HUMAN T-LYMPHOTROPIC VIRUS TYPES I AND II
ABBOTT HTLV-I/HTLV-II EIA

NAME AND INTENDED USE

ABBOTT HTLV-I/HTLV-II EIA IS AN *IN VITRO* ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETECTION OF ANTIBODIES TO HUMAN T-LYMPHOTROPIC VIRUS TYPE I AND/OR TYPE II (HTLV-I/HTLV-II) IN HUMAN SERUM OR PLASMA.

66-0450/R7

WARNING: A SOFTWARE UPGRADE AND/OR PROTOCOL EDITS WILL BE REQUIRED PRIOR TO IMPLEMENTING THIS ASSAY. CONTACT YOUR ABBOTT REPRESENTATIVE.

ABBOTT LABORATORIES
Diagnostics Division

CUSTOMER SUPPORT CENTER (USA)
1-800-323-9100

Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064
U.S. License No. 43
List No. 7A92

August, 1997

Printed in U.S.A.

NAME AND INTENDED USE

ABBOTT HTLV-I/HTLV-II EIA is an *in vitro* enzyme immunoassay for the qualitative detection of antibodies to Human T-Lymphotropic Virus Type I and/or Type II (HTLV-I/HTLV-II) in human serum or plasma. The ABBOTT HTLV-I/HTLV-II EIA is intended to be used as a screen for donated blood to prevent transmission of HTLV-I and HTLV-II to recipients of cellular blood components and as an aid in the clinical diagnosis of HTLV-I and HTLV-II infection and related diseases.

SUMMARY AND EXPLANATION OF TEST

HTLV-I, a human Type-C retrovirus,[1,2] has been etiologically associated with neoplastic conditions and a variety of demyelinating neurologic disorders including: adult T-cell leukemia (ATL),[3] tropical spastic paraparesis (TSP)[4,5] and/or HTLV-I associated myelopathy (HAM)[6], and more recently HTLV-I associated polymyositis, arthritis and infective dermatitis.[7,8,9] Antibodies to HTLV-I are found with high frequency in persons afflicted with these disorders. However, it is well established in studies from viral endemic areas that HTLV-I antibody negative ATL and TSP/HAM are seen.[10-12]

HTLV-I infection is endemic in the Caribbean,[11] southeastern Japan,[12] in some areas of Africa,[13] Central and South America,[14] and recently described in Melanesia[15,16] and central and northern Australia.[17,18] In the United States, HTLV-I has been identified in ATL patients, intravenous drug users, and in healthy individuals.[19-23] Transmission of HTLV-I (and HTLV-II) infection to transfusion recipients of infected cellular blood products is well documented.[24-29] Transmission also occurs via breast milk,[30] sexual contact,[31,32] and sharing of contaminated needles and syringes by intravenous drug users.[20,23,33]

HTLV-I causes ATL in only 2 to 4% of infected individuals and typically only after long latency periods.[34,35] The ATL syndrome appears to result from exposure early in life as occurs during maternal transmission via breast milk.[30] Approximately 20 to 25% of children exposed to HTLV-I through breast feeding develop antibodies to HTLV-I.[30,36-38] Perinatal transmission of HTLV-I occurs in approximately 5% of non-breast fed children born to infected mothers.[37,38] Following transfusion of cellular blood components in HTLV-I endemic areas, 44 to 63% of recipients seroconvert;[25,39] however, lower seroconversion rates (approximately 20%) have been reported in recipients of contaminated blood in the U.S.[27,28] Infection with HTLV-I during adult life results in TSP/HAM-like illness, and not in ATL.[9] The lifetime risk for TSP/HAM for those who live in an endemic area is estimated at less than 1%.[34,35] The presence of HTLV-I antibodies in an asymptomatic person indicates that the individual may be infected with the virus and should not donate blood,[26,40-41] but does not mean the individual has ATL or TSP/HAM or will develop ATL or TSP/HAM.[34,36,41] Consultation with appropriate medical personnel is recommended for discussion of additional concerns related to viral infection and its transmission.

HTLV-II was first isolated in 1982 from a patient having T-lymphocytic-hairy cell leukemia.[42,43] Association of HTLV-II with leukemia pathogenesis is not well established; however, some cases of neurologic diseases resembling TSP/HAM have been recently reported to be associated with HTLV-II infection.[44-47] Epidemiologic data suggest that HTLV-II is a new-world virus common among Amerindians in North, Central and South America.[14]

Transmission of HTLV-II, like HTLV-I, occurs via transfusion of cellular blood components, between needle-sharing intravenous drug users and through sexual contact,[14,23,48-51] but mother to child transmission of HTLV-II has recently been reported.[52] At least one-half of U.S. blood donors who are positive by supplemental testing for antibody following HTLV-I screening have been identified as HTLV-II positive rather than HTLV-I positive.[23,28,36,44,53]

Neither HTLV-I nor HTLV-II cause acquired immunodeficiency syndrome (AIDS) and the HTLV-I and HTLV-II viruses are only remotely related to the AIDS virus, HIV. No cross-reactivity with antibodies to HIV-1 or HIV-2 has been demonstrated for this assay. The finding of antibodies to HTLV-I/HTLV-II by this assay has no relationship to the presence of antibodies to HIV and does not imply any risk of AIDS.

The ABBOTT HTLV-I/HTLV-II EIA has been developed to detect antibodies to HTLV-I and HTLV-II in human serum or plasma. This detection is accomplished through the presence of HTLV-I and HTLV-II viral antigens on the solid phase. The ABBOTT HTLV-I/HTLV-II EIA does not discriminate between antibody reactivity to HTLV-I and HTLV-II.

Specimens with absorbance values greater than or equal to the Cutoff Value in ABBOTT HTLV-I/HTLV-II EIA are defined as initially reactive. Initially reactive specimens are to be retested in duplicate using the original sample. Reactivity in either or both of these duplicate tests (*i.e.*, repeatedly reactive), is highly predictive of the presence of HTLV-I and/or HTLV-II antibodies in individuals at increased risk for HTLV infection.

Additional, more specific tests, such as the Western Blot (WB) assay and the radioimmunoprecipitation assay (RIPA), are supportive in determining if repeatedly reactive specimens are positive for antibodies to HTLV.[36,41,54] A combination of such tests must be capable of identifying antibodies to HTLV core *(gag)* proteins (p24) and envelope *(env)* proteins (native gp46, gp61/68).[24,54]

An HTLV-I, HTLV-II, or dual infection can only be differentiated serologically by parallel testing using antigens from HTLV-I and HTLV-II in specific immunoassays.[55,56] Tests based on reactivities to HTLV-I and HTLV-II type specific proteins may enable viral typing. Nonserologic tests based on the presence of infected cells, or HTLV-I/HTLV-II DNA probe testing [*e.g.*, polymerase chain reaction (PCR)] may also be used in discrimination.[57]

Recommendation for appropriate use of additional, more specific tests may be issued periodically by the U.S. Public Health Service.[36,41]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

In the ABBOTT HTLV-I/HTLV-II EIA, human serum or plasma is diluted in a specimen diluent and incubated with a polystyrene bead coated with sonicated and detergent-inactivated HTLV-I and HTLV-II proteins. The HTLV-I is propagated in the chronically infected human T-lymphocyte cell line HUT 102.B2 and HTLV-II in the chronically infected B-lymphocyte cell line WIL-NRA. Specific antibody present in the sample binds to the antigens on the bead. Unbound materials are removed by washing the beads.

