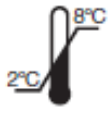
Avioq HTLV-I/II Microelisa System

Key Symbols Used

	Catalogue Number		Consult Instructions For Use
	Batch Code		In Vitro Diagnostic Medical Device
	Expiration Date		Positive Control
	Temperature Limit		Negative Control
	Latex Free		Contains Sufficient For <n> Tests
	Caution		Keep Away from Sunlight

Avioq HTLV-I/II Microelisa System

INTENDED USE

Avioq HTLV-I/II Microelisa System is a qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to Human T-Lymphotropic Virus Type I (HTLV-I) and/or Human T-Lymphotropic Virus Type II (HTLV-II) in human serum, plasma, and cadaveric samples. It is intended for screening individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HTLV-I/HTLV-II, and for use as an aid in clinical diagnosis of HTLV-I or HTLV-II infection and related diseases. It is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating and in testing specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens. In addition to being used as a Manual assay, the assay is also intended for use with the ORTHO® Summit System (OSS) for screening blood donors.

SUMMARY AND EXPLANATION OF THE TEST

HTLV-I, a human type C retrovirus, has been etiologically associated with Adult T-Cell Leukemia (ATL)[1-4] and with a demyelinating neurologic disorder termed Tropical Spastic Paraparesis, and/or HTLV-I Associated Myelopathy (TSP/HAM). Antibodies to HTLV-I are found with high frequency in persons affected with these disorders. However, it is well established in studies from viral endemic areas that virus negative ATL and TSP/HAM are seen. More recently, HTLV-I infection has been shown to be associated with B- and T-cell chronic lymphatic leukemia (CLL),[5,6] multiple myeloma,[7] some cases of non-Hodgkin's lymphoma (NHL),[8] polymyositis,[9] arthritis,[10,11] Kaposi's sarcoma,[12] uveitis,[13] strongyloidiasis[5] and mycosis fungoides.[14,15] HTLV-I is endemic in some Caribbean countries, Southern Japan, and possibly in some areas of Africa.[16-21] In the United States, HTLV-I has been identified in ATL patients, intravenous drug abusers, and in healthy individuals.

HTLV-II, a related virus, is endemic in several Amerindian tribes,[22-25] but has not been unequivocally proven to be a pathogen. A high rate of HTLV-II seropositives has been observed among intravenous drug abusers.[26-28] The first reported patients with HTLV-II infections presented with an atypical T-cell variant of hairy cell leukemia. More recent observations led to the assumption that HTLV-II may be associated with large granular lymphocyte leukemia (LGL),[29] leukopenic chronic T-cell leukemia,[30] T-prolymphocytic leukemia,[31] mycosis fungoides[14] and chronic neurodegenerative diseases[32,33] like myelopathy,[34] and spastic ataxia.[35] Antibodies to HTLV-II are significantly cross-reactive to HTLV-I antigens.

Transmission of HTLV-I and HTLV-II infections to transfusion recipients of infected cellular blood products is well documented. Other known modes of transmission include breast milk, sexual contact, and sharing of contaminated needles and syringes by intravenous drug abusers. Perinatal transmission is suspected but remains unproven.

PRINCIPLE OF THE TEST

Avioq HTLV-I/II Microelisa System is an enzyme-linked immunosorbent assay in which the solid phase (Microwells) is coated with a purified HTLV-I viral lysate, a purified HTLV-II viral lysate, and a recombinant HTLV-I p21E antigen.

With the addition of a diluted test sample containing antibodies to either HTLV-I or HTLV-II, complexes are formed by the interaction of the antibodies in the sample and the solid phase antigens. Following incubation, the sample is aspirated and the well is washed with buffer. Subsequently, goat anti-human immunoglobulins conjugated with horseradish peroxidase (HRP) are added which bind the antibody-antigen complex during a second incubation. Following a wash and incubation with TMB (Tetramethylbenzidine) substrate, a blue color is produced. The enzyme reaction is stopped by the addition of a sulfuric acid solution which changes the color to yellow. The amount of HTLV-I/or HTLV-II specific antibodies present in the sample is proportional to the color intensity.

