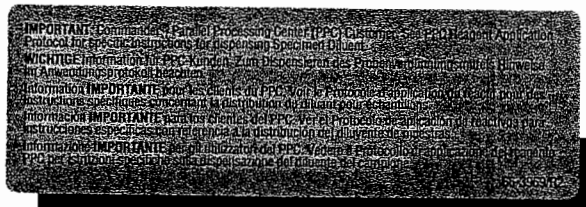
AIDS#37

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1
HIVAB™ HIV-1 EIA

NOTE CHANGES HIGHLIGHTED



NAME AND INTENDED USE

HIVAB™ HIV-1 EIA IS AN *IN VITRO* QUALITATIVE ENZYME IMMUNOASSAY FOR THE DETECTION OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) IN HUMAN SERUM OR PLASMA.

66-8805/R5

ELISA Test

ABBOTT LABORATORIES
Diagnostics Division

CUSTOMER SUPPORT CENTER (USA)
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HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ⊐HIVAB™ HIV-1 EIA

This product contains modifications to the ABBOTT HIV-1 EIA since January 16, 1987 which have improved sensitivity and includes the modification of March 27, 1989 that has made this product compatible with the COMMANDER® System. This product can be used with the Quantum™ II or Quantumatic™ without loss of sensitivity as an alternate procedure.

NAME AND INTENDED USE

⊐HIVAB HIV-1 EIA is an *in vitro* qualitative Enzyme Immunoassay for the Detection of Antibody to Human Immunodeficiency Virus Type 1 (HIV-1) in Human Serum or Plasma.

SUMMARY AND EXPLANATION OF THE TEST

Available data indicate that the acquired immunodeficiency syndrome (AIDS) is caused by a virus transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[1] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC), and from healthy persons at high risk for AIDS.[2] The incidence of antibodies specific for HIV-1 in AIDS and ARC patients and persons at increased risk for AIDS is high. The prevalence of HIV-1 infection in people not known to be at increased risk is not known. The HIV-1 antibody enzyme-linked immunoassay (EIA) (the abbreviation "ELISA" is equally acceptable) was developed to detect antibodies to HIV-1 and was developed to identify potentially infectious units of donated blood and plasma. It has been established that repeatably reactive units of blood and plasma should be eliminated from the blood supply.[3]

In order to afford maximum protection of the blood supply, the EIA was designed to be extremely sensitive. As a result, non-specific reactions may be seen in samples from some people who, for example, due to prior pregnancy, blood transfusion, or other exposure, have antibodies to the human cells or media in which the HIV-1 is grown for manufacture of the EIA.[4] Because of these and other nonspecific reactions, it is appropriate to investigate specimens found to be reactive on EIA in a manner that gives improved predictability that HIV-1 antibody, in fact, is present. When a specimen reacts in an initial test (is initially reactive), the EIA should be repeated in duplicate on the same sample source. Reactivity in either or both of these duplicate tests (repeatably reactive) is highly predictive of the presence of antibody in people at increased risk for HIV-1 infection (e.g., homosexual men, hemophiliacs, or intravenous drug users). Repeatably reactive specimens obtained from people at increased risk for HIV-1 infection are usually found to contain antibodies by additional more specific, or supplemental, testing. However, when the EIA is used to screen populations in which the prevalence of HIV-1 infection is low (e.g., blood donors), nonspecific reactions may be more common (see Table II). Information about prevalence of HIV-1 infections in persons in various categories of risk, as well as clinical and public health guidelines, are available in the publication Morbidity and Mortality Weekly Reports.

Although for all clinical and public health applications of the EIA both the degree of risk for HIV-1 infection of the person studied and the degree of reactivity of the serum may be of value in interpreting the test, these correlations are imperfect. Therefore, in most settings it is appropriate to investigate repeatably reactive specimens by additional more specific, or supplemental tests.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ⊐HIVAB HIV-1 EIA is manufactured from HIV-1 propagated in T-lymphocyte cell line H9/HTLV IIIB. The isolated virus is disrupted and inactivated with detergent and sonication. Two major viral proteins are purified from the lysate and then a combination of whole viral lysate and purified viral proteins is coated onto beads. Coated beads are incubated with a specimen diluent and human serum or plasma and appropriate controls. Any antibody to HIV-1 is bound to the HIV-1 antigens on the solid phase. After aspiration of the unbound material and washing of the bead, goat antibody to human IgG conjugated with horseradish peroxidase (Anti-Human IgG:HRPO) is incubated with the bead-antigen-antibody complex. Unbound enzyme conjugate is then aspirated and the beads washed. Next, o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the bead and, after incubation, a yellow-orange color develops in proportion to the amount of antibody to HIV-1 which is bound to the bead.