Goat antibodies directed against human immunoglobulins conjugated with horseradish peroxidase (goat anti-Hu IgG:HRPO) are incubated with the beads and, if specific antibody is present in the sample, the goat anti-Hu IgG:HRPO binds to it. Unbound conjugate is removed by washing the beads.

The beads are then incubated with o-Phenylenediamine (OPD) Substrate Solution containing hydrogen peroxide. The reaction of OPD Substrate Solution with HRPO yields a yellow-orange color. The intensity of the color formed is proportional to the amount of specific antibody in the sample. The enzyme reaction is stopped by the addition of 1 *N* Sulfuric Acid and the intensity of color developed is read using a spectrophotometer set at 492 nm.

REAGENTS

ABBOTT HTLV-I/HTLV-II EIA, 100/1000/5000 TESTS

① 1 Vial (100 Beads)/2 Vials (500 Beads each)/10 Vials (500 Beads each) Human T-Lymphotropic Virus Type I (Inactivated) and Human T-Lymphotropic Virus Type II (Inactivated) Coated Beads.

② 3 Vials (1 mL each)/3 Vials (5 mL each)/15 Vials (5 mL each) Conjugate Concentrate. Goat Antibody to Human IgG: Peroxidase (Horseradish). Minimum Concentration: 1.6 µg/mL in TRIS Buffer with 10% Animal Serum (Calf) and Red Dye No. 33. Preservatives: 0.1% ProClin™ 300 (Isothiazolone) and 0.01% Gentamicin.

③ 3 Vials (19 mL each)/3 Vials (95 mL each)/15 Vials (95 mL each) Conjugate Diluent containing 20% Animal Sera (Goat, Calf) in TRIS Buffer. Preservatives: 0.1% ProClin™ 300 (Isothiazolone) and 0.01% Gentamicin.

④ 1 Vial (2 mL)/2 Vials (2 mL each)/10 Vials (2 mL each) HTLV-I Positive Control. Inactivated Human Plasma reactive for antibody to HTLV-I, nonreactive by FDA licensed tests for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg, with bromophenol blue dye added. Minimum Titer: 1:5. Preservative: 0.1% Sodium Azide. HTLV-I Positive Control may be cross-reactive with antibody to HTLV-II.

⑤ 1 Vial (2 mL)/2 Vials (2 mL each)/10 Vials (2 mL each) Negative Control. Human Plasma nonreactive by FDA licensed tests for antibodies to HTLV-I, HIV-1/HIV-2 and HCV, and nonreactive for HBsAg. Preservative: 0.1% Sodium Azide.

⑥ 1 Vial (100 mL)/4 Vials (100 mL each)/20 Vials (100 mL each) Specimen Diluent containing 30% Animal Sera (Goat, Calf) in TRIS Buffer. Preservative: 0.1% Sodium Azide.

⑦ 1 Vial (2 mL)/2 Vials (2 mL each)/10 Vials (2 mL each) HTLV-II Positive Control. Inactivated Human Plasma reactive for antibody to HTLV-II, nonreactive by FDA licensed tests for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg. Minimum Titer: 1:5. Preservative: 0.1% Sodium Azide. HTLV-II Positive Control may be cross-reactive with antibody to HTLV-I.

• There is no reagent 8.

⑨ 1 Bottle (10 Tablets)/2 Bottles (40 Tablets each)/10 Bottles (40 Tablets each) OPD (o-Phenylenediamine•2HCl) 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•2HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

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The stopping reagent may be provided as an accessory to the ABBOTT HTLV-I/HTLV-II EIA Kit and consists of:

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

Use of acid other than 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® Parallel Processing Center (PPC) (all specific references to procedures for the COMMANDER System or the PPC alone will be highlighted in red). Use a Quantum™ II 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 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, acid 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".

ADDITIONAL REAGENTS AVAILABLE (Most International Locations):

- 1 *N* Sulfuric Acid, No. 7212-01 (110 mL).
- 6 *N* Sulfuric Acid, No. 7212-03 (110 mL).

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

Safety Precautions

CAUTION: This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the REAGENTS section of this package insert. Components sourced from human blood have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV, and nonreactive for HBsAg by FDA licensed tests. 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 material should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with OSHA Standard on Bloodborne Pathogens.[71] Biosafety Level 2[58] or other appropriate biosafety practices[59,60] should be used for materials that contain or are suspected of containing infectious agents. The HTLV-I and HTLV-II antigens have been inactivated by sonication and detergent-treatment prior to coating on beads. The HTLV-I and HTLV-II Positive Controls have been inactivated by heat treatment.[61] Although an EBV- transformed cell line was used to prepare viral lysate, no evidence exists that infectious EBV is present in the kit.

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 when handling specimens and kit reagents. Upon completion, remove gloves and wash hands thoroughly.
4. 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.
5. Clean and disinfect all spills of specimens and reagents using a suitable disinfectant,[62] such as 1% sodium hypochlorite for non-radioactive material[63] or 2% glutaraldehyde for spills containing radioactive material.[64]
6. Dispose of all materials that have come into contact with specimens and reagents in accordance with local, state and federal regulations.[65] Solid wastes may be incinerated or autoclaved for an appropriate period of time. Due to variations among autoclaves and in waste configuration, each user must verify the effectiveness of his decontamination cycle using biological indicators.[66]

   NOTE: Liquid waste containing acid must be neutralized prior to the addition of disinfectants and/or disposal.
7. This product contains Sodium Azide as a preservative. 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, flush drains thoroughly 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 (U.S.A.) recommends the

following: (1) siphon liquid from 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.

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 Economic Community (EEC) 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.
S2 Keep out the of reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
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 Sodium Carbonate. The OPD tablets are classified per applicable European Economic Community (EEC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) Phrases.

R40/22 Harmful: possible risks of irreversible effects if swallowed.
R36/38 Irritating to eyes and skin.
R43 May cause sensitization by skin contact.
S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S46 If swallowed, seek medical advice immediately and show this container or label.

The 6*N* Sulfuric Acid listed in the REAGENTS section of this package insert is classified per applicable European Economic Community (EEC) Directives as: Corrosive (C). The following are the appropriate Risk (R) and Safety (S) Phrases.

R35 Causes severe burns.
S1/2 Keep locked up and out of the reach of children.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately.