REAGENTS

Components in each Avioq HTLV-I/II Microelisa System kit

192 tests	576 tests	9600 tests	
2 stripholders	6 stripholders	100 stripholders	**HTLV-I/II Microelisa Strips** – Twelve per holder, each containing 8 wells coated with inactivated HTLV-I viral lysate, a recombinant HTLV-I antigen (rp21E), and inactivated HTLV-II viral lysate; contained in a foil pouch with silica gel desiccant.
1 vial (50 mg)	2 vials (50 mg each)	4 vials (50 mg each)	**EnzAbody® for HTLV-I/II (EnzAbody Concentrate)** – Horseradish Peroxidase Conjugated Goat Anti-human Immunoglobulin, ~0.06% w/w or 30µg; lyophilized with goat serum, sucrose, and non-fat dry milk.
1 bottle (55 ml)	2 bottles (55 ml each)	28 bottles (55 ml each)	**EnzAbody® Diluent** – Phosphate buffered saline containing 10% goat serum and non-ionic surfactants. Preservatives: 0.2% gentamicin sulfate and 0.02% cinnamaldehyde.
1 bottle (100 ml)	2 bottles (100 ml each)	16 bottles (100 ml each)	**Sample Diluent** – Phosphate buffered saline containing 10% goat serum, non-ionic surfactants, sodium chloride, 0.14% bovine serum albumin, non-fat dry milk and amaranth dye. Preservative: 0.03% (w/v) bromonitrodioxane.
1 bottle (22 ml)	2 bottles (22 ml each)	34 bottles (22 ml each)	**TMB Solution** – Citric acid containing 0.03% tetramethylbenzidine•2HCl.
1 bottle (22 ml)	2 bottles (22 ml each)	34 bottles (22 ml each)	**Peroxide Solution** – Citric acid/sodium citrate buffer containing 0.04% urea peroxide.

192 tests	576 tests	9600 tests	
1 vial (1.5 ml)	2 vials (1.5 ml each)	17 vials (1.5 ml each) 	**Negative Control Serum** – Human serum with protein stabilizers; nonreactive by FDA licensed tests for antibodies to HTLV-I, HTLV-II, HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-Ag. Preservative: 0.05% (w/v) bromonitrodioxane.
1 vial (1.0 ml)	1 vial (1.0 ml)	12 vials (1.0 ml each) 	**HTLV-I Positive Control Serum** – Inactivated human serum with protein stabilizers and Amaranth red dye; reactive for antibodies to HTLV-I; nonreactive by FDA licensed tests for antibodies to HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-Ag. May cross-react with HTLV-II antigen. Preservative: 0.05% (w/v) bromonitrodioxane.
1 vial (1.0 ml)	1 vial (1.0 ml)	12 vials (1.0 ml each) 	**HTLV-II Positive Control Serum**– Inactivated human serum with protein stabilizers and Patent blue dye; reactive for antibodies to HTLV-II; nonreactive by FDA licensed tests for antibodies to HIV-1, HIV-2, HCV, and nonreactive for HBsAg and HIV-Ag. May cross-react with HTLV-I antigen. Preservative: 0.05% (w/v) bromonitrodioxane.
1 each	1 each	5 each	**Clamp and Rod (or Equivalent)** – Closure for foil pack.
10 sheets	20 sheets	30 sheets	**Plate Sealers** – Adhesive.

Note: The Wash Buffer Concentrate is provided as an accessory to the kit.

Wash Buffer Concentrate, Product number 559879, consists of 1 bottle (100 ml)

Wash Buffer Concentrate, Product number 559880, consists of 4 bottles (4X100 ml)

Note: The required Stop Solution is 2N Sulfuric Acid and is not provided by Avioq. Do not use any other Stop Solution for this assay.