REAGENTS

No. 3A11, ⊐HIVAB HIV-1 EIA KIT (100/1000 TESTS)

① 100/1000 HIV-1 Antigen Coated Beads. (Inactivated).

② 1 Vial (20 mL)/2 Vials (100 mL each) Anti-Human Conjugate (Goat). Anti-Human IgG (Goat): Peroxidase (Horseradish). Minimum Concentration: 0.01 µg/mL in TRIS Buffer. Preservatives: 0.01% Thimerosal and 0.015% Gentamicin.

③ 1 Vial (1.3 mL)/2 Vials (1.3 mL each) Positive Control. Inactivated Human Plasma Positive for Antibody to HIV-1. Minimum titer: 1:2. Preservative: 0.1% Sodium Azide.

④ 1 Vial (1.0 mL)/2 Vials (1.0 mL each) Negative Control. Human Plasma Negative for Anti-HIV-1, Anti-HCV and HBsAg. Preservative: 0.1% Sodium Azide.

⑤ 2 Vials (20 mL each)/5 Vials (100 mL each) Specimen Diluent containing 0.1% Triton X-100, Bovine and Goat Sera (minimum concentration of 5%) and Human T-Lymphocyte Lysate (minimum titer of 1:7500). Preservative: 0.1% Sodium Azide.

*

⑨ 1 Bottle (10 Tablets)/2 Bottles (40 Tablets each) OPD (o-Phenylenediamine•2HCl) Tablets. OPD/Tablet: 12.8 mg.

⑩ 1 Bottle (55 mL)/2 Bottles (220 mL each) Diluent for OPD (o-Phenylenediamine•2HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

*There are no reagents 6, 7 and 8.

The stopping reagent is provided as an accessory to the ⊐HIVAB HIV-1 EIA Kit and consists of:

⑪ 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and some International Locations).

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, Sulfuric Acid must pass the following test each time it is prepared. The following test cannot be performed on the COMMANDER system. Use a Quantum II, Quantumatic, or suitable spectrophotometer to perform this test.

1. Pipette 300 µL of OPD Substrate Solution into 5 EIA reaction tubes or acid washed/distilled or deionized water rinsed tubes.
2. Add 2 mL of the 1 *N* Sulfuric Acid under test to each of the five tubes.
3. Measure the A_{492} of the OPD/Acid Solution against distilled or deionized water at "0 TIME" and "120 MIN".
4. Calculate the Mean Absorbance at "0 TIME" and "120 MIN".
5. To be acceptable, acid must exhibit:
   a. an A_{492} of less than 0.08 at "0 TIME" **and**
   b. a difference of less than 0.03 units in the values obtained at "0 TIME" and "120 MIN".

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

WARNING: FDA has licensed this test kit for use with human serum or plasma specimens only. Use of this licensed test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.

- Handle all ⊐HIVAB HIV-1 EIA biological materials as though capable of transmitting infection.[10] HIV-1 antigen coated on beads has been inactivated by detergent and sonication prior to coating. Positive Control has been inactivated by heat treatment.
- The Negative and Positive Controls are provided in prediluted form. They should not be diluted as specimens.

Safety Precautions

1. Do not pipette by mouth.
2. Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.
3. Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterward.
4. Avoid splashing or forming an aerosol.
5. Avoid contact of OPD and sulfuric acid with skin and mucous membranes. If these reagents come into contact with skin, wash thoroughly with water.
6. Dispose of all specimens and materials used to perform the test as if they contained infectious agents. The preferred method of disposal is autoclaving for a minimum of one hour at 121.5°C. Disposable materials may be incinerated. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite.[11] Allow 30 minutes for decontamination to be completed.