Handling Precautions

1. Do not use kit beyond the expiration date.
2. Do not mix reagents from different lots.
   NOTE: Any OPD Diluent lot, OPD Tablet lot, or 1 *N* Sulfuric Acid (No. 7212) lot may be used with any ABBOTT EIA kit.
3. Avoid microbial contamination of specimens, reagents, and water used for washing. Use of disposable pipette tips is recommended. Avoid chemical contamination of reagents and equipment.
4. Do not expose OPD reagents to strong light during storage or incubation.
5. Avoid contact of the OPD Substrate Solution and 1 *N* Sulfuric Acid with any oxidizing agent. Do not allow OPD Substrate Solution or 1 *N* Sulfuric Acid to come in contact with any metal parts. Prior to use, rinse glassware used with OPD Substrate Solution thoroughly with 1 *N* Acid (sulfuric or hydrochloric) using approximately 10% of the container volume followed by three rinses of distilled or deionized water at 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.
   CAUTION: Do not open the Bead bottle until it has reached room temperature.
7. USE A CLEAN DEDICATED DISPENSER FOR THE CONJUGATE SOLUTION TO AVOID NEUTRALIZATION.
8. The Negative and Positive Controls, as provided, should be dispensed and diluted in the same manner as specimens.
9. Ensure that the specimen is added to the reaction well according to the procedures outlined in this insert. If a specimen is inadvertently not added, the assay may yield an ERRONEOUS, nonreactive result.
10. Inadequate adherence to package insert instructions may result in ERRONEOUS results.
11. Use accurately calibrated equipment.

INSTRUCTIONS FOR PREPARATION OF CONJUGATE REAGENT

1. Bring Conjugate Concentrate and Conjugate Diluent to 15 to 30°C before mixing by allowing material to stand at room temperature.
2. Carefully empty the contents of a Conjugate Concentrate vial into a vial of Conjugate Diluent. This can ONLY be accomplished by slowly squeezing the small vial 2 to 3 times while maintaining the nozzle within the opening of the large vial. Avoid foaming. One vial of diluted conjugate is sufficient for up to 100 tests (100 Test Kit) or up to 500 tests (1000/5000 Test Kit).
3. Reseal the large vial. Mix thoroughly by slowly inverting the vial several times. Do not vortex.
4. **Write the date of dilution and expiration date in the space provided on the Conjugate Diluent label. Conjugate is stable for 14 days (though not exceeding the kit expiration date) after dilution when stored at 2 to 8°C.**
5. Allow newly diluted conjugate to equilibrate at room temperature for a minimum of 60 minutes prior to use.
6. If storing the diluted conjugate, store at 2 to 8°C. Bring to room temperature before use.
7. Do not combine vials of diluted conjugate. Separate Negative and Positive Controls must be run with each vial of diluted conjugate.

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 five minutes, but no longer than 60 minutes, prior to dispensing for Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine•2HCl) Tablet(s) in Diluent for OPD. DO NOT USE A TABLET THAT IS NOT INTACT.

Using clean pipettes and metal-free containers (such as plasticware or acid-washed and distilled or deionized 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 Substrate Solution bottle while the tablets are dissolving.

   NOTE: The OPD Substrate Solution **MUST** be used within 60 minutes of preparation and **MUST NOT** be exposed to strong light. Record the preparation time and expiration time of the OPD Substrate Solution.
3. Just prior to dispensing for Color Development, swirl container gently to obtain a homogeneous solution. Remove air bubbles from dispenser tubing, and prime dispenser prior to use.

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

NOTE: 300 µL of OPD Substrate Solution is required for each specimen or Control as well as for each substrate blank. Laboratories using the COMMANDER® Parallel Processing Center (PPC) will require additional OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

1. Store kit reagents and OPD diluent at 2 to 8°C. OPD Tablets and 1 *N* Sulfuric Acid may be stored at 2 to 30°C. Do not freeze kit reagents.
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 the Bead bottle or the OPD Tablet bottle until it has reached room temperature.

3. Retain desiccant in the Bead bottle and in the OPD Tablet bottle at all times during storage.
4. Reconstituted OPD Substrate Solution **MUST** be stored at room temperature and **MUST** be used within 60 minutes. Do not expose to strong 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 indicates that the reagent has been contaminated and must be discarded.

A value of less than 0.500 absorbance units for the difference between the HTLV-I Positive and Negative Control Means (PCN1-NCN1) or the HTLV-II Positive and Negative Control Means (PCN2-NCN1) may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such runs must be repeated.

SPECIMEN COLLECTION AND PREPARATION

ABBOTT HTLV-I/HTLV-II EIA test may be performed on human serum, plasma or recalcified plasma.

1. Either serum (including serum collected in serum separator tubes) or plasma collected in ACD, Sodium Citrate, CPDA-1, CPD, CP2D, Sodium EDTA, Potassium EDTA, Sodium Heparin or Potassium Oxalate based anticoagulant may be used in the test. The correct ratio of anticoagulant quantity to specimen volume, as recommended by the manufacturer of anticoagulant, is required. Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. No qualitative performance differences were observed when HTLV-I/HTLV-II EIA negative and reactive samples were tested with elevated levels of bilirubin (≤ 20mg/dL), hemoglobin (≤ 2000 mg/dL), or lipids (≤ 2200mg/dL). Specimens containing precipitate may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying.
2. Do not use heat-inactivated specimens.
3. Performance has not been established using cadaver specimens or body fluids other than serum or plasma, such as urine, saliva, pleural fluid, amniotic fluid or semen.
4. If specimens are to be stored, they may be stored at 2 to 8°C for up to 14 days. However, if storage periods greater than 14 days are anticipated, the serum or plasma specimens must be removed from the clot or red cells and should be stored frozen at -10°C or colder. More than three freeze-thaw cycles should be avoided. Specimens must be mixed thoroughly after thawing prior to testing.
5. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[67] Specimens may be shipped ambient, refrigerated (2 to 8°C) on wet ice, or frozen (-10°C or colder) on dry ice.
6. All glassware or plastic materials coming into contact with the specimen should be free of any residue from previous specimens, reagents, or cleaning compounds.

PROCEDURE

Materials Provided

No. 7A92 ABBOTT HTLV-I/HTLV-II EIA Kit, 100/1000/5000 Tests

(See REAGENTS for a complete listing)The list of accessories required for the COMMANDER® Flexible Pipetting Center (FPC) and Parallel Processing Center (PPC) are found in the appropriate COMMANDER® Operator's Manual(s). A combination of accessories is included with the COMMANDER® FPC and PPC. ABBOTT HTLV-I/HTLV-II EIA is designed to be compatible with the COMMANDER® FPC and PPC. The product may be used with a Quantum™ II.

An optimum combination of the following accessories may be provided:

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

Materials Required but not Provided

- Precision pipettes with disposable tips, EIA Pipetting Package, or similar equipment to deliver 10 µL, 200 µL, 300 µL, 400 µL (tolerance is ±5%), and 1 mL (tolerance is ±10%).
- Qwikwash® for washing beads.
- COMMANDER® Dynamic Incubator capable of maintaining temperature between 38 and 42°C.
- COMMANDER® PPC or Quantum™ II.
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Metal-free containers for the OPD Substrate Solution, can be plasticware or acid-washed, distilled or deionized water-rinsed glassware.
- Protective gloves.
- Disinfectant as described in Safety Precautions.
- * Nonmetallic forceps.
- * Bead Dispenser.
- Test tubes and rack for dilution of specimens.
- * Membrane Seal Puncture Tool for acid bottles.
- OPD Tray Covers (for COMMANDER® testing).
- Blanking Beads (for COMMANDER® testing).
- Distilled or deionized water.

* Included in EIA Pipetting Package

ABBOTT HTLV-I/HTLV-II EIA TEST PROCEDURE

Preliminary Comments

Laboratories using the COMMANDER® Flexible Pipetting Center (FPC), Dynamic Incubator (DI) or Parallel Processing Center (PPC) should refer to the appropriate COMMANDER® Operator's Manual(s) and note special COMMANDER® instructions below. When using other automated instrumentation to deliver Controls and specimens, follow the manufacturer's directions to achieve the appropriate volumes and dilutions required within the recommended time limits. Precise timing of enzyme immunoassays is critical.