WARNINGS AND PRECAUTIONS

1. **Caution: Handle all Avioq HTLV-I/II biological materials as though capable of transmitting infectious agents.** The antigens used to coat the microelisa wells have been inactivated for viruses by detergent disruption and the **HTLV-I** and **HTLV-II Positive Control Sera** have been inactivated by addition of detergent. The positive and negative controls are derived from human serum or plasma and have been tested for HIV-1 antigen, HBsAg, anti-HIV-1, anti-HIV-2, and anti-HCV, and have been found to be nonreactive by FDA licensed tests. As no test method can offer complete assurance that infectious agents are absent, all materials of human origin should be handled as though capable of transmitting infectious agents.
2. All test operators should adhere to the Occupational Safety and Health Administration (OSHA) regulations (29 CFR 1910.1030).
3. Keep testing area separate from areas in which blood or blood products for transfusion are stored.
4. Do not pipet any of the materials by mouth. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
5. Do not perform the test in the presence of reactive vapors (e.g., from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
6. Use disposable gloves and handle all materials used in the test (including samples, controls, wash solution, microelisa strips and stripholders, and pipets) cautiously as though capable of transmitting infectious agents. Consult a physician immediately in the event that materials are ingested or come in contact with open lacerations, lesions or other breaks in the skin, mucous membranes, or eyes.
7. Immediately clean up any spillage of material containing antigen or antibody using a 1:10 dilution of 5% sodium hypochlorite (final concentration 0.5%) or equivalent disinfectant to decontaminate. Dispose of cleaning material by an acceptable method.
8. Dispose of all materials that have come into contact with specimens and reagents in accordance with local, state, and federal regulations.[37] Solid wastes may be incinerated or autoclaved for an appropriate period of time. Due to variations among autoclaves and in waste configurations, each user must verify the effectiveness of this decontamination cycle using biological indicators.[38]

   Note: Liquid waste containing acid must be neutralized prior to the addition of disinfectants and/or disposal.
9. Some components of this kit contain small concentrations of hazardous chemicals (Wash Buffer Concentrate, Peroxide Solution, TMB Solution). Refer to the Material Safety Data Sheet (MSDS) for more specific information. Contact Avioq Inc. to obtain an MSDS.
10. **2N Sulfuric Acid** - Sulfuric Acid is corrosive and should be handled with care to prevent exposure to skin and eyes. If this reagent comes into contact with skin or eyes, wash thoroughly with water.
11. Use caution when assembling microplates for partial plate runs (mixing coated and uncoated strips). Some analyzers may not be able to differentiate between coated and uncoated wells and may produce results for any well position with an assigned ID number or control.

REAGENT PREPARATION

Prepare the following reagents before or during the assay procedure. Reagents and samples should be at room temperature (15-30°C) before dilution/preparation and before beginning the test and can remain at room temperature during testing. Sufficient working reagents must be prepared to complete the desired number of tests. Return reagents to 2-8°C after use.

Preparation of EnzAbody Concentrate

1. Pipet 4.0 ml **EnzAbody Diluent** into 1 vial of lyophilized **EnzAbody Concentrate**. Mix the contents thoroughly. Avoid excessive foaming. Allow **EnzAbody Concentrate** to rehydrate a minimum of 30 minutes after reconstitution. Do not handle **EnzAbody Concentrate** vial with gloves that have come in contact with serum or plasma.
2. Record preparation date and expiration date on the vial. The reconstituted EnzAbody Concentrate is stable for 5 weeks when stored at 2-8°C.

Preparation of the EnzAbody Working Solution

1. Clean, preferably disposable, polypropylene vessels should be used. **Do not use polystyrene containers**. When using an automated microplate processor, refer to the manufacturer's recommendations for container use. Transfer an appropriate amount of **EnzAbody Diluent** to the vessel and add an appropriate amount of reconstituted **EnzAbody Concentrate** to make a 1:251 **EnzAbody Working Solution** (see table below). Ensure the reconstituted **EnzAbody Concentrate** is well mixed before use. Return the unused reconstituted **EnzAbody Concentrate** to 2-8°C. Do not combine vials of **reconstituted EnzAbody Concentrate**. More **EnzAbody Working Solution** may be needed depending upon the reagent dispenser used.

Preparation of the EnzAbody Working Solution

Number of Microelisa Strips	Volume of Reconstituted EnzAbody Concentrate	Volume of EnzAbody Diluent
2	10 µl	2.5 ml
3	20 µl	5.0 ml
6	25 µl	6.25 ml
9	30 µl	7.5 ml
12	50 µl	12.5 ml

Number of Plates	Volume of Reconstituted EnzAbody Concentrate	Volume of EnzAbody Diluent
1	50 µl	12.5 ml
2	100 µl	25.0 ml
4	200 µl	50.0 ml
6	300 µl	75.0 ml
10	500 µl	125.0 ml

2. Once prepared, **EnzAbody Working Solution** is stable for four hours at room temperature. Record the preparation and expiration times of the **EnzAbody Working Solution**. Discard any unused **EnzAbody Working Solution** after completion of the assay.