NOTE: Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.[12]

7. SPILLS: Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution.

   Iodophor Disinfectant:

   - Iodophor disinfectant should be used at a dilution providing at least 100 ppm available iodine. Hy Sine, Ioprep, Mikroklene and Wescodyne are proprietary iodophors; the latter may be used at approximately a 1:100 to 1:150 final dilution in water. Neutralization of acid-containing spills is not required.[13-15]

   Sodium Hypochlorite:

   - Non acid-containing spills should be wiped up thoroughly with a 5% sodium hypochlorite solution.
   - Acid-containing spills should be wiped dry. Spill areas should then be wiped with a 5% sodium hypochlorite solution.

   Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.

Handling Precautions

1. DO NOT MIX REAGENTS FROM DIFFERENT LOTS. Any OPD tablet, Diluent for OPD, or Sulfuric Acid lot, however, may be used with any ⊐HIVAB HIV-1 EIA kit lot.
2. Do not use kit beyond the expiration date.
3. DO NOT EXPOSE OPD REAGENTS TO STRONG LIGHT DURING INCUBATION OR STORAGE.
4. Avoid contact of the OPD Substrate Solution and 1 *N* Sulfuric Acid with any oxidizing agent. Do not allow Substrate Solution or 1 *N* Sulfuric Acid to come into contact with any metal parts. Prior to use, thoroughly rinse glassware used for OPD Solution with 1 *N* Sulfuric Acid using approximately 10% of the container volume. Follow with three washes of distilled water at the same volume.
5. Avoid microbial contamination of reagents when removing aliquots from the reagent vials. Use of disposable pipette tips is recommended.
6. USE A CLEAN DEDICATED DISPENSER FOR THE CONJUGATE SOLUTION TO AVOID NEUTRALIZATION.
7. Water for washing should be stored in clean containers to prevent contamination of water with HRPO inactivating substances.
8. If the desiccant obstructs the flow of beads, remove from bead bottle prior to dispensing beads. Replace desiccant in bottle and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

The OPD (o-Phenylenediamine•2 HCl) Tablet must be dissolved in Diluent for OPD 5 to 10 minutes prior to use. The Solution must not be stored longer than 60 minutes before use.

Five to ten minutes prior to Color Development, prepare the OPD Substrate Solution as follows:

NOTE: 300 µL of OPD Substrate Solution is required for each test specimen as well as for two reagent blanks.

CAUTION: Use pipettes and containers known to be metal free such as plastic ware or acid washed/distilled or deionized water rinsed glassware.

1. Using a clean pipette, transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer OPD Tablet(s) from bottle into container with Diluent for OPD using a nonmetallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet, and close bottle tightly. Allow tablet(s) to dissolve. Do not use a tablet that is not intact.
3. Just prior to dispensing for the final incubation of the assay, swirl gently to obtain a homogeneous solution.

OPD PREPARATION CHART

No. Tests	Tablets	Diluent
13	1	5 mL
28	2	10 mL
43	3	15 mL
58	4	20 mL
73	5	25 mL
88	6	30 mL
103	7	35 mL
118	8	40 mL
133	9	45 mL
148	10	50 mL

STORAGE INSTRUCTIONS

1. Store kit reagents at 2 to 8°C. OPD Tablets and 1 *N* Sulfuric Acid may be stored at 2 to 30°C.
2. Bring all reagents to room temperature (15 to 30°C) before use (approximately 30 minutes) and return to storage conditions indicated above immediately after use.

CAUTION: Do not open OPD tablet bottle until it is at room temperature.

3. Retain desiccant bags in OPD Tablet bottle at all times during storage.
4. Reconstituted OPD Solution MUST be stored at room temperature and MUST be used within 60 minutes. Do not expose to light.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The OPD Substrate Solution (OPD plus Diluent for OPD) should be colorless to pale yellow. A yellow-orange color of the solution indicates that the reagent has been contaminated and must be discarded.

A value of less than 0.400 absorbance units for the difference between the Positive and Negative Control Means (P-N) may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such runs should be repeated.

SPECIMEN COLLECTION AND PREPARATION

HIVAB™ HIV-1 EIA may be performed on human serum or plasma.

1. If specimens are to be stored, they may be stored as serum or plasma at 2 to 8°C for up to 5 days. However, if storage periods greater than 5 days are anticipated, the specimens should be stored frozen. Avoid multiple freeze-thaw procedures. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.
2. Either serum or plasma may be used in the test. Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. When possible, clear, nonhemolyzed specimens should be used. Specimens containing fibrin or any type of precipitate may give inconsistent test results. Such specimens should be clarified prior to assaying.