1. Assay two Negative Controls, two HTLV-I Positive Controls, and two HTLV-II Positive Controls with each run of specimens. Controls should be dispensed and diluted in the same manner as specimens. An assay run is defined as a minimum of two Negative Controls, two HTLV-I Positive Controls, two HTLV-II Positive Controls and one specimen on one 20-well or 60-well tray or a maximum of two Negative Controls, two HTLV-I Positive Controls, two HTLV-II Positive Controls and 494 specimens on 20-well or 60-well trays using a full bottle of diluted conjugate (500 tests). Use one preparation of working reagents per run. Ensure that all reaction trays containing Controls and/or specimens are subjected to the same processing and incubation times. This may require 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 specimen and Control in order to avoid cross-contamination.

2. Prior to beginning the assay procedure, bring all reagents to room temperature (15 to 30°C) and mix gently. Adjust the Dynamic Incubator to 40°C.
3. Identify the reaction tray wells for each Control and specimen.
4. Make certain that sufficient diluted conjugate is available for the test. If necessary, prepare additional conjugate reagent. (See **INSTRUCTIONS FOR PREPARATION OF CONJUGATE REAGENT** Section.) Do not combine bottles of diluted Conjugate.
   NOTE: Each bottle of diluted conjugate requires a separate run in which all assay calculations are performed based upon the Controls in that run.
5. **After each step, visually verify the presence of solution and bead in each well.**
6. The exact order of specimen and reagent additions as described in this test procedure must be followed.

PROCEDURAL NOTES

SAMPLE PIPETTING AND DILUTION

Assays performed on the COMMANDER® Parallel Processing Center (PPC) must not have undiluted specimen added to the reaction tray well. This may be accomplished by 1) using the Flexible Pipetting Center (FPC) with Assay Update Diskette Version 2.5.1 or higher or 2) adding a diluted specimen (200 µL) when performing manual pipetting of specimen and Specimen Diluent. **Failure to add Specimen Diluent to each reaction tray well prior to the addition of undiluted Control or specimen is not a validated test procedure if the reaction tray is processed on the COMMANDER® PPC.**

1. When using the ABBOTT COMMANDER® Flexible Pipetting Center (FPC) and Assay Update Diskette Version 2.5.1 or higher for Control or specimen dilution for PPC processing or Quantum™ II processing. Use the appropriate FPC assay protocol, HTLV-I/II PPC D0-a, or HTLV-I/II QT D0-a for Control or specimen dilution. This protocol pipettes 105 µL of Specimen Diluent, followed by 5 µL of Controls or specimen plus 105 µL of Specimen Diluent for a final dilution of 1:41. The dilution for the FPC is numerically greater than 1:41; however, due to additional specimen adhering to the outside of the pipette tip, additional volume of Specimen Diluent is required to achieve a final dilution of 1:41.
   NOTE: Do not dispense Specimen Diluent into trays prior to pipetting on the FPC.
2. When using a manual method of sample dilution, follow the steps in the "Dilution of Specimen" section of the ASSAY PROCEDURE.
   NOTE: To dispense specimen and diluent using a manual pipette, hold the dispenser tip over the center of each reaction well, perpendicular to the tray and slightly above the bottom of the well. Dispense. Ensure that the tip does not contact the side of the well and that the dispensed liquid does not splash onto the side of the well.

NOTE: Assays that are processed using the COMMANDER® Parallel Processing Center (PPC) must NOT have Controls or specimens and Specimen Diluent pipetted using automated equipment designed to dispense 5 µL of Control or specimen followed by 200 µL of Specimen Diluent, as these devices do not dispense specimen diluent prior to undiluted specimen.

ASSAY SELECTION ON THE PPC

1. Insert tray and select the appropriate assay number for HTLV-I/HTLV-II 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.
2. Verify reagent dispenser assignment:

STATION	REAGENT	DISPENSER VOLUME
2	Conjugate	200 µL
4	OPD Solution	300 µL
5	Acid	300 µL

3. BLANKING (COMMANDER® 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 batch.

GENERAL NOTES

1. Specimen dilutions may be performed in either tubes or trays.
2. 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 cause test interference.
3. **Verify that the dispensing equipment delivers specified volume and appropriate dilutions for each procedure.**
4. When using a Bead Dispenser, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells.
5. Ensure cover seals adhere tightly to all wells.
6. When washing beads, follow the directions provided with the washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.
7. When using the COMMANDER® Dynamic Incubator, **select the ROTATION incubation method** and the incubation temperature and time(s) designated in the ASSAY PROCEDURE section which follows. **Use the ROTATION incubation method throughout the assay.** Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operator's Manual may result in incorrect assay results. The Dynamic Incubator is to remain in the ROTATION incubation method while inserting and removing trays.

COLOR DEVELOPMENT

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.
   NOTE: Conjugate and OPD Substrate dispensers must be rinsed with distilled or deionized water after each use. Refer to dispenser inserts for cleaning procedure.

READING (QUANTUM™ II)

1. Remove air bubbles prior to reading absorbance.
2. Visually inspect blank tubes and discard those that are contaminated (indicated by a yellow-orange color). If both blanks are contaminated, the entire run must be repeated.
3. Determine the absorbance of the substrate blank. In Mode 0, blank the instrument with the water tube and read the substrate blank as a sample. Stop the Mode 0 assay. 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.
4. If the substrate blank is valid, use it to blank the instrument. Read Negative and Positive Controls, then specimens. If the substrate blank is not valid, repeat Steps 3 and 4 using the alternate substrate blank. If the alternate substrate blank is invalid, the entire run must be repeated.
5. If there is an interruption during the reading of samples, reblank the instrument with the substrate blank using the alternate substrate blank if necessary. Continue reading specimens.

ASSAY PROCEDURE (See Preliminary Comments and Procedural Notes)

Laboratories using the COMMANDER® Flexible Pipetting Center (FPC) and Parallel Processing Center (PPC) should follow procedures in the appropriate COMMANDER® Operator's Manual(s). When using other automated instrumentation to deliver Controls and specimens, follow the manufacturer's directions to achieve the appropriate volumes required. The following assay procedure should be used with the Quantum™ II when pipetting manually.

DILUTION OF SPECIMEN

1. a. Pipette 10 µL of each Control or specimen into the bottom of an individual test tube, dilution tray well or equivalent.
   - Pipette two (2) Negative Control replicates followed by two (2) HTLV-I Positive Control replicates and two (2) HTLV-II Positive Control replicates. Kit controls are to be positioned on the first EIA reaction tray of the run prior to specimens.

   b. Add 400 µL of Specimen Diluent to each tube, dilution tray well or equivalent.
   c. Ensure adequate mixing.
   d. Transfer 200 µL of each diluted Control or specimen into appropriate well of reaction tray.

FIRST INCUBATION

2. Add one Bead to each well containing a Control or specimen.
3. Apply cover seal.
4. Incubate at 40±2°C for 60±5 minutes in a Dynamic Incubator in the **ROTATION** mode.
5. Remove and discard cover seal. Wash each bead immediately.