Preparation of Wash Buffer

Important! The **Wash Buffer Concentrate** is formulated specifically for the Avioq HTLV-I/II assay. Do not use any other wash buffer for this assay.

1. Check the **Wash Buffer Concentrate** for the presence of crystals or precipitate. If crystals or precipitate have formed in the solution, resolubilize by warming it at 37°C until crystals or precipitate dissolve. Mix the **Wash Buffer Concentrate** before diluting.
2. Dilute the **Wash Buffer Concentrate** 1:25 with purified water,[39] according to the table below.

Preparation of Wash Buffer

Number of Microelisa Strips	Volume of Wash Buffer Concentrate	Volume of Purified Water	Total Volume of Wash Buffer
1-6	7 ml	168 ml	175 ml
7-12	14 ml	336 ml	350 ml

Number of Plates	Volume of Wash Buffer Concentrate	Volume of Purified Water	Total Volume of Wash Buffer
1	14 ml	336 ml	350 ml
2	28 ml	672 ml	700 ml
4	56 ml	1344 ml	1400 ml
6	84 ml	2016 ml	2100 ml
10	140 ml	3360 ml	3500 ml

The total volume of **Wash Buffer** does not include any additional volume required for an automated washer (priming, dead volume, etc.). Refer to the manufacturer's instructions for the microelisa plate washer.

3. **Wash Buffer** is stable for two weeks when stored at 2-30°C. Record the date of preparation and the expiration date.

Preparation of TMB Substrate 

Prepare **TMB Substrate** in a clean, preferably disposable, polypropylene container. **Do not use polystyrene containers**. Transfer a sufficient amount of **Peroxide Solution** to a container, add an appropriate amount of **TMB Solution** to **Peroxide Solution** and mix thoroughly prior to use (see table below).

Each microwell plate requires at least 10 ml of **TMB Substrate**. More **TMB Substrate** may be needed depending upon the reagent dispenser used. See the instrument manufacturer's instructions for additional reagent requirements.

Preparation of TMB Substrate

Number of Microelisa Strips	Volume of TMB Solution	Volume of Peroxide Solution
2	1 ml	1 ml
3	2 ml	2 ml
6	3 ml	3 ml
9	5 ml	5 ml
12	6 ml	6 ml

Number of Plates	Volume of TMB Solution	Volume of Peroxide Solution
1	6 ml	6 ml
2	12 ml	12 ml
4	24 ml	24 ml
6	36 ml	36 ml
10	60 ml	60 ml

The **TMB Substrate** is stable for 6 hours when held at room temperature and should be colorless when used. Record the preparation and expiration times. If it is noticeably blue in color, discard and prepare more **TMB Substrate** as required.

Note: **TMB Solution** and **TMB Substrate** should be protected from exposure to light. Avoid contact with metal or metal ions as it may result in unwanted blue color formation.

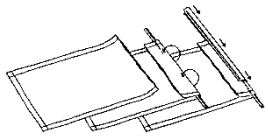
KIT STORAGE INSTRUCTIONS

Store all components at 2-8°C when not in use. Expiration date printed on the kit indicates the date beyond which the product should not be used. Stability of kit reagents after reconstitution or dilution is listed in "REAGENT PREPARATION." Do not store frozen.

HTLV-I/II MICROELISA STRIPS

The foil pack should be brought to room temperature (15-30°C) before opening to prevent condensation on the **Microelisa Strips**. After the airtight foil pack has been opened, the **Strips** are stable for 4 weeks at 2-8°C, if the foil pack is resealed with the clamp and rod provided or equivalent. Record the date of opening and the expiration date on the foil pack. **The silica gel bag must not be removed.**

Figure 1: Foil pack closure.



1 2 3
Fold open end of foil pack over rod.
Apply clamp.

CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY

Alterations in the physical appearance of test kit materials may indicate instability or deterioration. Expiration dates shown on kit reagent labels indicate the date beyond which the product should not be used.

If **TMB Substrate** is noticeably blue in color, discard and prepare more **TMB Substrate** as required.