PROCEDURE

Materials Provided

No. 3A11, HIVAB HIV-1 EIA Kit, 100/1000 Tests
(See REAGENTS for complete listing.)

The list of accessories required for the COMMANDER® System is found in the COMMANDER Manual. A combination of accessories is included with the COMMANDER System. The HIVAB HIV-1 EIA is designed to be compatible with the COMMANDER System; this product may also be used with a suitable spectrophotometer, Quantum™ II or Quantumatic.™

An optimum combination of the following accessories for Quantum II and Quantumatic is provided for performance of the tests ordered.

- Reaction Trays.
- Cover Seals (Tear along perforation for use with 20 well trays).
- Assay Tubes with Identifying Cartons (for transfer of beads from reaction trays).
- 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and some International locations).

Materials Required but not Provided (for Quantum II and Quantumatic)

- Precision pipettes, EIA Pipetting Package (No. 7186), or similar equipment to deliver 200µL, 300µL and 1 mL.
- Precision pipette with disposable tip to deliver 10µL.
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Device for delivery of rinse solution such as a Gorman-Rupp™ Dispensing Pump, Heidolph Dispensing Pump (European customers only) or equivalent.
- An aspiration device for washing beads such as a Pentawash,® with a vacuum source such as a Gast® Vacuum Pump, or equivalent, and a double trap for retaining the aspirate and maintaining adequate vacuum, or use Qwikwash®.
- Water bath or equivalent capable of maintaining temperature at 39 to 41°C.
- Quantum Analyzer or spectrophotometer capable of reading absorbance at 492 nm.
- Single Bead Dispenser for dispensing one bead at a time from a 100 bead bottle.
- Multi-Bead Dispenser for dispensing twenty beads at a time from a 500 bead bottle.
- Nonmetallic forceps.
- Membrane Seal Puncture Tool for acid bottles.
- 1 *N* Sulfuric Acid.
- Predilution trays, test tubes and racks, or equivalent for predilution of samples.

*Included in EIA Pipetting Package

ADDITIONAL REAGENTS AVAILABLE

1. Abbott OPD (o-Phenylenediamine•2 HCl) Reagent No. 6172.
2. 1 *N* Sulfuric Acid, No. 7212, (Most U.S. and some International Locations).

ASSAY PROCEDURE

Preliminary Comments

Laboratories using the COMMANDER system should follow procedures in the COMMANDER Manual and the Reagent Applications Protocol as appropriate. The following assay procedure should be used with the Quantum II and Quantumatic.

DILUTION OF SPECIMEN*

1a. Dispense 10 µL of each specimen into bottom of a predilution tray, test tube or equivalent. Add 200 µL of Specimen Diluent to each tube. Mix thoroughly. **DO NOT INCLUDE CONTROLS IN THIS STEP.**

1b. Dispense 10 µL of Diluted Specimen into bottom of appropriate wells of reaction tray.

1c. Dispense 200 µL of Specimen Diluent to each well containing 10µL diluted specimen.

DILUTION OF CONTROLS*

2a. Dispense 10 µL of each control into bottom of appropriate wells of reaction tray (2 Negative and 3 Positive Controls).

2b. Dispense 200 µL of Specimen Diluent to each well containing a control.

FIRST INCUBATION

3. Carefully add one bead to each well containing a control or diluted specimen.
4. Apply cover seal. Gently tap the tray to cover beads and remove any trapped air bubbles.
5. Incubate at 40°± 1°C for 1 hour ± 5 minutes.
6. Remove and discard cover seal. If a PENTAWASH® System is used, aspirate the liquid and wash each bead with 12 to 18 mL of distilled or deionized water. If an alternate system is used, consult your washing apparatus manual for appropriate washing procedures.

SECOND INCUBATION

7. Pipette 200µL of Conjugate into each well containing a bead.
8. Apply new cover seal. Gently tap the tray to cover beads and remove any trapped air bubbles.
9. Incubate at 40° ± 1°C for 2 hours ± 10 minutes.
10. Remove and discard cover seal. Aspirate the liquid and wash each bead as in FIRST INCUBATION.