SECOND INCUBATION

6. Add 200 µL of Conjugate to each reaction well.
7. Apply new cover seal.
8. Incubate at 40±2°C for 30±2 minutes in a Dynamic Incubator in the **ROTATION** mode.
9. Remove and discard cover seal. Wash each bead immediately.

COLOR DEVELOPMENT

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

PREPARATION OF WATER TUBE

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

READING

16. In Mode 0, blank the instrument with the water tube. (See Operator's Manual for running Mode 0).
17. Determine the absorbance of the substrate blank. To be valid, 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.
18. Select the Mode for processing HTLV-I/HTLV-II EIA.
19. Blank the instrument with the valid substrate blank.
20. Determine absorbance of Controls and specimens within 2 hours of addition of acid.

READING RESULTS

INSTRUMENTS

Performance of the ABBOTT HTLV-I/HTLV-II 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. SHOULD SOFTWARE NEED TO BE INSTALLED OR RELOADED, ANY EDITED ASSAY PROTOCOLS MUST BE RECREATED.

1. Laboratories using the COMMANDER® Parallel Processing Center (PPC) must use software version 8.00 or above. Laboratories using software version 8.00, 8.10, 8.01 and 8.11 must create an edited assay protocol. If PPC Assay Protocol #24 is not present, contact your Abbott Representative to make the assay protocol available on the software.

   Laboratories using software greater than 8.11 should use the assay protocol as provided.

   PPC version 8.00, 8.10, 8.01 and 8.11 users: Proceed to edit PPC Assay Protocol #24 to change the following parameters:

Line 02	Assay Name	HTLV-I HTLV-II 7A92
Line 06	Assay Procedure	PN
Line 67	Min. Cntl. Diff. (P-N):	0.500
Line 69	Min. PC2 Diff.	0.500

   No other assay protocol parameters require edits. Verify that all other assay protocol parameters of the edited protocol match the assay protocol #24.

   When pipetting with FPC version 2.5.1 or higher, the Assay List Number and Assay Procedure Code must match that contained in the appropriate edited PPC Assay Protocol for the ABBOTT HTLV-I/HTLV-II EIA. When configuring Assay Protocols in the FPC, ensure the assay procedure code is specified as "PN".

2. Laboratories using the Quantum™ II should read this assay as follows:

   Laboratories using a Quantum™ II, Module A, List Number greater than 4045-97 should process ABBOTT HTLV-I/HTLV-II EIA using the HTLV-I HTLV-II EIA protocol as provided in the software without editing. Laboratories using the Quantum™ II, Module A, List Number 4045-96 or 97 must create an edited assay protocol.

   Edit mode 1.27 or 1.28 to match the following assay parameters. Verify that the edited protocol values and assay name match the protocol values and assay name below.

   NAME: HTLV-I HTLV-II EIA
   FILTERS = 492:600
   PATH LENGTH = 1.11
   NEGATIVE CONTROLS
   REPLICATION = 2
   MINIMUM ΔA = 0.020
   MAXIMUM ΔA = 0.200
   ABERRANT VALUE OPTION = 1
   ABERRANT CUTOFF = 42.00
   POSITIVE CONTROLS
   REPLICATION = 2
   MINIMUM ΔA = 0.700
   MAXIMUM ΔA = 1.700
   ABERRANT VALUE OPTION = 1
   ABERRANT CUTOFF = 32.00
   PC-NC
   MINIMUM VALUE = 0.500
   UNKNOWNS
   REPLICATION = 1
   CUTOFF = A+NC+B+PC+C
   A = 0.000
   B = 0.400
   C = 0.000
   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
   MINIMUM SAMPLE REACTIVITY ABS
   MIN SAMP REACT ΔA = 0.005
   POSITIVE-2 CONTROLS
   REPLICATION = 2
   MINIMUM ΔA = 0.700
   MAXIMUM ΔA = 1.700
   ABERRANT VALUE OPTION = 1
   ABERRANT CUTOFF = 32.00
   MINIMUM CONTROL DIFF. = 0.500

QUALITY CONTROL PROCEDURES[68,69]

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 the 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® Parallel Processing Center (PPC) according to the procedure described in the PPC Operator's Manual. If the run is invalid, technique errors in preparation of the OPD Substrate Solution are suspect and the run must be repeated.
2. Negative Control Calculations and Acceptance Criteria
   a. Calculation of Results - When a COMMANDER® PPC or Quantum™ II is used, all calculations below are performed automatically.
      1) Calculation of Negative Control Mean

      Absorbance ($NCN1\bar{x}$)

      Determine the Mean of the Negative Control Values.

      Example:

      Negative Control

Sample No.	Absorbance
1	0.103
2	0.094
TOTAL	0.197

      $$(NCN1\bar{x}) = \frac{\text{Total Absorbance}}{2} = \frac{0.197}{2} = 0.099$$

   b. Negative Control Acceptance Criteria

      Individual Negative Control values must meet the following criteria:

      1) Individual Negative Control values must be less than or equal to 0.200 and greater than or equal to 0.020.
      2) Individual Negative Control values must be within the range 0.58 to 1.42 times the Negative Control Mean.

      Each Negative Control value must meet the above criteria or the run is invalid and must be repeated.
3. HTLV-I Positive Control Calculations and Acceptance Criteria
   a. Calculation of Results - When a COMMANDER® PPC or Quantum™ II is used, all calculations below are performed automatically.
      1) Calculation of HTLV-I Positive Control Mean

      Absorbance ($PCN1\bar{x}$)

      Determine the Mean of the HTLV-I Positive Control Values.

      Example:

      HTLV-I Positive Control

Sample No.	Absorbance
1	1.315
2	1.203
TOTAL	2.518

      $$(PCN1\bar{x}) = \frac{\text{Total Absorbance}}{2} = \frac{2.518}{2} = 1.259$$

   b. HTLV-I Positive Control Acceptance Criteria

      Individual HTLV-I Positive Control values must meet the following criteria:

      1) Individual HTLV-I Positive Control values must be less than or equal to 1.700 and greater than or equal to 0.700.
      2) Individual HTLV-I Positive Control values must be within the range 0.68 to 1.32 times the HTLV-I Positive Control Mean.

      Each HTLV-I Positive Control value must meet the above criteria or the run is invalid and must be repeated.
4. HTLV-II Positive Control Calculations and Acceptance Criteria
   a. Calculation of Results - When a COMMANDER® PPC or Quantum™ II is used, all calculations below are performed automatically.
      1) Calculation of HTLV-II Positive Control Mean Absorbance ($PCN2\bar{x}$)

      Determine the Mean of the HTLV-II Positive Control Values.

      Example:

      HTLV-II Positive Control

Sample No.	Absorbance
1	1.430
2	1.400
TOTAL	2.830

      $$(PCN2\bar{x}) = \frac{\text{Total Absorbance}}{2} = \frac{2.830}{2} = 1.415$$

   b. HTLV-II Positive Control Acceptance Criteria

      Individual HTLV-II Positive Control values must meet the following criteria:

      1) Individual HTLV-II Positive Control values must be less than or equal to 1.700 and greater than or equal to 0.700.
      2) Individual HTLV-II Positive Control values must be within the range 0.68 to 1.32 times the HTLV-II Positive Control Mean.