SPECIMEN COLLECTION, STORAGE AND PREPARATION

Living Donor Specimens

Collection: No special preparation or fasting of the patient is necessary. Serum or plasma derived from heparin, citrate, CPD, CPDA-1, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used. Refer to instructions provided by the manufacturer of sample collection tubes for information regarding the correct ratio of specimen volume to anticoagulant to be used. Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. Samples from serum separator tubes and segmented blood bag tubing may be used. The Avioq HTLV-I/II Microelisa System is not affected by elevated levels of lipids (3000 mg/dl), total bilirubin (20 mg/dl), rheumatoid factor, or hemoglobin (3051 mg/dl). Samples may be heat inactivated for 30 minutes at 56°C with no loss of reactivity. Performance has not been established for other specimen types, including pleural fluid, saliva, oral fluid, dried blood spot eluates, and non-human specimens.

Storage: Specimens should be free of microbial contamination and may be stored at 2-8°C for up to 14 days. For long term storage, specimens should be frozen at -20°C. Specimens should be mixed after thawing. Specimens may be frozen and thawed once with no loss of reactivity. However, specimens repeatedly frozen and thawed or those containing particulate matter may give erroneous results.

Shipment: Specimens to be shipped must be packaged in compliance with applicable regulations governing the transport of etiologic agents. Specimens may be shipped ambient, refrigerated (2-8°C), or frozen (-20°C or colder). Upon receipt, specimens should be stored at the recommended storage temperature described above.

Cadaveric Donor Specimens

Collection: Cadaveric specimens may be collected from serum, serum-separator tubes, or EDTA plasma**.** Clear non-hemolyzed samples are preferred. Precipitates in specimens should be removed by centrifugation.

Storage: Cadaveric specimens may be stored up to 14 days at 2-8°C and at -20°C undergoing 4 freeze/thaw cycles. Mix thoroughly after thawing and before testing.

Shipment: Specimens to be shipped must be packaged in compliance with applicable regulations governing the transport of etiologic agents. Specimens may be shipped at -20C to 30C. Upon receipt, specimens should be stored at the recommended storage temperatures not to exceed a total of 14 days, shipping time included.

AVIOQ HTLV I/II MICROELISA TEST SYSTEM PROCEDURE

Materials provided

HTLV-I/II Microelisa Strips

EnzAbody® for HTLV-I/II

EnzAbody® Diluent

Sample Diluent

TMB Solution

Peroxide Solution

Negative Control Serum

HTLV-I Positive Control Serum

HTLV-II Positive Control Serum

Clamp and Rod

Plate Sealers

Additional materials required but not provided

Instruments/Equipment

Note: For any instrument, the manual provided by the manufacturer should be reviewed for additional information regarding the following:

a) Installation and special requirements.
b) Operation principles, instructions, precautions, and hazards.
c) Manufacturer's specifications and performance capabilities.
d) Service and maintenance information.
e) Quality control.

Automated diluter/dispenser system or equivalent

Aspiration/wash system

The aspiration/wash system must be capable of dispensing a minimum volume of 300 µl, and capable of performing a 30 ± 5 second soak cycle. Aspirated waste must be contained in a closed system.

Adjustable multi-channel variable volume pipet system capable of delivering 50 – 300 µl ± 5 %, and tips

Micropipet(s) capable of delivering 20 µl ± 5%, 100 µl ± 5%, and tips

Incubator - A dry incubator, heating block or equivalent, capable of maintaining 37 ± 2 ºC.

Microelisa plate reader

Any microelisa reader capable of transmitting light at 450 nm ± 5 nm or dual wavelength 450 nm ± 5 nm and 620/630 nm ± 5 nm as reference, with a linear absorbance range of 0 to 2.000, a drift of less than 0.005% AU/hr, and a bandwidth at half height of 10 ± 2 nm may be used.

Timer

Graduated cylinder, 50 ml and 1-2.5L or equivalent

Reagents/Disposables

Wash Buffer Concentrate (Product number 559879)

2N Sulfuric Acid

Purified water,[39] USP, NCCLS Type I[41] or equivalent

Stripholder with uncoated wells (Product number 259576)

Absorbent paper

V-shaped disposable troughs or equivalent

Disposable gloves

Sodium hypochlorite solution (5%), liquid bleach, or equivalent disinfectant

Appropriate biohazard waste containers for materials potentially contaminated with infectious agents

Disposable polypropylene capped tubes (15 or 50 ml) or equivalent

Materials available from Avioq Inc.