COLOR DEVELOPMENT

11. Immediately transfer beads to properly identified assay tubes.
12. Pipette 300µL of freshly prepared OPD Substrate Solution into two empty tubes (Substrate Blanks) and then into each tube containing a bead.
    NOTE: Prime dispenser immediately prior to dispensing OPD Substrate Solution.
13. Cover and incubate at room temperature for 30 ± 2 minutes.
14. Add 1 mL of 1 *N* Sulfuric Acid to each tube.

READING

15. Blank Spectrophotometer with a Substrate Blank at 492 nm.
16. Determine absorbance of Controls and Specimens at 492 nm.

*An automated procedure may be used to obtain an equivalent final dilution. Volume of diluted specimen and controls may vary from 200 to 420 µL without affecting the performance of the test.

READING RESULTS

INSTRUMENTS: 1) Laboratories using the COMMANDER System should follow procedures in the COMMANDER manual and the HIV-1 Reagent Applications Protocol as appropriate. 2) Laboratories using Quantum II or Quantumatic should read absorbances as outlined below.

Quantum II	Quantumatic
Use Mode 1.17 in Module A containing the HIVAB HIV-1 EIA protocol.	Use the following assay protocol for HIV-1 Cutoff Value = 1.000 ($NC\bar{x}$) + 0.100 ($PC\bar{x}$) + 0.000 P > N No. of Neg. Controls = 2
Line 10 of A Modules with List No. 4045-06 must be edited to read: 10: Max. Delta A = 1.999	Min. Neg. Control = 0.010 Max. Neg. Control = 0.100 Neg. Aberrant Cutoff = 50.0% No. of Pos. Control = 3 Min. Pos. Control = 0.400 Max. Pos. Control = 1.999 Pos. Aberrant Cutoff = 50.0% Min. Control Diff. = 0.400 No. of Patient Replicates = 1

*A Modules with List Nos. 4045-01 through 4045-04 may be edited to run HIVAB HIV-1 EIA by contacting your ABBOTT Representative.

When using an edited procedure, the following checks must be manually performed:

1. Individual Negative Control values should be less than or equal to 0.100 and greater than or equal to 0.010.
2. Individual Negative Control values must be within the range 0.5 to 1.5 times the Negative Control Mean.
3. Individual Positive Control values should be less than or equal to 1.999 and greater than or equal to 0.400.
4. Individual Positive Control values must be within the range of 0.5 to 1.5 times the Positive Control Mean.

Refer to CALCULATION OF RESULTS for further detail.

For Instruments other than the Quantum: Following the reading of any specimen as outlined above in steps 1 through 4, cuvettes should be thoroughly rinsed with distilled or deionized water prior to reading the next tube. For calculations see RESULTS section.

RESULTS

When a COMMANDER or Quantum Analyzer is used, refer to the appropriate Operator's Manual to determine which calculations are performed automatically. If a COMMANDER or Quantum Analyzer is not used, perform the following calculations on the assay data.

The presence or absence of antibody to HIV-1 is determined by relating the absorbance of the specimen to the Cutoff Value. The Cutoff Value is the absorbance of the Negative Control Mean plus 0.1 times the Positive Control Mean. For the run to be valid, the difference between the Means of the Positive and Negative Controls (P - N) should be 0.400 or greater. If not, technique may be suspect and the run must be repeated. If the P - N Value is consistently low, deterioration of reagents may be suspect.

Calculation of Results

1. Calculation of Negative Control Mean Absorbance ($NC\bar{x}$).
   Determine the Mean of the Negative Control Values.

   Example:

Negative Control Sample No.	Absorbance
1	0.060
2	0.056
TOTAL	0.116

$$\frac{\text{Total Absorbance}}{2} = \frac{0.116}{2} = 0.058\ (NC\bar{x})$$

Individual Negative Control Values must be less than or equal to 0.100 and greater than or equal to 0.010.

Individual Negative Control Values must be within the range 0.5 to 1.5 times the Negative Control Mean. If one value is outside this range, the test must be repeated. If more than an occasional value falls outside this range, technique problems should be investigated.

2. Calculation of Positive Control Mean Absorbance ($\overline{PC}$).
Determine the Mean of the Positive Control Values.

Example:

Positive Control Sample No.	Absorbance
1	0.835
2	0.925
3	1.015
TOTAL	2.775

$$\frac{\text{Total Absorbance}}{3} = \frac{2.775}{3} = 0.925\ (\overline{PC})$$

Individual Positive Control Values must be less than or equal to 1.999 and greater than or equal to 0.400.