      Each HTLV-II Positive Control value must meet the above criteria or the run is invalid and must be repeated.
5. Assay Run Validity Criteria

   For the run to be valid, the difference between the mean absorbance of the HTLV-I Positive Control and the Negative Control (PCN1-NCN1) and the HTLV-II Positive Control and the Negative Control (PCN2-NCN1) must be 0.500 or greater. If not, technique or deterioration of reagents may be suspect and the run must be repeated.

   Calculations for Determining PCN1-NCN1 and PCN2-NCN1

   Example:

   $NCN1\bar{x}$ = 0.099

   $PCN1\bar{x}$ = 1.259

   PCN1-NCN1 = (1.259 - 0.099) = 1.160

   $PCN2\bar{x}$ = 1.415

   PCN2-NCN1 = (1.415 - 0.099) = 1.316

RESULTS

When a COMMANDER® PPC or Quantum™ II is used, all calculations below are performed automatically.

Calculation of the Cutoff Value

The Cutoff Value is the mean absorbance of the HTLV-I Positive Control multiplied by 0.4.

Calculation of the Cutoff Value:

Example:

PCN1$\bar{x}$ = 1.259

Cutoff Value = 0.4 X PCN1$\bar{x}$

= 0.4 X 1.259 = 0.504

The presence or absence of antibody to HTLV- I and/or HTLV-II is determined by relating the absorbance of the specimen to the Cutoff Value. If the absorbance of the specimen is greater than or equal to the Cutoff Value, it is considered reactive by the criteria of ABBOTT HTLV-I/HTLV-II EIA.

INTERPRETATION OF RESULTS

1. Specimens with absorbance values equal to or greater than 0.005 but less than the Cutoff Value are considered nonreactive by the criteria of ABBOTT HTLV-I/HTLV-II EIA and may be considered negative for antibodies to HTLV-I and negative for antibodies to HTLV-II. Further testing is not required.
2. Specimen results having absorbance values below 0.005 must be retested singly using the original sample with the ABBOTT HTLV-I/HTLV-II EIA to verify the initial test result as technique may be suspect. If the specimen has an absorbance value less than the Cutoff Value when retested, the specimen may be considered negative for antibodies to HTLV-I and HTLV-II by the criteria of the ABBOTT HTLV-I/HTLV-II EIA. Further testing is not required.
3. Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive by the criteria of ABBOTT HTLV-I/HTLV-II EIA but before interpretation, the original sample must be retested in duplicate with the ABBOTT HTLV-I/HTLV-II EIA. If either or both duplicate retests are reactive, the specimen is considered to be repeatedly reactive for antibodies to HTLV-I and/or HTLV-II by the criteria of ABBOTT HTLV-I/HTLV-II EIA.
4. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative by the criteria of the ABBOTT HTLV-I/HTLV-II EIA. Further testing is not required.
5. If the specimen is repeatedly reactive, the probability that antibodies to HTLV-I and/or HTLV-II are present is high, especially in specimens obtained from individuals at increased risk for HTLV infection. In most settings it is appropriate to investigate repeatedly reactive specimens by additional more specific tests. Specimens found repeatedly reactive by EIA and positive by these supplemental tests should be considered positive for antibodies to HTLV-I and/or HTLV-II. The interpretation of results of specimens found repeatedly reactive by EIA and negative or indeterminate on additional testing is unclear; further clarification may be obtained by testing another specimen from the same patient taken three to six months later. Guidelines for interpretation of results are issued periodically by the U.S. Public Health Service.[36,41]

LIMITATIONS OF THE PROCEDURE

The ABBOTT HTLV-I/HTLV-II EIA PROCEDURE and the INTERPRETATION OF RESULTS must be closely followed when testing serum or plasma specimens for the presence of antibodies to HTLV-I and/or HTLV-II. This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens must not be used as the accuracy of their test results has not been validated. Performance has not been established using cadaver specimens or body fluids other than serum or plasma such as urine, saliva, pleural fluid, amniotic fluid or semen. Do not use heat-inactivated specimens. A test result that is negative does not exclude the possibility of exposure to or infection with HTLV-I and/or HTLV-II. Negative results in this assay in individuals with prior exposure to HTLV-I and/or HTLV-II may be due to antibody levels below the limit of detection of this assay or lack of antibody reactivity to the HTLV antigens used in this assay.

Failure to add specimen in the PROCEDURE could result in a falsely negative test result. Repeat testing should be considered where there is clinical suspicion of HTLV-I or HTLV-II infection.

ABBOTT HTLV-I/HTLV-II EIA detects antibodies to HTLV-I and HTLV-II in blood and thus is useful as a diagnostic screening procedure. The use of this test to screen blood for transfusion may prevent transmission of HTLV-I and/or HTLV-II infection.

Guidelines published by the U.S. Public Health Service recommend that repeatedly reactive specimens be investigated by additional more specific tests such as Western Blot (WB) and radioimmunoprecipitation assay (RIPA). These supplemental tests should be used in addition to type-specific peptide or probe tests for HTLV-I and HTLV-II discrimination. Interpretation of such tests should be consistent with these published guidelines.

An individual whose serum or plasma is found to react in both the EIA and additional more specific tests for antibodies to HTLV-I and/or HTLV-II is presumed to be infected with one or both of these viruses. The medical implications of HTLV-II seropositivity are unknown. Appropriate counseling and medical evaluation should be offered. Such an evaluation should be considered an important part of HTLV-I and HTLV-II antibody testing and should include test result confirmation on a freshly drawn sample. ATL and TSP/HAM are clinical syndromes and their diagnosis can only be established clinically. ABBOTT HTLV-I/HTLV-II EIA testing alone cannot be used to diagnose HTLV-associated conditions, even if the recommended investigation of reactive specimens confirms the presence of HTLV-I and/or HTLV-II antibodies. A negative result at any point in the serologic investigation does not preclude the possibility of exposure to or infection with HTLV-I and/or HTLV-II.

False-positive test results can be expected with a test kit of this nature. The proportion of reactives that are falsely reactive will depend upon the sensitivity and specificity of the test kit and on the prevalence of HTLV-I and/or HTLV-II antibodies in the population being screened.

EXPECTED RESULTS

SPECIFIC PERFORMANCE CHARACTERISTICS

ASSAY REPRODUCIBILITY

Assay reproducibility was determined by assaying a 28 member panel consisting of four replicates each of three diluted HTLV-I antibody containing specimens, three diluted HTLV-II antibody containing specimens, and one specimen nonreactive for antibody to HTLV-I or HTLV-II. The panel was tested over a minimum of five days for each of three master lots at a total of eight sites.

The intra- and inter-assay standard deviation (S.D.) and percent coefficient of variation (%CV) were analyzed with a variance components analysis, using a nested analysis of variance model[70] (Table I).

Mean S/CO is defined as the mean sample absorbance divided by the calculated Cutoff Value.