Wash Buffer Concentrate 100 ml bottle (Product number 559879)

Wash Buffer Concentrate 4 X 100 ml bottles (Product number 559880)

Procedural notes

1. Inadequate adherence to package insert instructions may result in erroneous results or invalid assays.
2. **Microelisa Strips, EnzAbody for HTLV-I/II, EnzAbody Diluent, Sample Diluent, TMB Solution, Peroxide Solution,** and **Controls** used in an assay must be of the same master lot number. The Wash Buffer is not master kit lot number specific and may be used with any master kit lot number. Materials should be used before the expiration date shown on the package label. Components and test specimens should be at room temperature (15-30°C) and mixed (as applicable) before testing begins. Return the reagents to 2-8°C after use. Do not store frozen.
3. **Strips** of the microelisa plate are removable. Store unused **Strips** as described in "KIT STORAGE INSTRUCTIONS." Before testing begins, inspect the microelisa stripholder and ensure that all strips are secure. Stripholders should be handled with care to ensure that no strip is dislodged during testing. **Microelisa Strips** may be numbered to ensure re-insertion should strips become dislodged.
4. **Microelisa Strips** and **Plate Sealers** may be used only once.
5. To avoid contamination, do not touch the top of the **Strips** or the edge of the wells with fingers or pipet tips.
6. All reagents and specimens must be mixed well before use. The **Positive** and **Negative Controls** may be vortexed before pipetting. The **Positive** and **Negative Controls** should be dispensed and diluted in the same manner as specimens. One of each **Positive Control** and three **Negative Controls** must be run on each plate (stripholder). If more than one stripholder is processed, you must ensure that all specified incubation times are met.
7. All pipetting steps should be performed with the utmost care and accuracy. Cross-contamination between reagents will invalidate test results. Use micropipets for quantitative delivery of samples and reagents. For the manual pipetting of controls and specimens, use individual, disposable specimen tips to prevent carryover of specimens. Avoid microbial or any other contamination of reagents.
8. If a specimen is inadvertently not added in this assay, e.g., a well is missed; the assay results for this specimen may be incorrectly interpreted as nonreactive.
9. Refrain from opening the door of the incubator (37°C) during the incubation time.
10. Avoid chemical contamination of reagents and equipment. Routine maintenance of the aspiration/wash system is strongly recommended to prevent carryover of antibody from highly reactive specimens to nonreactive specimens.
11. Microplate washers should be flushed with copious amounts of water upon completion of the final wash of the assay. Refer to manufacturer's recommendations for the maintenance of the liquid handling system for automated microplate processors.
12. Manual plate washing should be validated before use. Use of an automated plate washer is recommended (refer to **Additional materials required but not provided** for automated washer requirements). Incomplete washing will adversely affect the test outcome.
13. The assay run must proceed to completion without interruption and within the time limits set in the package insert.
14. Do not return leftover reagents to their original bottles.

15. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells.
16. Ensure that the **Microelisa Strips** are level in the Microelisa stripholder during the test procedure. If necessary, wipe the bottom of the **Microelisa Strips** carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading. If necessary, dried buffer may also be removed from the bottom of the **Microelisa Strips** by wiping the bottom of the **Microelisa Strips** with a soft cloth dampened with water, then with a dry, soft, lint-free tissue before reading.
17. Negative or positive control values which are not within the expected range (refer to Quality Control section) may indicate a technique problem or product deterioration.
18. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
19. The microelisa plate reader may contain a 620 nm or 630 nm reference filter. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched or irregular may cause inaccurate readings.
20. Bubbles in the **Microelisa Strip** wells may cause inaccurate microwell readings. Care should be taken to make sure no bubbles are present.
21. Use only properly calibrated equipment.

Wash procedure

1. Incomplete washing will adversely affect the test outcome. **Wash Buffer** must be at room temperature (15-30°C) before use.
2. Aspirate the well contents into a waste flask. Then fill the wells (approximately 0.3 ml) with **Wash Buffer** and allow to soak 30 ± 5 seconds, unless otherwise validated. Aspirate and repeat the wash and soak procedure three additional times for a total of four washes.
3. Ensure the **Microelisa Strips** are completely aspirated after the final aspiration. Invert the stripholder and tap firmly on a clean paper towel to absorb excess **Wash Buffer**, if necessary.

Test procedure

1. Fit the stripholder with the required number of **Microelisa Strips.** If fewer than twelve **Strips** are needed, use uncoated strips to complete the plate when using a 96-well washer.
2. Prepare a 1:5 dilution of each test sample and **Control** using one of the procedures listed below. Include three wells of the **Negative Control**, one well of the **HTLV-I Positive Control** and one well of the **HTLV-II Positive Control** on each plate regardless of the number of strips used. Mix **Controls** thoroughly (e.g. vortexing) before pipetting or aliquoting for automated use.

   Caution: Do not allow the **Microelisa Strip** wells to dry once the assay has begun.

Direct Sample Addition

Manual method: Using a calibrated pipet, add 80 µl **Sample Diluent** into each microelisa test well. Add 20 µl sample or **Control** with a disposable micropipet tip. Mix the sample with **Sample Diluent** by repeatedly aspirating and dispensing the sample (at least 3-4 times) with each addition.

Automated method: The calibrated diluter/dispenser should be programmed to deliver a 1:5 dilution, typically 20 µl sample or **Control** with 80 µl **Sample Diluent** into each **Microelisa Strip** well. Pipette tip retention should be calculated, verified and built into the programming.

Note: Sample may be added to **Sample Diluent** as described in the manual method.

Indirect Sample Addition

Manual method: Pipet 120 µl **Sample Diluent** into a clean test tube followed by 30 µl sample or **Control**. Mix the contents well. Diluted samples in capped tubes may be stored up to 24 hours at 2-8°C but must be at room temperature (15-30°C) at the time of testing. Pipet 100 µl of the diluted sample into each **Microelisa Strip** well.

3. Cover the **Strips** with adhesive plate sealers or equivalent. If using **Plate Sealers**, ensure that all wells are covered. Within 30 minutes, incubate at 37 ± 2°C for 60 ± 5 minutes.
4. After incubation, discard plate sealer after use, if applicable. Do not reuse. Wash and soak each well four times with **Wash Buffer** (refer to "Wash Procedure").
5. Pipet 100 µl of **EnzAbody Working Solution** into each well. (Refer to "REAGENT PREPARATION" for instructions).

   Caution: Do not allow reconstituted **EnzAbody Concentrate** or **EnzAbody Working Solution** to contaminate **TMB Substrate**. If the same equipment is used to add both reagents, new disposable tips must be used.

6. Cover the **Strips** with a new **Plate Sealer** or equivalent. If using **Plate Sealers**, ensure that all wells are covered. Incubate at 37 ± 2°C for 60 ± 5 minutes.

   When used with the ORTHO® Summit System (OSS), incubate at 37 ± 2°C for 30 ± 5 minutes.
7. After incubation, discard **Plate Sealer** after use, if applicable. Do not reuse. Wash and soak each well four times with **Wash Buffer**. Refer to "Wash Procedure."
8. Pipet 100 µl of **TMB Substrate** into each well. Do not cover with an adhesive plate sealer. (Refer to "REAGENT PREPARATION" for instructions.)
9. Incubate at room temperature (15-30°C) for 30 ± 5 minutes.
10. Stop the reaction by adding 100 µl of **2N Sulfuric Acid** to each well (maintain the same sequence and time intervals used for **TMB Substrate** addition). **Plates should be read within two hours.**
11. Blank the microelisa reader on air (without stripholder and **Strips**) and read the absorbance of the solution in each well at 450 nm ± 5 nm (single wavelength) or 450 nm ± 5 nm and 620/630 nm ± 5 nm as reference (dual wavelength).

Quality Control

Qualification of Negative Control (NC) values: Absorbance of NC must be greater than or equal to 0.000 and less than or equal to 0.120. Eliminate any NC value outside this range. If two or more values are less than 0.000 or greater than 0.120, the run is invalid and should be repeated. Calculate the NC mean (NC*X*) of the remaining control values.

Qualification of HTLV-I (PC-I) and HTLV-II (PC-II) Positive Control values: Absorbance of PC-I and PC-II must be greater than or equal to 0.500. If the PC-I value and/or the PC-II value is below the specification, the run is invalid and must be repeated.