Individual Positive Control Values must be within the range 0.5 to 1.5 times the Positive Control Mean. If one value is outside the acceptable range, discard this value and recalculate the Mean. If two values are outside the range, the test should be repeated.

3. Calculation of the Cutoff Value
Cutoff Value = $\overline{NC} + (0.1 \times \overline{PC})$
Example:
$\overline{NC} = 0.058$
$\overline{PC} = 0.925$
Cutoff Value = $0.058 + (0.1 \times 0.925) = 0.151$

4. Calculations for Determining P - N
Example:
$\overline{NC} = 0.058$
$\overline{PC} = 0.925$
P - N = (0.925 - 0.058) = 0.867
For the run to be valid, the P - N Value should be 0.400 or greater. If not, technique or deterioration of reagents may be suspect and the run should be repeated.

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the cutoff value are considered not reactive by the criteria of ⊐HIVAB™ HIV-1 EIA and may be considered negative for the antibody. Further testing is not required.
2. Specimens with absorbance values equal to or greater than the cutoff value are considered reactive (initially reactive) by the criteria of ⊐HIVAB HIV-1 EIA, but before interpretation, the original sample source should be retested in duplicate. If either duplicate retest is reactive, the specimen is considered repeatably reactive.
3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV-1.
4. If the specimen is repeatably reactive, the probability that antibodies to HIV-1 are present is high, especially in specimens obtained from subjects at increased risk for HIV-1 infection or in specimens with very high absorbance values.[5] In most settings it is appropriate to investigate repeatably reactive specimens by additional more specific, or supplemental tests. Specimens found repeatably reactive by EIA and positive by additional more specific, or supplemental testing are considered positive for antibodies to HIV-1. The interpretation of results of specimens found repeatably reactive by EIA and negative on additional more specific testing is unclear; further clarification may be obtained by testing another specimen taken three to six months later.

LIMITATIONS OF THE PROCEDURE

The ⊐HIVAB HIV-1 EIA antibody procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to HIV-1 in plasma or serum from individual subjects. Because the EIA was designed to test individual units of blood or plasma, most data regarding its interpretation were derived from testing individual samples. Insufficient data are available to interpret tests performed on other body specimens, pooled blood or processed plasma, and products made from such pools; testing of these specimens is not recommended.

⊐HIVAB HIV-1 EIA detects antibodies to HIV-1 in blood and thus is useful in screening blood and plasma donated for transfusion and further manufacture, in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-1. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV-1.[6] For most uses it is recommended that repeatably reactive specimens be investigated by an additional more specific, or supplemental test. A person who has antibodies to HIV-1 is presumed to be infected with the virus, except that 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. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate. Such an evaluation should be considered an important part of HIV-1 antibody testing and should include test result confirmation on a freshly drawn sample.

AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.[7] EIA testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1 is present. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1. The risk of an asymptomatic person with a repeatably reactive serum sample developing AIDS or an AIDS-related condition is not known.[8]

Data obtained from testing persons both at increased and at low risk for HIV-1 infection suggest that repeatably reactive specimens with high absorbance on EIA are more likely to demonstrate the presence of the HIV-1 antibodies by additional more specific, or supplemental testing.[9] Reactivity at or only slightly above the cut-off value is more frequently nonspecific, especially in samples obtained from persons at low risk for HIV-1 infection; however, the presence of antibodies in some of these specimens can be demonstrated by additional more specific, or supplemental testing.

EXPECTED RESULTS

A. SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Assay reproducibility was determined by assaying five specimens in replicates of three on four consecutive runs over a period of four days using the same lot of material at three sites.

The Intra-assay and Inter-assay Standard Deviation (SD) and Coefficient of Variation (%CV) were calculated (Table I). Mean S/CO is defined as the Mean Sample Absorbance divided by the Cutoff. S/CO ratios greater than or equal to 1.00 are Reactive, and S/CO ratios less than 1.00 are Negative. Mean absorbance is defined as the Mean of all controls tested.

TABLE I
⊐HIVAB HIV-1 EIA Reproducibility

Specimen	Mean S/CO	Intra-assay SD	Intra-assay % CV	Inter-assay SD	Inter-assay % CV
1	0.52	0.060	11.6	0.075	14.4
2	1.81	0.150	8.3	0.185	10.2
3	3.43	0.256	7.5	0.289	8.4
4	5.80	0.459	7.9	0.617	10.6
5	9.79	0.802	8.2	0.863	8.8

Control	Mean Absorbance	Intra-assay SD	Intra-assay % CV	Inter-assay SD	Inter-assay % CV
Negative	0.037	0.0080	21.6	0.0080	21.6
Positive	1.250	0.0643	5.1	0.1315	10.5

Sensitivity and Specificity

At present there is no recognized standard for establishing the presence and absence of HIV-1 antibody in human blood. Therefore sensitivity was computed based on the clinical diagnosis of AIDS and specificity based on random donors.

All data in this package insert were obtained with the COMMANDER® System.

The ABBOTT studies show that:

1. Sensitivity based on an assumed 100% prevalence of HIV-1 antibody in AIDS patients is estimated to be 100% (144 patients tested).
2. Specificity based on an assumed zero prevalence of HIV-1 antibody in random donors is estimated to be 99.9%* (4777 random donors tested).

*In these calculations, three samples of the eight total repeatably reactive specimens that confirmed have been excluded.

B. REACTIVITY IN RANDOM DONOR POPULATIONS

The ability of ⊐HIVAB HIV-1 EIA to detect antibody to HIV-1 in blood bank donor specimens is shown in Table II. The data include 4777 serum and plasma samples obtained from blood donors at three geographically distinct blood banks. The number of specimens found repeatably reactive for antibody to HIV-1 by ⊐HIVAB HIV-1 EIA with testing performed in the automated mode using the COMMANDER System was 0.17%. The percent of repeatably reactive specimens obtained from blood donors at three geographically distinct blood and plasma centers was 0.00 to 0.30%.

TABLE II
Detection of Antibody to HIV-1 in Plasma (P) Samples (Sites 1 and 3) and Serum (S) Samples (Sites 2 and 3) from Blood and Plasma Donors.

Site	Number Tested	⊐HIVAB HIV-1 EIA Nonreactive	Initially Reactive	Repeatably Reactive
1(P)	1048	1047 (99.90%)	1 (0.10%)	0 (0.00%)
2(S)	993	990 (99.70%)	3 (0.30%)	3* (0.30%)
3(P)	1679	1674 (99.70%)	5 (0.30%)	5** (0.30%)
3(S)	1057	1055 (99.81%)	2 (0.19%)	0 (0.00%)
Total	**4777**	**4766 (99.77%)**	**11 (0.23%)**	**8 (0.17%)**

*In these studies, the three repeatably reactive samples were indeterminate on the FDA licensed western blot.

**In these studies, three of five repeatably reactive samples were positive on the FDA licensed western blot. Two of five repeatably reactive samples were indeterminate on the FDA licensed western blot.

C. REACTIVITY IN PATIENT POPULATIONS

The reactivity of ⊐HIVAB HIV-1 EIA was determined by testing serum and plasma specimens from patients diagnosed as having AIDS, AIDS-related complex (including patients with lymphadenopathy and idiopathic thrombocytopenia purpura) and high risk groups. (See Table III).

TABLE III
Detection of Antibody to HIV-1 in Serum and Plasma Samples from Patients with AIDS, AIDS-Related Complex (ARC) and High Risk Groups

Group	No. of Specimens Tested	⊐HIVAB HIV-1 EIA COMMANDER System No. Reactive	⊐HIVAB HIV-1 EIA COMMANDER System (% Reactive)
AIDS	144	144	(100%)
ARC	84	84	(100%)
*High Risk Groups	150	150	(100%)

*Previously tested and found repeatably reactive by ⊐HIVAB HIV-1 EIA (List 3036 and 2A95) and positive by the FDA licensed western blot.

A total of 50 samples from patients with medical conditions unrelated to AIDS were tested at two centers. None of the samples was reactive. Medical conditions including 25 positive RPR patients, 9 with other viral infections, 1 with toxoplasmosis, 3 with systemic lupus erythematosus, 3 with rheumatoid arthritis, 7 with hypergammaglobulinemia, and 2 with autoimmune conditions.

