The Determine™ HIV 1/2 Ag/Ab Combo assay was evaluated with a total of 25 samples. This included 5 commercial samples obtained from Zeptometrix and 20 previously tested patient samples. The 5 commercial samples were comprised of 4 positive (1 HIV-1 Ag, 1 HIV-1 Ab, 1 HIV-2 Ab, and 1 HIV-1 Ag/Ab) and 1 nonreactive sample. Eight of the patient samples were positive for HIV-1 Ab and 12 were negative for HIV1/2 Ab. All 8 of the Ab-positive samples had been confirmed by Western Blot. No patient samples positive for HIV-1 Ag were available from PAML or ARUP.

The Determine™ HIV 1/2 Ag/Ab Combo assay detected all commercial and patient samples positive for HIV Ab. Table 1 summarizes the results for detection of HIV Ab. Unfortunately, clinical samples containing HIV-1 Ag were unavailable for this verification study. The assay detected both commercial samples containing HIV-1 Ag. No false-positive or false-negative results were encountered during the verification.

Table 1: HIV 1/2 Ab Detection

	Negative EIA	Positive EIA/WB	Total
Negative Combo	14	0	14
Positive Combo	0	11	11
Total	14	11	25

Overall Agreement	100% (86.7 to 100%)
Sensitivity	100% (74.1 to 100%)
Specificity	100% (78.5 to 100%)
Positive Agreement	100%
Negative Agreement	100%
Predicitve Value Positive	100%
Predicitve Value Negative	100%

Precision testing is currently ongoing by daily testing of external controls. The data will be summarized and added here once it has been completed.

14.0 References

1. Package insert: Alere Determine™ HIV 1&2 Ag/Ab Combo, Rev. 04, 09/30/2013

15.0 Document Control History

Reviewed by director (AR) 12/03/2014

Reviewed by supervisor (JC) 12/03/2014

Changes and updates: