For Informational Use ONLY. Not to be used for performing assay. Refer to most current package insert accompanying kit.

HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2: (*E. COLI, B. MEGATERIUM*, RECOMBINANT ANTIGEN) HIVAB™ HIV-1/HIV-2 (rDNA) EIA

NOTE CHANGES HIGHLIGHTED

NAME AND INTENDED USE

HIVAB HIV-1/HIV-2 (rDNA) EIA IS AN *IN VITRO* ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUSES TYPE 1 AND/OR TYPE 2 (HIV-1/HIV-2) IN HUMAN SERUM, PLASMA, OR CADAVERIC SERUM.

34-3284/R16

WARNING: A SOFTWARE UPGRADE AND/OR PROTOCOL EDITS MAY BE REQUIRED PRIOR TO IMPLEMENTING THIS ASSAY. PLEASE CONTACT YOUR LOCAL CUSTOMER SUPPORT CENTER.

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Abbott Park, IL 60064

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NAME AND INTENDED USE

HIVAB HIV-1/HIV-2 (rDNA) EIA is an *in vitro* enzyme immunoassay for the qualitative detection of antibodies to human immunodeficiency viruses type 1 and/or type 2 (HIV-1/HIV-2) in human serum, plasma, or cadaveric serum.

SUMMARY AND EXPLANATION OF THE TEST

Epidemiologic data suggest that the Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two types of human immunodeficiency viruses, collectively designated HIV. Human immunodeficiency virus type 1 (HIV-1), the first discovered AIDS virus, has been isolated from patients with AIDS and AIDS-related complex (ARC), and from healthy persons at high risk for AIDS.[1,2,3] HIV-1 is transmitted by sexual contact, by exposure to blood or certain blood products, or from an infected mother to her fetus or child.[4] The prevalence of HIV-1 antibodies in AIDS and ARC patients and persons at risk is high[5] and the virus can be isolated from nearly 90% of all seropositive individuals.[6]

In 1986 a second HIV virus, HIV-2, was isolated from patients with AIDS in West Africa.[7] HIV-2 infections have also been identified in:

1. Europeans who have lived in West Africa or have had sexual relations with individuals from this region.[8,9,10]
2. Homosexual men with sexual partners from endemic areas.[11,12]
3. 31 cases of HIV-2 infection have been reported in the United States.[13,14,15,16]
4. One patient attending a sexually transmitted disease clinic in London.[17]

Retrospectively diagnosed cases of AIDS due to HIV-2 infection acquired in the late 1960's have been reported in France[18] and the UK[19] and the first examples of transfusion associated HIV-2 infection have been documented recently.[20] Today HIV-2 seems to be endemic only in West Africa. However, based on experience with HIV-1, it is likely that HIV-2 will spread to other parts of the world.

The HIV-2 virus is similar to the HIV-1 virus in its morphology, cell tropism, interaction with the CD_4 cellular receptor, *in vitro* cytopathic effect on CD_4 cells, overall genomic structure and its ability to cause AIDS.[21]

However, HIV-2 differs from HIV-1 as indicated by the following results:

1. HIV-1 DNA probes constructed with the entire genome fail to hybridize with HIV-2 RNA under medium or high stringency conditions.[21]
2. There is less than 60% amino acid identity for the *gag and pol* gene products and only 37% for *env* encoded proteins.[21]
3. HIV-1 antibody containing sera have no neutralizing effect on HIV-2 isolates contrasting with the cross-neutralization activity of HIV-2 antisera on HIV-1 strains.[22]

Serologic studies indicate that HIV-1 and HIV-2 share multiple common epitopes in their core antigens but the envelope glycoproteins are much less cross reactive.[21] This limited cross reactivity of the envelope antigens could explain the failure of currently used serologic assays for HIV-1 to react with certain sera from individuals with antibody to HIV-2.[23]

Use of recombinant DNA derived antigens corresponding to three viral proteins, HIV-1 core and envelope and HIV-2 envelope, allows for the detection of anti-HIV-1 and/or anti-HIV-2 positive specimens.

The HIVAB HIV-1/HIV-2 (rDNA) EIA was developed to allow the simultaneous detection of IgG and IgM antibodies to HIV-1 and HIV-2 and to identify potentially infectious units of donated blood and plasma. It has been established that repeatedly reactive units of blood and plasma should be eliminated from the blood supply.[24]

In order to provide maximum protection of the blood supply, enzyme immunoassays were designed to be extremely sensitive. As a result, EIAs based on whole virus or virus lysate were sometimes found to yield non-specific reactions with specimens from individuals with autoimmune diseases, history of multiple pregnancies, anti-HLA, EBV infections or hypergammaglobulinemia due to antibodies reactive with human cell proteins present in the antigen preparations. This problem is largely avoided in the HIVAB HIV-1/HIV-2 (rDNA) EIA by the use of proteins which correspond to viral proteins derived by bacterial recombinant DNA technology.

A specimen found to be initially reactive by HIVAB HIV-1/HIV-2 (rDNA) EIA should be retested in duplicate using a sample from the original source. Reactivity in either or both of these duplicate tests (i.e., repeatedly reactive), is highly predictive of the presence of HIV-1 and/or HIV-2 antibodies in people at increased risk for HIV infection. However, because of possible non-specific reactions due to other causes, particularly when testing low prevalence populations (e.g. blood donors), it is appropriate to further investigate specimens found to be repeatedly reactive in the HIVAB HIV-1/HIV-2 (rDNA) EIA to prove that HIV antibodies are indeed present. Repeatedly reactive specimens obtained from people at increased risk for HIV infection are usually found to contain antibodies by supplemental tests (WB, IFA, RIPA).

HIVAB HIV-1/HIV-2 (rDNA) EIA does not discriminate between HIV-1 and HIV-2 reactivity. An HIV-1, HIV-2, or dual infection can only be confirmed serologically by parallel testing against both antigens using specific immunoassays,[25] immunoblotting, immunoprecipitation assays or a combination thereof. Certain specimens may require HIV-1/HIV-2 DNA probe testing or culture to ensure discrimination. A full differential diagnostic work-up for the diagnosis of AIDS and AIDS-related conditions necessarily includes an examination of the patient's immune status and a clinical history. Recommendation for appropriate use of such additional tests may be issued periodically by the U.S. Public Health Service.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

In the HIVAB HIV-1/HIV-2 (rDNA) EIA, human serum, plasma, or cadaveric serum is diluted in a specimen diluent and incubated with a polystyrene bead coated with Recombinant HIV-1 *env* and *gag* and HIV-2 *env* proteins. Specific antibody present in the sample reacts with the antigens on the coated bead. After removal of the unbound materials and washing of the bead, specific immunoglobulins remaining bound to the solid phase are detected by incubating the bead-antigen-antibody complex with a solution containing HIV-1 *gag* and *env* together with HIV-2 *env* Recombinant proteins labeled with horseradish peroxidase (HRPO).

Unbound enzyme conjugate is then removed and the beads are washed. Next, o-Phenylenediamine (OPD) solution containing hydrogen peroxide is then added to the bead and, after incubation, a yellow-orange color develops in proportion to the amount of anti-HIV-1 and/or anti-HIV-2 which is bound to the bead. The enzyme reaction is stopped by the addition of 1 *N* Sulfuric Acid and the intensity of color developed is read using a spectrophotometer.

REAGENTS

HIVAB HIV-1/HIV-2 (rDNA) EIA, 100/1000/5000 Tests

① 1 Vial (100)/2 Vials (500 each)/10 Vials (500 each) HIV-1/HIV-2 (*E. coli, B. megaterium*, Recombinant) Antigen Coated Beads.

② 2 Vials (0.65 mL each)/3 Vials (5 mL each)/15 Vials (5 mL each) HIV-1/HIV-2 (*E. coli*, Recombinant) 20X Antigen: Peroxidase (Horseradish) Conjugate Concentrate. Minimum concentration: 0.01 µg/mL in TRIS Buffer with red dye. Preservatives: Quinolone (Serafloxin HCl) 0.0005%, Nipasept (Sodium Alkyl Paraben) 0.1%, Gentamycin Sulfate 0.01%.

③ 2 Vials (12.35 mL each)/3 Vials (95 mL each)/15 Vials (95 mL each) Conjugate Diluent. Contains 20% Bovine Serum. Preservatives: Quinolone (Serafloxin HCl) 0.0005%, Nipasept (Sodium Alkyl Paraben) 0.1%, Gentamycin Sulfate 0.01%.

④ 1 Vial (3 mL)/3 Vials (3 mL each)/15 Vials (3 mL each) HIV-1 Positive Control (Human). Inactivated recalcified plasma reactive for anti-HIV-1, reactive or nonreactive for HIV-1 NAT or nonreactive for HIV-1 Ag, and nonreactive for HBsAg and anti-HCV. Minimum Titer 1:2. Preservative: 0.1% Sodium Azide.

⑤ 1 Vial (4 mL)/3 Vials (4 mL each)/15 Vials (4 mL each) Negative Control (Human). Recalcified plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV, anti-HIV-1/HIV-2, and anti-HBs. Preservative: 0.1% Sodium Azide.

⑥ 1 Vial (15 mL)/1 Vial (90 mL)/5 Vials (90 mL each) Specimen Diluent. Contains 2.5% Tween-20. (Concentration of *E. coli* lysate 5-10%.) Preservatives: Quinolone (Serafloxin HCl) 0.0005%, Nipasept (Sodium Alkyl Paraben) 0.1%.

⑦ 1 Vial (3 mL)/3 Vials (3 mL each)/15 Vials (3 mL each) HIV-2 Positive Control (Mouse Monoclonal). Contains 10% Bovine Serum. Preservatives: Quinolone (Serafloxin HCl) 0.0005%, Nipasept (Sodium Alkyl Paraben) 0.1%.

*

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

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

*There is no component 8.

The Stopping Reagent may be provided as an accessory to the HIVAB HIV-1/HIV-2 (rDNA) EIA and consists of:

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

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, 1 *N* 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, or suitable spectrophotometer to perform this test.

1. Pipette 300 µL OPD Substrate Solution into 5 EIA reaction tubes or acid-washed/distilled or deionized water rinsed tubes.
2. Add 1 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, the acid solution must exhibit:
   a. an A_{492} of less than 0.040 at "0 TIME" *and*
   b. a difference of less than 0.030 units in the values obtained at "0 TIME" and "120 MIN".

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

Safety Precautions

CAUTION: This product contains human sourced and/or potentially infectious components. Some components sourced from human blood have been tested and found to be reactive for anti-HIV-1 by FDA licensed tests. Refer to **REAGENTS** section for details. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[26] Biosafety Level 2[27] or other appropriate biosafety practices[28,29] should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
4. Clean and disinfect all spills of specimens or reagents using a tuberculocidal disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.[30,31]
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.[32,33,34]

WARNING: FDA has licensed this test kit for use with human serum, plasma, or cadaveric serum 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.

1. Avoid contact of OPD and Sulfuric Acid with skin and mucous membranes. If these reagents come into contact with skin, wash thoroughly with cool water.
2. Some components of this product contain Sodium Azide as a preservative. For a specific listing, refer to the **REAGENTS** section of this package insert. Sodium Azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of solutions containing Sodium Azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health (USA) recommends the following: (1) siphon liquid from drain trap using a rubber or plastic hose, (2) fill with 10% Sodium Hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

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Some components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R22	Harmful if swallowed.
R32	Contact with acids liberates very toxic gas.
S35	This material and its container must be disposed of in a safe way.
S36	Wear suitable protective clothing.
S46	If swallowed, seek medical advice immediately and show this container or label.

The OPD tablets listed in the **REAGENTS** section of this package insert contain o-Phenylenediamine • 2 HCl and are classified per applicable European Community (EC) Directives as: Harmful (Xn) and Dangerous for the environment (N). The following are the appropriate Risk (R) and Safety (S) phrases.

R22	Harmful if swallowed.
R36	Irritating to eyes.
R40	Limited evidence of a carcinogenic effect.
R43	May cause sensitization by skin contact.
R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
R68	Possible risks of irreversible effects.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35	This material and its container must be disposed of in a safe way.
S36/37	Wear suitable protective clothing and gloves.
S46	If swallowed, seek medical advice immediately and show this container or label.
S61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Handling Precautions

1. Do not use kit beyond the expiration date.
2. Do not mix reagents from different lots.

   NOTE: Any OPD reagent lot or 1 *N* Sulfuric Acid lot may be used with any ABBOTT EIA kit.
3. Avoid microbial contamination of reagents when removing aliquots from the reagent vials. Use of disposable pipette tips is recommended.
4. Do not expose OPD reagents to strong light during storage or incubation.
5. Avoid contact of the OPD Substrate Solution or 1 *N* Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution to come in contact with any metal parts. Prior to use, rinse glassware to be used for OPD Substrate Solution thoroughly with 1 *N* Acid (sulfuric or hydrochloric) using approximately 10% of the container volume followed by three washes of distilled water of the same volume.
6. 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.
7. **Use a clean dedicated dispenser for the diluted conjugate to avoid contamination.**
8. The Negative and HIV-1 Positive Controls, and the HIV-2 Positive Control, as provided, should be treated the same way as specimens.

INSTRUCTION FOR PREPARATION OF DILUTED CONJUGATE

1. Conjugate Concentrate and Conjugate Diluent should be brought to room temperature before mixing.
2. Carefully empty the contents of a Conjugate Concentrate vial into a vial of Conjugate Diluent. This can be done most efficiently by slowly squeezing the small vial 2 to 3 times while maintaining the nozzle within the opening of the large vial. Avoid foaming. *Write the date of dilution in the space provided on the Conjugate Diluent label.*
3. Reseal the large vial. Mix thoroughly by slowly inverting the vial several times. Do not vortex.
4. Allow diluted conjugate to equilibrate at room temperature for approximately 30 minutes before use.
5. One vial of diluted conjugate is sufficient for up to 100 tests (100 Test Kit) or up to 500 tests (1000 and 5000 Test Kit). Conjugate is stable for 21 days (though not exceeding kit expiration date) after dilution when stored at 2 to 8°C. Bring to room temperature before using.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (15 to 30°C).

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

At least 5 minutes, but not more than 60 minutes prior to Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine • 2 HCl) tablet(s) in Diluent for OPD. **DO NOT USE A TABLET THAT IS NOT INTACT.**

Using clean pipettes and metal-free containers (such as plastic ware or acid-washed and distilled water-rinsed glassware) follow the procedure below:

1. Transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of 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 cap or stopper the OPD Substrate Solution bottle while the tablets are dissolving. THE OPD SUBSTRATE SOLUTION MUST BE DISPENSED WITHIN 60 MINUTES OF PREPARATION AND MUST NOT BE EXPOSED TO STRONG LIGHT.
3. Just prior to dispensing for Color Development, swirl container gently to obtain a homogeneous solution, remove air bubbles from tubing, and prime dispenser prior to use.

OPD PREPARATION CHART

No. of 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

NOTE: 300 µL of OPD Substrate Solution is required for each Control, and specimen as well as for each substrate blank. **Laboratories using the COMMANDER Parallel Processing Center (PPC™) will require approximately an additional 3 mL of OPD Substrate Solution for instrument priming.**

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) for use and return them 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. The OPD Substrate Solution **MUST** be stored at room temperature and **MUST** be dispensed within 60 minutes. Do not expose to strong light.
5. Replace desiccant in bead bottle, and cap bottle for storage.

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 indicates that the reagent has been contaminated and must be discarded.

An absorbance value of less than 0.500 for either HIV-1 Positive Control replicate and/or an absorbance value of less than 0.350 for either HIV-2 Positive Control replicate may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such runs must be repeated.

SPECIMEN COLLECTION AND PREPARATION

HIVAB HIV-1/HIV-2 (rDNA) EIA may be performed on either human serum, plasma, or cadaveric serum.

1. If serum or plasma specimens are to be stored, they may be stored at 2 to 8°C for a maximum of 14 days. For long term storage, the specimens should be stored frozen. Samples have been tested after three freeze/thaw cycles and no performance difference was seen.
2. If cadaveric serum specimens are to be stored, they may be stored at 2 to 8°C for a maximum of five days. For long term storage, the specimens should be stored frozen. Samples have been tested after three freeze/thaw cycles and no performance difference was seen.
3. If serum, plasma, or cadaveric serum specimens are to be shipped, they should be packaged and labeled in compliance with federal and international regulations covering the transportation of clinical specimens and etiologic agents.
4. The HIVAB HIV-1/HIV-2 (rDNA) EIA can be used with serum (including serum collected in serum separator tubes), plasma containing heparin, EDTA, citrate and CPDA-1, or cadaveric serum.
5. Clear, nonhemolyzed serum, plasma, and cadaveric serum specimens should be used. Separate serum, plasma, and cadaveric serum from the clot or red cells as soon as possible to avoid hemolysis.
6. For cadaveric serum specimens, no performance differences were observed in ten negative and ten low-level reactive specimens both tested with or without elevated levels of bilirubin (≤ 20 mg/dL) or triglycerides (≤ 3000 mg/dL).
7. Serum, plasma, or cadaveric serum specimens containing precipitates may give inconsistent test results. Such specimens should be clarified prior to testing.
8. Do not use heat-inactivated serum, plasma, or cadaveric serum specimens.
9. Serum, plasma, or cadaveric serum specimens with obvious microbial contamination should not be tested.

PROCEDURE

Materials Provided

No. 3A77 HIVAB HIV-1/HIV-2 (rDNA) EIA 100/1000/5000 Tests
(See REAGENTS for a complete listing)

The list of accessories required for the COMMANDER System is found in the COMMANDER Operations Manual(s). A combination of accessories is included with the COMMANDER System. HIVAB HIV-1/HIV-2 (rDNA) EIA is designed to be compatible with the COMMANDER System; the product may be used with a suitable spectrophotometer or Quantum II.

An optimum combination of the following accessories for Quantum II is provided for performance of the tests ordered:

- Reaction Trays.
- Cover Seals.
- Assay Tubes with Identifying Cartons.
- 1 *N* Sulfuric Acid, No. 7212 (Most U.S. and International Locations).

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Materials Required but not Provided

- Precision pipettes or similar equipment to deliver 50 µL, 150 µL, 200 µL, 300 µL (tolerance is ± 5%) and 1 mL (tolerance is ± 10%).
- QwikWash®; or device for washing beads with a vacuum source and a double trap for retaining the aspirate and maintaining minimum vacuum of 21 inches of mercury to deliver a total wash volume of 11-18 mL per well.
- COMMANDER Dynamic Incubator (DI).
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Nonmetallic forceps.
- Quantum II or spectrophotometer capable of reading absorbance at 492 nm with a band width of 10 nm ± 2 nm.
- Bead Dispenser.
- Membrane Seal Puncture Tool or non-metallic tool to open acid bottles.

Additional Reagents Available

1. Abbott OPD (o-Phenylenediamine • 2 HCl) Reagent, No. 6172.
2. 1 *N* Sulfuric Acid, No. 7212.

HIVAB HIV-1/HIV-2 (rDNA) EIA TEST PROCEDURE

Laboratories using the COMMANDER System should refer to the appropriate COMMANDER Operations Manual(s) and note special COMMANDER instructions below.

Preliminary Comments

1. Assay three Negative and two HIV-1 Positive Controls, and two HIV-2 Positive Controls with each run of specimens. Ensure that all reaction trays are subjected to the same process and incubation times. This requires maintenance of specific time intervals between processing trays. Once the assay has been started, complete all subsequent steps without interruption.

   CAUTION: Use a separate disposable pipette tip for each control and specimen in order to avoid cross-contamination.

2. **If using Flexible Pipetting Center (FPC™) the Specimen Diluent must be dispensed using the Bottle (D1/D2) diluter.**
3. Prior to beginning the assay procedure, bring all reagents to room temperature (15 to 30°C) and mix gently.
4. Make certain that sufficient diluted conjugate is available for the test. If necessary, prepare additional diluted conjugate.
5. Identify the reaction tray wells for each specimen and Control .
6. After each step, visually verify the presence of solution and bead in each well.

Procedural Notes

Sample Pipetting and Dilution

1. When using a manual method of sample dilution follow the instructions in the FIRST INCUBATION section of the ASSAY PROCEDURE.
2. When pipetting with the COMMANDER Flexible Pipetting Center (FPC), use System Software Version 2.5 or higher. Use the appropriate Assay Update Diskette and assay protocol as follows:
   a. When using U.S. Assay Update Diskette software, use Version 2.5.1 or higher and pipette using the appropriate assay protocol as follows:

      -A-HIVAB-1-2 PPC or -A-HIVAB-1-2-QT

   b. When using Non-US (Rest of World [ROW]) Assay Update Diskette software, use Version 2.5.2 or above.

      When using ROW Version 2.5.2, create an edited assay protocol. Use the protocol for List Number 1D89, HIV 1-2 gO PPC or HIV 1-2 gO QT as the template from which the edited protocol is created. Change the following items and save the new assay protocol.

      1. In the General Information section change the assay name to HIV 1-2 3A77 PPC or HIV 1-2 3A77 QT as appropriate.
      2. In the General Information section change the List Number to 3A77.
      3. In the General Information section change the Analyzer Test Number to 71.
      4. In the Pipetting Section for the PCN2, change the Component Location to 12 mL bottle.

      Verify that all other assay protocol parameters match the Abbott provided assay protocol for List Number 1D89 except the changes previously identified.

      When using ROW versions greater than 2.5.2, use the assay protocol provided in the software for List Number 3A77 without editing.

When pipetting with the FPC, the Assay List Number and Assay Procedure Code must match that contained in the appropriate PPC Assay Protocol for HIVAB HIV-1/HIV-2 (rDNA) EIA. When configuring the Assay Protocols in the FPC, ensure the Assay Procedure Code matches the Assay Procedure Code specified in the PPC Assay Protocol.

ASSAY SELECTION ON THE PPC

1. Insert tray and select the appropriate assay number for the HIVAB HIV-1/HIV-2 (rDNA) EIA. An operator-edited version may be used if the edited lines are consistent with the assay package insert specifications and are supported by documentation at the time of edit. Follow the instructions on the instrument display board.

   When using an automated pipetting device, such as a COMMANDER Flexible Pipetting Center, verify that the correct PPC assay protocol has been selected for processing on the Instrument display board.

2. Verify the reagent dispenser assignment on the PPC.

Station	Reagent	Dispenser
2	Conjugate	200 µL
4	OPD Solution	300 µL
5	Acid	300 µL

3. **BLANKING (PPC only)**

   NOTE: Use ABBOTT COMMANDER Reagent Blanking Beads only.

   a. During the conjugate incubation step, prepare a "blanks" tray using a separate tray. Place one blanking bead into each of the five wells, A1 through A5.
   b. At the conclusion of the conjugate incubation step, press the Blank key and insert the "blanks" tray, followed immediately by the first assay tray.
   c. At the conclusion of the OPD incubation step, insert the "blanks" tray as the first tray of the final read pass.

First and Second Incubation

1. Do not splash specimen or conjugate outside of well or high up on well rim as it may not be removed in subsequent washings and may be transferred to the tubes causing test interference.
2. When dispensing beads, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells of the reaction tray as directed in the Bead Dispenser inserts.
3. Ensure cover seals adhere tightly to all wells.
4. When washing beads, follow the directions provided in the manual for your washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.
5. **When using the COMMANDER Dynamic Incubator, select the ROTATION incubation method. The same incubation method should be used throughout the assay (i.e., the first and second incubation must be in the ROTATION mode). After inserting the first tray of the batch, the Dynamic Incubator must remain in the ROTATION incubation method while inserting and removing trays of the same batch. Select the incubation temperature and time(s) designated in the ASSAY PROCEDURE section which follows.**

   CAUTION: Failure to use the Dynamic Incubator for incubation in the manner described in the Dynamic Incubator Operations Manual may result in incorrect assay results.

6. **When inserting the tray into the PPC at the conjugate addition step, select the appropriate PPC Assay Protocol number for -A-HIVAB HIV-1 HIV-2.**

Color Development (QUANTUM II and SPECTROPHOTOMETER)

1. When transferring beads from wells to assay tubes, align inverted carton of tubes over their respective wells in the reaction tray. Press the tubes tightly over the wells and invert tray and tubes together so that beads fall into corresponding tubes. Blot excess water from top of tube carton.
2. Avoid strong light during Color Development.
3. Dispense acid in same tube sequence as OPD Substrate Solution.
4. Do not allow acid or OPD Substrate Solution to contact metal.

Reading (QUANTUM II and SPECTROPHOTOMETER)

1. Air bubbles should be removed prior to reading absorbance.
2. Visually inspect both blanks and discard those that are contaminated (indicated by a yellow-orange color). If both blanks are contaminated, the entire run must be repeated.
3. A determination of the absorbance of the substrate blank must be made. The absorbance value of the substrate blank relative to that of the water tube must be greater than or equal to –0.020 and less than or equal to 0.040 in order for the assay to be valid. In Mode 0, blank the instrument with the water tube and read the substrate blank as a sample. (Mode 0 refers to Mode 0 on the QUANTUM II). Check the blank absorbance for assay validity. Stop the Mode 0 assay.
4. If the substrate blank is valid, use it to blank the instrument, read Negative, Positive Control 1 and Positive Control 2, then specimens. If the substrate blank is not valid, repeat steps 3 and 4 using the alternate substrate blank.
5. If there is an interruption during the reading of samples, reblank the instrument and continue reading specimens.

ASSAY PROCEDURE (See Preliminary Comments and Procedural Notes)

Laboratories using the COMMANDER Flexible Pipetting Center or Parallel Processing Center should follow procedures in the appropriate Operations Manual(s). When using other automated instrumentation to deliver Controls and specimens ensure instrumentation is compatible with this assay. Follow manufacturer's directions to achieve the appropriate volumes and dilutions required. The following FIRST INCUBATION and SECOND INCUBATION instructions should be used when processing assays on the QUANTUM II, PPC, and SPECTROPHOTOMETER. The following COLOR DEVELOPMENT and READING instructions should be used when reading on a QUANTUM II or SPECTROPHOTOMETER. For PPC color development and reading instructions, refer to the PPC Operations Manual.

CAUTION: Verify that dispensing equipment is calibrated according to manufacturer's recommendation to ensure delivery of specified sample and/or reagent volumes and does not introduce cross-contamination.

FIRST INCUBATION

1. Pipette 150 µL of each Control into the appropriate reaction tray wells (3 Negative Controls followed by 2 HIV-1 Positive Controls followed by 2 HIV-2 Positive Controls).
2. Pipette 150 µL of specimen into the appropriate wells.
3. Add 50 µL of Specimen Diluent to each well containing a Control or specimen.
4. Add one bead to each well containing a Control or specimen.
5. Apply cover seal; tap tray gently to cover beads and remove any trapped air bubbles.
6. Incubate at 40 ± 1°C for 30 ± 2 minutes in a Dynamic Incubator in the **ROTATION** mode.
7. Remove and discard cover seal. Wash each bead.

SECOND INCUBATION

8. Add 200 µL of diluted conjugate to each reaction well.
9. Apply new cover seal; tap tray gently.
10. Incubate at 40 ± 1°C for 30 ± 2 minutes in a Dynamic Incubator in the **ROTATION** mode.
11. Remove and discard cover seal. Wash each bead.

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COLOR DEVELOPMENT (QUANTUM II and SPECTROPHOTOMETER)

12. Immediately transfer beads to assay tubes.
13. Prime OPD Dispenser immediately prior to dispensing OPD Substrate Solution.
14. Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.
15. Cover and incubate at room temperature (15 to 30°C) for 30 ± 2 minutes.
16. Add 1 mL of 1 *N* Sulfuric Acid to each tube. If necessary, agitate to mix.

PREPARATION OF THE WATER TUBE

17. Pipette approximately 2 mL of distilled or deionized water into an empty tube.

READING (QUANTUM II and SPECTROPHOTOMETER)

18. In Mode 0, blank the instrument with the water tube. (See appropriate Operations Manual for running Mode 0.)
19. Determine the absorbance of the substrate blank. The substrate blank must be greater than or equal to –0.020 and less than or equal to 0.040. Stop the Mode 0 assay.
20. Select mode for processing HIVAB HIV-1/HIV-2 (rDNA) EIA.
21. Blank the instrument with the valid substrate blank.
22. Determine absorbance of Controls and specimens (within 2 hours after addition of acid).

READING RESULTS

INSTRUMENTS

Performance of the HIVAB HIV-1/HIV-2 (rDNA) EIA requires the use of a precision spectrophotometer (i.e., COMMANDER PPC or Quantum II). REFER TO THE APPROPRIATE INSTRUMENT MANUAL FOR PROPER OPERATION AND CALIBRATION.

1. Laboratories using the COMMANDER Parallel Processing Center (PPC) must use Software Version 9.00/9.10 or higher. Use the assay protocol -A-HIVAB HIV-1 HIV-2 as provided in the software without editing. When using the PPC with the COMMANDER Flexible Pipetting Center (FPC), the assay list number and the assay procedure code in the PPC assay protocol must match the assay list number and the assay procedure code configured on the FPC.
2. Laboratories must use Quantum II Module A, List Number 4045-96 or 4045-97.
   a. Laboratories using Quantum II Module A List Number 4045-97, should read the HIVAB HIV-1/HIV-2 (rDNA) EIA using the assay protocol -A-HIVAB HIV-1 HIV-2 (mode 1.28) as provided in the software without editing.
   b. Laboratories using Quantum II Module A 4045-96, should read the HIVAB HIV-1/HIV-2 (rDNA) EIA using an edited assay protocol. Edit mode 1.27 by editing, deleting, or inserting the following lines into the assay protocol:

      Edit the following lines :

      Edit Line 5: MAXIMUM ΔA to 0.100

      Edit Line 9: MINIMUM ΔA to 0.500

      Line 13 must be deleted (Delete by pressing 13 DASH CLEAR ENTER)

      Insert the following lines by using the Edit function:

      Edit Line 26: MIN SAMP REACT ΔA TO -0.015

      Edit Line 27: REPLICATION to 2

      Edit Line 28: MINIMUM ΔA to 0.350

      Edit Line 30: ABERRANT VALUE OPTION to 1

      Edit Line 31: ABERRANT CUTOFF to 50

      Save to a new Mode number and name the protocol -A-HIVAB HIV-1 HIV-2. Verify that the edited protocol values and assay name match the protocol values and assay name below:

      Name: -A-HIVAB HIV-1 HIV-2
         FILTERS = 492:600
         PATH LENGTH = 1.11
      NEGATIVE CONTROLS
         REPLICATION = 3
         MINIMUM ΔA = -0.010
         MAXIMUM ΔA = 0.100
         ABERRANT VALUE OPTION = 1
         ABERRANT CUTOFF = 50.00
      POSITIVE CONTROLS
         REPLICATION = 2
         MINIMUM ΔA = 0.500
         ABERRANT VALUE OPTION = 1
         ABERRANT CUTOFF = 50.00
      UNKNOWNS
         REPLICATION = 1
      CUTOFF = A*NC + B*PC +C
         A = 1.000
         B = 0.000
         C = 0.100
         REACTIVE GRAY ZONE = 0.00%
         NEGATIVE GRAY ZONE = 0.00%
      DISTINCTION:
         REACTIVE >= CUTOFF (0)
         REACTIVE <= CUTOFF (1)
         DISTINCTION = 0
      FLAGGING
         REACTIVE UNKNOWNS (0)
         NEGATIVE UNKNOWNS (1)
         FLAG = 0
      MIN SAMPLE REACTIVITY ABS
         MIN SAMP REACT ΔA = -0.015
      POSITIVE-2 CONTROLS
         REPLICATION = 2
         MINIMUM ΔA = 0.350
         ABERRANT VALUE OPTION = 1
         ABERRANT CUTOFF = 50.00
3. Laboratories using an instrument other than a Quantum II or PPC should read this assay as follows:
   - Read the absorbances for each Control and specimen at 492 nm.
   - Rinse cuvettes thoroughly with distilled or deionized water after reading each specimen.

QUALITY CONTROL PROCEDURES

1. Substrate Blank Acceptance Criteria
   a. **Quantum II users**: An assay run is considered valid with respect to the substrate blank if the blank has an absorbance value that is greater than or equal to -0.020 and less than or equal to 0.040. The determination of assay validity due to substrate blank must be done by user.

      The substrate blank value is an indication of the integrity of the OPD Substrate Solution. If the substrate blank absorbance falls outside the acceptable range, the preparation of the substrate is in question and the alternate substrate blank may be used. If the alternate substrate blank is unacceptable, the assay is invalid, and the run must be repeated.
   b. **COMMANDER users**: Quality control with respect to the substrate blank is determined automatically by the COMMANDER PPC instrument according to the procedure described in the PPC Operations Manual. If the run is invalid, technique errors in preparation of the OPD substrate solution are suspect and the run must be repeated.
2. Control Calculations and Acceptance Criteria

   PPC and Quantum II Software will perform all calculations. Refer to the appropriate manual for specific PPC and QUANTUM II calculations performed. If PPC or Quantum II are not used, perform the following calculations on the assay data.

   An absorbance value of less than 0.500 for either HIV-1 Positive Control replicate and/or an absorbance value of less than 0.350 for either HIV-2 Positive Control replicate may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such runs must be repeated. The presence or absence of antibodies to HIV-1 and/or HIV-2 is determined by relating the absorbance of the specimen to the Cutoff Value. The Cutoff Value is the Negative Control Mean Absorbance plus 0.100.

   a. Calculation of Negative Control Mean Absorbance ($NC\bar{x}$)

      Determine the Mean of the Negative Control Values.

      Example:

Negative Control Sample No.	Absorbance
1	0.010
2	0.018
3	0.014
TOTAL	0.042

$$\frac{\text{Total Absorbance}}{3} = \frac{0.042}{3} = 0.014\ (NC\bar{x})$$

   Individual Negative Control Values must be less than or equal to 0.100 and greater than or equal to -0.010 and within the range 0.5 to 1.5 times the Negative Control Mean ($NC\bar{x}$). When the $NC\bar{x}$ is below 0.012, the calculation of 0.5 to 1.5 times the $NC\bar{x}$ may be discarded. In such cases, all negative control values should be within the range $NC\bar{x} \pm 0.006$. If one value is outside the acceptable range, discard this value and recalculate the $NC\bar{x}$. If more than one value is outside this range, the test must be repeated. If more than an occasional value falls outside this range, contact Customer Service for assistance.

   b. Calculation of HIV-1 Positive Control Mean Absorbance ($PC1\bar{x}$)

      Determine the Mean of the HIV-1 Positive Control Values.

      Example:

HIV-1 Positive Control Sample No.	Absorbance
1	0.835
2	0.925
TOTAL	1.760

$$\frac{\text{Total Absorbance}}{2} = \frac{1.760}{2} = 0.880\ (PC1\bar{x})$$

   Individual HIV-1 Positive Control values should be greater than or equal to 0.500 and within the range 0.5 to 1.5 times the $PC1\bar{x}$.

   Each replicate must meet both of the above criteria or the test must be repeated.

   c. Calculation of HIV-2 Positive Control Mean Absorbance ($PC2\bar{x}$)

      Determine the Mean of the HIV-2 Positive Control Values.

      Example:

HIV-2 Positive Control Sample No.	Absorbance
1	0.653
2	0.557
TOTAL	1.210

$$\frac{\text{Total Absorbance}}{2} = \frac{1.210}{2} = 0.605\ (PC2\bar{x})$$

   Individual HIV-2 Positive Control values should be greater than or equal to 0.350 and within the range 0.5 to 1.5 times the $PC2\bar{x}$.

   Each replicate must meet both of the above criteria or the test must be repeated.

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RESULTS

1. Calculation of the Cutoff Value

   Cutoff Value = $NC\bar{x}$ + 0.100

   Example:

   $NC\bar{x}$ = 0.014

   Cutoff Value = 0.014 + 0.100 = 0.114

2. Calculation of the Unknown

   The presence or absence of antibody to HIV-1 and/or HIV-2 is determined by relating the absorbance of the unknown specimen to the Cutoff Value. If the absorbance of the unknown specimen is greater than or equal to the Cutoff Value, it is considered reactive by the criteria of HIVAB HIV-1/HIV-2 (rDNA) EIA.

INTERPRETATION OF RESULTS

1. Specimens with absorbance values equal to or greater than -0.015 but less than the Cutoff Value are considered not reactive, and may be considered negative for antibodies to HIV-1 and HIV-2.
2. Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive and should be retested in duplicate before interpretation using the original sample.

   If either or both duplicate retests is (are) reactive, the specimen is considered repeatedly reactive.
3. Initially reactive specimens which do not react in either of the duplicate retests are considered negative for antibodies to HIV-1 and HIV-2.
4. A specimen having absorbance values less than -0.015 must be retested to verify the initial result, as technique may be suspect. If the specimen has an absorbance value less than the cutoff when retested, the specimen may be considered negative for antibodies to HIV-1 and HIV-2. Further testing is not required.
5. Specimens found to be repeatedly reactive by HIVAB HIV-1/HIV-2 (rDNA) EIA must be investigated by additional, more specific supplemental tests. If also found to be reactive with these tests, the specimens are considered positive for antibodies to HIV-1 and/or HIV-2.
6. The interpretation of results of specimens found to be repeatedly reactive by HIVAB HIV-1/HIV-2 (rDNA) EIA and negative or indeterminate on additional more specific supplemental 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/HIV-2 (rDNA) EIA procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to HIV in plasma, serum, or cadaveric 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/HIV-2 (rDNA) EIA detects antibodies to HIV-1 and/or HIV-2 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. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV.[35] It is recommended that repeatedly 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 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.[36] EIA testing cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests that the antibodies to HIV are 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. The risk of an asymptomatic person with a repeatedly reactive serum sample developing AIDS or an AIDS-related condition is not known. However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.[37,38]

Data obtained from testing persons both at increased and at low risk for HIV infection suggest that repeatedly reactive specimens with high absorbance on EIA are more likely to demonstrate the presence of the HIV antibodies by additional, more specific or supplemental testing.[39] Reactivity at or only slightly above the Cutoff Value is more frequently nonspecific, especially in samples obtained from persons at low risk for HIV 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 OF SERUM AND PLASMA TESTING

Precision

Assay reproducibility was determined at seven sites in two separate studies. In each study, five specimens were assayed in replicates of three in four consecutive runs over a period of two days.

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.

TABLE I
HIVAB HIV-1/HIV-2 (rDNA) EIA REPRODUCIBILITY

Study 1

		Intra-assay		Inter-assay	
Specimen	Mean S/CO	SD	%CV	SD	%CV
1	0.124	0.032	25.8	0.040	32.7
2	1.258	0.046	3.7	0.098	7.8
3	3.141	0.105	3.3	0.253	8.1
4	4.207	0.154	3.7	0.325	7.7
5	5.392	0.256	4.7	0.557	10.3

	Mean	Intra-assay		Inter-assay	
Control	Absorbance	SD	%CV	SD	%CV
Negative	0.014	0.003	20.7	0.004	32.1
HIV-1 Positive	0.959	0.052	5.4	0.093	9.7

Study 2

		Intra-assay		Inter-assay	
Specimen	Mean S/CO	SD	%CV	SD	%CV
1	0.173	0.031	17.9	0.035	20.1
2	1.899	0.056	2.9	0.139	7.3
3	3.281	0.123	3.7	0.245	7.5
4	6.561	0.225	3.4	0.476	7.3
5	10.014	0.336	3.4	0.679	6.8

	Mean	Intra-assay		Inter-assay	
Control	Absorbance	SD	%CV	SD	%CV
Negative	0.020	0.005	23.3	0.006	30.6
HIV-1 Positive	1.267	0.051	4.0	0.086	6.8
HIV-2 Positive	0.632	0.053	8.4	0.053	8.4

Sensitivity and Specificity

At present, there is no recognized standard for establishing the presence or absence of antibodies to HIV-1 and HIV-2 in human blood.

Sensitivity for HIV-1 antibodies was computed based on the clinical diagnosis of AIDS. For HIV-2, sensitivity was expressed in terms of detection rate using investigational confirmation assay results as a basis for comparison.

Specificity is based on assay of blood donations from random donors. Sensitivity for HIV-1 antibody was shown to be equivalent to a previously licensed test based on comparative studies in various clinical groups including AIDS, ARC, and High Risk.

The ABBOTT studies show that:

1. Specificity based on an assumed zero prevalence of antibody to HIV-1 and/or HIV-2 in random donors (17037 out of 17054) is estimated to be 99.90%* with a 95% confidence interval: 99.83-99.94% (Table II).

   *In these calculations, one sample of the eighteen total repeatedly reactive specimens was confirmed by Western Blot and has been excluded.
2. HIV-1 sensitivity was equivalent to a previously licensed test in a population of 352 AIDS patients with known antibodies (352 out of 352 detected for an estimated sensitivity of 100% with a 95% confidence interval: 99.15 - 100%, Table III). Similarly the HIV-1 sensitivity was equivalent to a previously licensed test for 1042 known positive samples from other groups with ARC, High Risk or clinical status unknown (1042 out of 1042 detected for an estimated sensitivity of 100% with a 95% confidence interval: 99.71 - 100%, Table III).
3. HIV-2 antibody detection rate in a population of 246 HIV-2 confirmed antibody positive individuals (246 out of 246) is estimated to be 100% with a 95% confidence interval: 98.79 - 100%, compared to a previously licensed HIV-2 EIA which is 99.59% (244 out of 245, Table IV).

B. REACTIVITY IN RANDOM DONOR POPULATION

The results of testing specimens from random blood donors for antibodies to HIV-1 and/or HIV-2 are shown in Table II. The data include 17054 samples obtained from random blood donors at six geographically distinct blood banks and one plasmapheresis center.

TABLE II
Detection of Antibodies to HIV-1 and/or HIV-2 in Serum Specimens and Plasma Specimens from Blood and Plasma Donors

	HIVAB HIV-1/HIV-2 (rDNA) EIA	
Number Tested	Initially Reactive	Repeatedly Reactive
Serum from Volunteer Blood Donors (3 sites)		
Total 6510	11(0.17%)	7(0.11%)
Plasma from Volunteer Blood Donors (4 sites)		
Total 6549	10(0.15%)	5(0.08%)
Plasma from Plasmapheresis Donors (1 site)		
Total 3995	6(0.15%)	6(0.15%)
TOTAL 17054 (7 sites)	27(0.16%)	18(0.11%)

C. REACTIVITY IN PATIENT POPULATIONS

1. Retrospective Studies

   The reactivity of HIVAB HIV-1/HIV-2 (rDNA) EIA was determined by testing specimens from patients clinically diagnosed as having AIDS, AIDS related complex, asymptomatic individuals who were HIV-1 antibody positive, patients treated with AZT, and specimens from HIV-1 antibody positive individuals for whom the clinical status was unknown (Table III).

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TABLE III
Detection of Antibodies to HIV-1 and/or HIV-2 in Specimens from Individuals Preselected as Positive for HIV-1 Antibodies

Group	Number Tested	HIVAB HIV-1/HIV-2 (rDNA) EIA Number Reactive	(%Reactive)	HIV-1 EIA (Previously Licensed) Number Reactive	(%Reactive)
AIDS	352*	352	(100.00)	344	(99.71)
ARC	270	270	(100.00)	270	(100.00)
Asymptomatic	162	162	(100.00)	162	(100.00)
Patients treated with AZT	49	49	(100.00)	49	(100.00)
Unknown Clinical Status	561	561	(100.00)	561	(100.00)
TOTAL	1394**	1394	(100.00)	1386	(99.93)

* 345 specimens tested by the licensed HIV-1 EIA due to insufficient volume of seven samples.

** 1387 specimens tested by the licensed HIV-1 EIA due to insufficient volume of seven samples.

The ability of HIVAB HIV-1/HIV-2 (rDNA) EIA to detect antibodies to HIV-2 in 246 samples from a total of 246 symptomatic and asymptomatic individuals, including persons from Europe and West Africa, is shown in Table IV.

TABLE IV
Detection of Antibodies to HIV-1 and/or HIV-2 in Specimens from Individuals Preselected as Positive for HIV-2 Antibodies and Confirmed by Western Blot

Group	Number Tested	HIVAB HIV-1/HIV-2 (rDNA) EIA Number Reactive	(%Reactive)	HIV-2 EIA (Previously Licensed) Number Reactive	(%Reactive)
AIDS	8	8	(100.00)	8	(100.00)
ARC	24	24	(100.00)	24	(100.00)
Asymptomatic	83	83	(100.00)	83	(100.00)
Other	131*	131	(100.00)	129	(99.23)
TOTAL	246**	246	(100.00)	244	(99.59)

* 130 specimens tested by the licensed HIV-2 EIA due to insufficient volume of one sample.

** 245 specimens tested by the licensed HIV-2 EIA due to insufficient volume of one sample.

2. Prospective Studies

The results of testing for antibodies to HIV-1 and/or HIV-2 in 100 specimens from 100 individuals at high risk for HIV-1 infection is shown in Table V.

TABLE V
Detection of Antibodies to HIV-1 in Specimens from Individuals at High Risk for HIV Infection in the United States

Number Tested	HIVAB HIV-1/HIV-2 (rDNA) EIA Repeatedly Reactive	HIV-1 EIA (Previously Licensed) Repeatedly Reactive
100	16*	15

* 15/16 confirmed for the HIVAB HIV-1/HIV-2 (rDNA) EIA using an investigational Western Blot. 15/15 confirmed for the previously licensed HIV-1 EIA. The same 15 samples were reactive in both test methods.

To demonstrate the ability of HIVAB HIV-1/HIV-2 (rDNA) EIA to detect antibody to HIV-1 and/or HIV-2 in an HIV-2 endemic area, 488 unselected specimens from the Cape Verde Islands were initially tested with both HIV-1 and HIV-2 Western Blots. All specimens were then tested with HIVAB HIV-1/HIV-2 (rDNA) EIA. The results are shown in Table VI.

TABLE VI
Detection of Antibodies to HIV-2 in Unselected Specimens from an HIV-2 Endemic Area

Number Tested	No. HIV Western Blot Positive	No. HIVAB HIV-1/HIV-2 (rDNA) EIA Reactive	No. HIV-2 EIA (Previously Licensed) Reactive
488	8(1.6%)*	18(3.7%)	10 (2.1%)

* 8/488 samples confirmed by Western Blot (WB): 1 specimen was HIV-1 WB positive, 2 specimens were HIV-1 and HIV-2 WB positive, 5 specimens were HIV-2 WB positive. 8/8 WB positives were reactive in the HIVAB HIV-1/HIV-2 (rDNA) EIA. 7/8 WB positives were reactive in a previously licensed HIV-2 EIA. The nonreactive sample was HIV-1 WB positive only.

D. REACTIVITY OF SEROCONVERTING DONORS

Evidence for improved sensitivity of the HIVAB HIV-1/HIV-2 (rDNA) EIA was obtained from studies of seroconversion sera (see Table VII). Nine seroconversion panels were obtained retrospectively from plasmapheresis donors with no known risk factors. All samples were tested by an FDA licensed Western Blot. All series showed *gag* and *env* gene product bands which are characteristic of HIV-1 infection. These donors (with the exception of 72593, C1066, and H831) have been described previously.[40] Each sample was tested in four separate assays, except 46402, which was tested in three assays. The HIVAB HIV-1/HIV-2 (rDNA) EIA detected the presence of HIV-1 antibody at the same time or earlier than the FDA licensed ABBOTT HIVAB HIV-1 EIA.

TABLE VII
Performance of the HIVAB HIV-1/HIV-2 (rDNA) EIA on Seroconverting Sera versus the Current HIVAB HIV-1 EIA and a FDA licensed Western Blot

Donor/	(No.)	Days of Donation	HIVAB HIV-1/HIV-2 (rDNA) EIA	HIVAB HIV-1 EIA	Western Blot Bands
6977	(1)	1	+	+	24
	(2)	6	+	+	24
	(3)	10	+	+	24,41,160
	(4)	16	+	+	24,41,120,160
13078	(1)	1	-	-	None
	(2)	6	+	+/-[1]	None
	(3)	8	+	+	None
	(4)	14	+	+	24
	(5)	17	+	+	24,41,55
103524	(1)	1	+	+	24,41,66
	(2)	5	+	+	24,41,51,55,66
46402	(1)	1	+	+	24
	(2)	8	+	+	24,160
	(3)	10	+	+	24,160
	(4)	17	+	+	24,51,66,160
3988	(1)	1	-	-	None
	(2)	3	-	-	None
	(3)	8	+	+/-[2]	24
	(4)	10	+	+	24
	(5)	17	+	+	24,41,160
6108	(1)	1	+	+	24
	(2)	5	+	+	24,55
	(3)	9	+	+	17,24,55
	(4)	12	+	+	17,24,41,55
	(5)	16	+	+	17,24,41,55,120,160
	(6)	19	+	+	17,24,41,55,66,120,160
72593	(1)	1	+	+	24
	(2)	3	+	+	24
	(3)	10	+	+	24,120,160
	(4)	16	+	+	24,120,160
C1066	(1)	1	-	-	None
	(2)	5	+/-[3]	-	None
	(3)	16	+	+	24,120,160
	(4)	21	+	+	24,120,160
H831	(1)	1	-	-	None
	(2)	4	-	-	None
	(3)	15	-	-	None
	(4)	22	-	+/-[4]	None
	(5)	52	+	+/-[5]	24,55,120

[1] Three of seven replicates reactive
[2] One of seven replicates reactive
[3] Five of seven replicates reactive
[4] One of seven replicates reactive
[5] Six of seven replicates reactive

PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING REPRODUCIBILITY

Inter-assay reproducibility of HIVAB HIV-1/HIV-2 (rDNA) EIA was assessed using 20 postmortem and 20 normal donor sera. These sera specimens were spiked with human plasma reactive for anti-HIV to create low-level reactive specimens. Each of the specimens was tested once on six different days on each of three lots of HIVAB HIV-1/HIV-2 (rDNA) EIA at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 3.4% to 9.6% for the low-level reactive postmortem specimens and from 3.4% to 14.2% for the low-level reactive normal donor specimens. For inter-lot reproducibility the %CV ranged from 6.8% to 16.1% for the low-level reactive postmortem specimens and from 10.0% to 16.6% for the low-level reactive normal donor specimens.

SPECIFICITY

Specificity was evaluated using 50 postmortem and 50 normal donor specimens. Each of the specimens was tested once on each of three lots of HIVAB HIV-1/HIV-2 (rDNA) EIA. The mean sample to cutoff (S/CO) ratio for 150 postmortem replicates (50 specimens with three reagent lots) was 0.131 and the mean S/CO ratio for 150 normal donors replicates (50 specimens with three reagent lots) was 0.114. Results are presented in Table VIII.

TABLE VIII
Reactivity with HIVAB HIV-1/HIV-2 (rDNA) EIA

Population	No. of Specimens	No. of Replicates	Mean S/CO	Nonreactive	Initially Reactive
Postmortem	50	150	0.131	150 (100.0%)	0 (0.0%)
Normal Donor	50	150	0.114	150 (100.0%)	0 (0.0%)

The reactivity of HIVAB HIV-1/HIV-2 (rDNA) EIA has an estimated specificity of 100% (100%; binomial confidence interval[41] = [97.57%, 100%]) for postmortem specimens.

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SENSITIVITY

Sensitivity was evaluated using 50 postmortem and 50 normal donor specimens that were pre-screened for HIV-1 and HIV-2 and found to be negative. Different aliquots of the 50 postmortem and 50 normal donor specimens were spiked with human plasma reactive for HIV-1 or HIV-2 to create low-level reactive specimens. Each of the specimens was tested once with each of three lots of HIVAB HIV-1/HIV-2 (rDNA) EIA. The mean sample/cutoff (S/CO) for the 150 postmortem HIV-1 replicates (50 specimens with three reagent lots) was 4.075, and the mean S/CO ratio for the 150 normal donor HIV-1 replicates (50 specimens with three reagent lots) was 4.140. The mean S/CO ratio for the 150 postmortem HIV-2 replicates (50 specimens with three reagent lots) was 3.571, and the mean S/CO ratio for the 150 normal donor HIV-2 replicates (50 specimens with three reagent lots) was 3.324. The mean S/CO for the 300 postmortem HIV-1 and HIV-2 replicates (50 HIV-1 and 50 HIV-2 specimens with three reagent lots) was 3.823 and the mean S/CO ratio for the 300 normal donor HIV-1 and HIV-2 replicates (50 HIV-1 and 50 HIV-2 specimens with three reagent lots) was 3.732. The calculated difference between the combined mean S/CO of the postmortem HIV-1 and HIV-2 specimens and the mean S/CO of the normal donor HIV-1 and HIV-2 specimens was 0.091, which was determined by the F test analysis not to be statistically significant (p-value 0.341).

TABLE IX
Reactivity with HIVAB HIV-1/HIV-2 (rDNA) EIA

Population	No. of Specimens	No. of Replicates	Mean S/CO	Non reactive	Initially Reactive
Postmortem Spiked HIV-1	50	150	4.075	0 (0.0%)	150 (100.0%)
Normal Donor Spiked HIV-1	50	150	4.140	0 (0.0%)	150 (100.0%)
Postmortem Spiked HIV-2	50	150	3.571	0 (0.0%)	150 (100.0%)
Normal Donor Spiked HIV-2	50	150	3.324	0 (0.0%)	150 (100.0%)
Postmortem Combined (Spiked HIV-1 and Spiked HIV-2)	100	300	3.823	0 (0.0%)	300 (100.0%)
Normal Donor Combined (Spiked HIV-1 and Spiked HIV-2)	100	300	3.732	0 (0.0%)	300 (100.0%)

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