TABLE I
ABBOTT HTLV-I/HTLV-II EIA Reproducibility

Specimen	Number of Replicates	Mean S/CO*	Intra-assay S.D.	Intra-assay %CV	Inter-assay S.D.	Inter-assay %CV
1	472	3.294	0.2544	7.7	0.3496	10.6
2	472	1.998	0.1480	7.4	0.2099	10.5
3	472	1.260	0.1085	8.6	0.1365	10.8
4	472	3.328	0.2309	6.9	0.3612	10.9
5	472	2.380	0.2274	9.6	0.2928	12.3
6	472	1.400	0.1349	9.6	0.1666	11.9
7	472	0.330	0.0372	11.3	0.0482	14.6

*Cutoff = 0.4 X PCN1 Mean

Controls	Number of Replicates	Mean O.D.	Intra-assay S.D.	Intra-assay %CV	Inter-assay S.D.	Inter-assay %CV
NCN1	236	0.093	0.0115	12.3	0.0149	16.0
PCN1	236	1.135	0.0801	7.1	0.1144	10.1
PCN2	236	1.236	0.0953	7.7	0.1294	10.5

O.D. = Optical Density
NCN1 = Negative Control
PCN1 = HTLV-I Positive Control
PCN2 = HTLV-II Positive Control

ASSAY SPECIFICITY

The specificity of the ABBOTT HTLV-I/HTLV-II EIA was estimated from screening tests of random U.S. blood donors (plasma, serum, and plasmapheresis specimens). Specificity was estimated by the following formula[a]:

$$\frac{(\text{total donations screened} - \text{total \# repeatedly reactive donations})}{(\text{total donations screened} - \text{\# repeatedly reactive, supplemental test-positive donations}^{b})} \times 100$$

[a] Although specificity calculated in this manner represents a variation of the single test specificity, it is used because of the testing protocol requirement for repeat testing of initially reactive serum or plasma specimens.

[b] A positive result was defined by the presence of antibodies to both *gag* (p24) and *env* (native gp46 or gp61/67/68) antigens in investigational Western Blot and/or RIPA.

A total of 15,215 serum and plasma specimens from volunteer blood donors and plasmapheresis donors were collected from five geographically distinct U.S. blood centers (Table II). Three sites tested a total of 5,909 serum specimens and had an overall initial reactive rate of 0.32% (19/5,909) and repeat reactive rate of 0.30% (18/5,909) by the ABBOTT HTLV-I/HTLV-II EIA. Of the 18 repeatedly reactive serum specimens, four (22.22%) tested positive, 11 were indeterminate, and three were negative by investigational Western Blot and RIPA. Two sites tested a total of 6,292 plasma specimens and had an overall initial reactive rate of 0.37% (23/6,292) and repeat reactive rate of 0.35% (22/6,292) by the ABBOTT HTLV-I/HTLV-II EIA. Of the 22 repeatedly reactive plasma specimens, one tested positive, 13 were indeterminate, and eight were negative by investigational Western Blot and RIPA. One site tested a total of 3,014 plasmapheresis specimens and had an initial reactive rate of 0.40% (12/3,014) and repeat reactive rate of 0.36% (11/3,014). Of the 11 repeatedly reactive specimens, five (45.45%) tested positive, five were indeterminate and one was negative by investigational Western Blot and RIPA.

Specificity of the ABBOTT HTLV-I/HTLV-II EIA estimated from screening tests in U.S. random blood donors was 99.73% (15,164/15,205) based on an assumed zero prevalence of HTLV-I and HTLV-II antibodies. Ten repeatedly reactive specimens that tested positive by supplemental testing were excluded from these calculations. Therefore, of 15,205 donations presumed seronegative for HTLV-I and HTLV-II, the ABBOTT HTLV-I/HTLV-II EIA has an estimated specificity of 99.63% to 99.81% by the binomial distribution at 95% confidence.

Three sites evaluated 639 specimens from medical conditions unrelated to HTLV-I or HTLV-II. Twenty-six specimens (4.07%) were initially reactive and 26 (4.07%) were repeatedly reactive by the ABBOTT HTLV-I/HTLV-II EIA (Table II). Five of the 26 specimens tested positive by investigational Western Blot or RIPA. No particular medical condition unrelated to HTLV-I or HTLV-II was significantly associated with false positive reactivity.

HTLV type differentiation of the 15 repeatedly reactive, supplemental test-positive specimens in the specificity study indicated that seven specimens were positive for antibodies to HTLV-I and eight specimens were positive for antibodies to HTLV-II. (Table II).

TABLE II
Reactivity in Low-Risk Populations and in Medical Conditions Unrelated to HTLV-I or HTLV-II Infection

Group/Type	Total Number of Specimens Tested	ABBOTT HTLV-I/HTLV-II EIA: Initially Reactive (% of Total)	ABBOTT HTLV-I/HTLV-II EIA: Repeatedly Reactive (RR) (% of Total)	Number of EIA Repeatedly Reactive, Not Positive by Supplemental Testing (% of Total)	Number of EIA Repeatedly Reactive, Positive by Supplemental Testing (% of RR)[a]	HTLV Type Differentiation of RR Supplemental Test-Positive Specimens[b]: HTLV-I	HTLV-II
Blood Donors							
Serum	5,909	19 (0.32)	18 (0.30)[c]	14 (0.24)	4 (22.22)	1	3
Plasma	6,292	23 (0.37)	22 (0.35)[d]	21 (0.33)	1 (4.56)	0	1
Plasmapheresis Donors	3,014	12 (0.40)	11 (0.36)[e]	6 (0.20)	5 (45.45)	3	2
Total Random Donors	15,215	54 (0.35)	51 (0.34)	41 (0.27)	10 (19.61)	4	6
Medical Conditions Unrelated to HTLV-I or HTLV-II[f]	639	26 (4.07)	26 (4.07)[g]	21 (3.28)[h]	5 (19.23)[i]	3	2

[a] A positive result in these studies was defined by the presence of antibodies to two gene products (*gag*, p24 and *env*, native gp46 or gp61/67/68) using investigational Western Blot and/or RIPA.

[b] HTLV-I and HTLV-II type differentiation was determined using the following investigational use assays: reactivity to the recombinant gp41-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, and/or PCR (using specific primers to the *tax* and *pol* regions).

[c] Four of the 18 repeatedly reactive specimens tested positive, 11 were indeterminate, and three were negative by investigational Western Blot and RIPA.

[d] One of the 22 repeatedly reactive plasma specimens tested positive, 13 were indeterminate and eight were negative by investigational Western Blot and RIPA.

[e] Five of the 11 repeatedly reactive specimens tested positive, five were indeterminate and one was negative by investigational Western Blot and RIPA.

[f] Included the following specimen groups: anti-CMV Positive (38); anti-EBV Positive (60); anti-HBs Positive (20); HBsAg Positive (20); anti-HCV Positive (43); anti-HIV-1

Positive (150); anti-HIV-2 Positve (10); anti-HSV Positive (20); anti-Toxoplasmosis Positive (10); Other Bacterial Infections (15); Other Diseases (9); SLE Patients (20); ANA Positive (20); Rheumatoid Arthritis (15); Hypergammaglobulinemia (10); Elevated Bilirubin (58); Elevated Hemoglobin (10); Elevated Triglycerides (15); Influenza and Tetanus Vaccine Recipients (10); Multiple Sclerosis Patients (4); Animal Handlers (5); Non-HTLV Leukemia (2); and IgM Nonspecific specimens from influenza vaccinated blood donors (10).

g Included specimens from the following specimen groups: anti-HCV Positive (3); anti-HIV-1 Positive (7); anti-HIV-2 Positive (1); Other Bacterial Infection (1); Elevated Bilirubin (6); Influenza Vaccine Recipients (5); Non-HTLV Leukemia (2); and IgM Nonspecific specimens from influenza vaccinated blood donor (1).

h Included non-supplemental test-positive specimens from the following groups: anti-HCV Positive (3); anti-HIV-1 Positive (4); anti-HIV-2 Positive (1); Elevated Bilirubin (6); Influenza Vaccine Recipients (4); Non-HTLV Leukemia (2); and IgM Nonspecific specimens from influenza vaccinated blood donor (1).

i Specimens that tested positive for antibodies to HTLV-I and/or HTLV-II by investigational Western Blot and/or RIPA include: anti-HIV-1 Positive (3); Other Bacterial Infection (1); and Influenza Vaccine Recipient (1).

ASSAY SENSITIVITY

A total of 1,272 specimens were tested with the ABBOTT HTLV-I/HTLV-II EIA, including patients with HTLV-I and/or suspected HTLV-II associated disease and their contacts, populations at increased risk of HTLV-I and/or HTLV-II infection, populations from HTLV-I and/or HTLV-II endemic areas, and known HTLV-I or HTLV-II positive specimens. Of the 1,272 specimens tested, 1,233 tested positive by investigational Western Blot and/or RIPA, of which 590 specimens were HTLV-I positive, 506 specimens were HTLV-II positive, and 137 specimens were untypeable HTLV-I/II (Table III). The ABBOTT HTLV-I/HTLV-II EIA has an estimated sensitivity of 100% (interval of 99.76% to 100%) for 1,233 supplemental test-positive specimens by the binomial distribution at 95% confidence. Prospective studies were performed on a total of 7,650 specimens from populations at increased risk of HTLV-I or HTLV-II infection and from populations in HTLV-I or HTLV-II endemic areas with the ABBOTT HTLV-I/HTLV-II EIA (Table IV). One hundred percent of these 528 supplemental test-positive samples were repeatedly reactive on the ABBOTT HTLV-I/HTLV-II EIA, of which 219 were HTLV-I positive, 242 were HTLV-II positive, and 67 were untypeable HTLV-I/II positive.

TABLE III
Comparison of the Reactivity of the ABBOTT HTLV-I/HTLV-II EIA and the ABBOTT HTLV-I 2.0 EIA with Supplemental Test-Positive HTLV-I or HTLV-II Specimens

Group	Number of Specimens Positive by Supplemental Testing[d]	HTLV Type Differentiation of Supplemental Test-Positive Specimens[d]			Number Supplemental Test-Positive that are EIA Repeatedly Reactive (% of Supplemental Test-Positive)	
		HTLV-I	HTLV-II	Untypeable HTLV-I/II	ABBOTT HTLV-I/HTLV-II EIA	ABBOTT HTLV-I 2.0 EIA
Populations with HTLV-I and/or Suspected HTLV-II Associated Disease[a]	144	141	3	0	144 (100.00)[f]	143 (99.31)
Populations at Increased Risk for HTLV-I or HTLV-II Infection and Populations from HTLV-I or HTLV-II Endemic Areas[b]	97	40	55	2	97 (100.00)[g]	96 (98.97)
Known HTLV-I or HTLV-II Positive Populations[c]	992	409	448	135	992 (100.00)	992 (100.00)
TOTAL	1,233	590	506	137	1,233 (100.00)	1,231 (99.84)

a Included the following specimen groups: ATL patients (50); TSP/HAM patients (54); Non-Hodgkin's Lymphoma (24); Southwest U.S. TSP/HAM patients infected with HTLV-II (2); and an HTLV Disease State Panel including one TSP/HAM patient infected with HTLV-II (14 members).

b Included the following specimen groups: Japanese Family Members of known HTLV-I infected individuals (36); and Specimens from a Southwest U.S. Endemic Area (61).

c Included 409 HTLV-I positive specimens, 448 HTLV-II positive specimens, and 135 untypeable HTLV-I/HTLV-II positive specimens.

d The number of positive specimens was based on supplemental test results from investigational HTLV-I/HTLV-II Western Blot and, in some cases, HTLV-I RIPA. A positive result in these studies was defined by the presence of antibodies to two gene products (*gag*, p24 and *env*, native gp46 or 61/67/68) using Western Blot and/or RIPA.

e HTLV-I and HTLV-II type differentiation was determined using the following investigational use assays: reactivity to the recombinant gp46-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, and/or PCR (using specific primers to the *tax* and *pol* regions).

f The additional sample detected by the ABBOTT HTLV-I/HTLV-II EIA was supplemental test-positive for anti-HTLV-I (Non-Hodgkin's Lymphoma Patient).

g The additional sample detected by the ABBOTT HTLV-I/HTLV-II EIA was supplemental test-positive for anti-HTLV-II (Southwestern U.S. Endemic Population).

TABLE IV
Comparison of the Reactivity of the ABBOTT HTLV-I/HTLV-II EIA and the ABBOTT HTLV-I 2.0 EIA with Specimens from Populations at Increased Risk of HTLV-I or HTLV-II Infection and Populations in HTLV-I or HTLV-II Endemic Areas

Group	Total Number of Specimens Tested	Number of Specimens Positive by Supplemental Testing[c]	HTLV Type Differentiation of Supplemental Test-Positive Specimens[d]			Number Supplemental Test-Positive that are EIA Repeatedly Reactive (% of Supplemental Test-Positive)	
			HTLV-I	HTLV-II	Untypeable HTLV-I/II	ABBOTT HTLV-I/HTLV-II EIA	ABBOTT HTLV-I 2.0 EIA
Populations at Increased Risk of HTLV-I and/or HTLV-II Infection[a]	1,594	269	8	230	31	269 (100.00)	269 (100.00)
Populations from HTLV-I or HTLV-II Endemic Areas[b]	6,056	259	211	12	36	259 (100.00)	259 (100.00)
TOTAL	7,650	528	219	242	67	528 (100.00)	528 (100.00)

a Included the following specimen groups: U.S. IV Drug Users (1,456) and specimens from Multiple Transfusion Recipients (138).

b Included the following specimen groups: Random Blood Donors from Central America (2,145); Amerindians from Central America (638); West Africans (20); Central Africans (38); Florida Migrant Workers predominantly from Haiti (276); Random Japanese Blood Donors (600); Random Jamaican Blood Donors (1,262); Hawaiians of Japanese Descent (215); Martinique Blood Donors (796); and Jamaican Hematology Clinic Patients (66).

c The number of supplemental test-positive specimens was based on HTLV-I/HTLV-II Western Blot, HTLV-I RIPA and HTLV-II RIPA investigational test results of any specimen that was repeatedly reactive or repeatedly within a 30% negative gray zone by EIA. A positive result in these studies was defined by the presence of antibodies to two gene products (*gag*, p24 and *env*, native gp46 or 61/67/68) using Western Blot and/or RIPA.

d HTLV-I and HTLV-II type differentiation was determined using the following investigational use assays: reactivity to the recombinant gp46-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, and/or PCR (using specific primers to the *tax* and *pol* regions).

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