D. REACTIVITY OF SEROCONVERSION DONORS

Evidence for improved sensitivity of the current kit (List 3A11) was obtained from studies of seroconversion sera tested at Abbott Laboratories (See Table IV). Ten seroconversion series, comprising 56 samples, were obtained from donors who were plasmapheresed regularly. No risk factors are known. All the samples tested by FDA licensed western blot were negative or indeterminate. None of the eight series had a band at p31; however all series showed other bands characteristic of an HIV-1 infection. These donors (with the exception of 72593, C1066 and H831) have been described previously.[16] Each sample was tested in triplicate in two separate assays on two separate days. The current kit (List 3A11) run on the COMMANDER System detected the presence of HIV-1 antibody earlier than the previously FDA licensed ABBOTT HIV-1 EIA (List 2A95).

TABLE IV
Performance on Seroconversion Sera of the Current ☐HIVAB™ HIV-1 EIA (List 3A11) Compared with the ABBOTT HIV-1 EIA Kit (List 2A95) Prior to Modification and the FDA licensed western blot

Donor/(No.)		Days of Donation	Replicates Reactive* HIV-1 EIA (List 2A95) COMMANDER	HIV-1 EIA (List 3A11) COMMANDER	Bands Found on FDA Licensed Western Blot
3988	(1)	1	0/3	0/3	None
	(2)	3	0/3	0/3	None
	(3)	8	0/3	0/3	24
	(4)	10	0/3	0/3	24
	(5)	17	3/3	3/3	24,41,160
	(6)	20	3/3	3/3	24,41,160
6108	(1)	1	0/3	3/3	24
	(2)	5	0/3	3/3	24,55
	(3)	9	1/3	3/3	17,24,55
	(4)	12	1/3	3/3	17,24,41,55
	(5)	16	3/3	3/3	17,24,41,55 120,160
	(6)	19	3/3	3/3	17,24,41,55,66 120,160
	(7)	23	3/3	3/3	17,24,41,51,55 66,120,160
	(8)	26	3/3	3/3	17,24,41,51,55, 66,120,160
6977	(1)	1	3/3	3/3	24
	(2)	6	3/3	3/3	24
	(3)	10	3/3	3/3	24,41,160
	(4)	16	3/3	3/3	24,41,120,160
	(5)	20	3/3	3/3	24,41,120,160
13078	(1)	1	0/3	0/3	None
	(2)	6	0/3	0/3	None
	(3)	8	0/3	2/3	None
	(4)	14	3/3	3/3	24
	(5)	17	3/3	3/3	24,41,55
	(6)	21	3/3	3/3	24,41,55
	(7)	24	3/3	3/3	24,41,55
46320	(1)	5	3/3	3/3	17,24,51,66 120,160
	(2)	9	3/3	3/3	17,24,51,66 120,160
46402	(1)	1	0/3	2/3	24
	(2)	8	0/3	3/3	24,160
	(3)	10	3/3	3/3	24,160
	(4)	17	3/3	3/3	24,51,66,160
	(5)	22	3/3	3/3	24,51,66,160
103524	(1)	1	1/3	3/3	24,41,66
	(2)	5	3/3	3/3	24,41,51,55,66
	(3)	8	3/3	3/3	24,41,51,55,66
72593	(1)	1	3/3	3/3	24
	(2)	3	1/3	3/3	24
	(3)	10	3/3	3/3	24,120,160
	(4)	16	3/3	3/3	24,120,160
	(5)	22	3/3	3/3	24,120,160
	(6)	24	3/3	3/3	24,120,160
C1066	(1)	1	0/3	0/3	None
	(2)	5	0/3	0/3	None
	(3)	16	1/3	3/3	24,120,160
	(4)	21	2/3	3/3	24,120,160
	(5)	23	3/3	3/3	24,120,160
	(6)	28	3/3	3/3	24,120,160
	(7)	30	3/3	3/3	24,120,160
H831	(1)	1	0/3	0/3	None
	(2)	4	0/3	0/3	None
	(3)	15	0/3	0/3	None
	(4)	22	0/3	0/3	None
	(5)	52	0/3	2/3	24,55,120
	(6)	56	0/3	3/3	24,55,120
	(7)	60	0/3	3/3	24,55,120
Overall Percent Detected of 56 Samples			58	76	

*A total of 3 replicates per sample were tested, table entries represent number of replicates reactive/number of replicates tested.