Test Validity: A test run is valid if the Positive and Negative Control values are qualified and

(PC-I) - NC*X* ≥ 0.380 (PC-II) - NC*X* ≥ 0.380

If results do not meet these criteria, technique may be suspect and the run is invalid and must be repeated.

RESULTS

Calculations

Calculations must be performed separately for each stripholder.

Cutoff Value: If the test run is valid, calculate the Cutoff Value as follows:

Cutoff Value = NC*X* + 0.330

A test sample is nonreactive if sample absorbance is greater than or equal to 0.000 and less than the Cutoff Value.

A test sample is reactive if sample absorbance is greater than or equal to the Cutoff Value.

Sample calculations

Absorbance (Single Wavelength)

NC	=	0.065, 0.070, 0.075
NC*X*	=	0.070
PC-I	=	1.110
PC-II	=	1.050

Absorbance (Dual Wavelength)

NC	=	0.034, 0.030, 0.035
NC*X*	=	0.033
PC-I	=	1.073
PC-II	=	1.013

Acceptance Criteria

Eliminate any Control absorbance values not meeting the following criteria:

0.000 ≤ NC ≤ 0.120	None eliminated
PC-I ≥ 0.500	None eliminated
PC-II ≥ 0.500	None eliminated

Ensure that the following is within specified acceptance criteria.

(Single Wavelength)

(PC-I) - NC*X* ≥ 0.380

(PC-II) - NC*X* ≥ 0.380

1.110 - 0.070 = 1.040	Pass
1.050 - 0.070 = 0.980	Pass

(Dual Wavelength)

1.073 – 0.033 = 1.040	Pass
1.013 – 0.033 = 0.980	Pass

Test Validity (Single Wavelength)

Pass

Test Validity (Dual Wavelength)

Pass

Calculate Cutoff Value (Single Wavelength)

Cutoff Value	=	NC*X* + 0.330
	=	0.070 + 0.330
	=	0.400

Calculate Cutoff Value (Dual Wavelength)

Cutoff Value	=	NC*X* + 0.330
	=	0.033 + 0.330
	=	0.363

INTERPRETATION OF RESULTS

1. Specimens with absorbance values equal to or greater than 0.000 and less than the Cutoff Value are considered nonreactive by the criteria of Avioq HTLV-I/II and may be considered negative for antibodies to HTLV-I and HTLV-II. A negative test result does not exclude the possibility of exposure to or infection with HTLV-I/II.
2. Specimen results having absorbance values below 0.000 must be retested singly to verify the initial result. 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 Avioq HTLV-I/II.
3. Specimens with absorbance values equal to or greater than the Cutoff Value are considered initially reactive by the criteria of Avioq HTLV-I/II, but before interpretation the sample should be retested in duplicate using the Avioq HTLV-I/II assay. If either duplicate retest is reactive, the specimen is considered repeatedly reactive for antibodies to HTLV-I and/or HTLV-II by the criteria of Avioq HTLV-I/II Microelisa System.
4. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HTLV-I/II.
5. In most settings, it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests (**LIMITATIONS OF PROCEDURE**). Specimens found repeatedly reactive by ELISA and positive by additional, more specific testing are considered positive for antibodies to HTLV-I and/or HTLV-II. The interpretation of results of specimens found repeatedly reactive by ELISA and negative or indeterminate on additional more specific testing is unclear; further clarification may be obtained by testing another specimen taken from the same individual three to six months later.

LIMITATIONS OF PROCEDURE

The Avioq HTLV-I/II Microelisa System "TEST PROCEDURE" and "INTERPRETATION OF RESULTS" must be followed closely when testing for the presence of antibodies to HTLV-I and/or HTLV-II in plasma or serum from individual subjects. This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens and from serum or plasma specimens collected from cadaveric donors. Performance characteristics have not been established for other specimen types (i.e., pleural fluid, saliva, oral fluid, dried blood spot eluates, non-human specimens etc.), pooled blood or processed plasma, and products made from such pools.

Failure to add specimen as instructed in the "TEST PROCEDURE" could result in a falsely negative test.

The Avioq HTLV-I/II Microelisa System detects antibodies to HTLV-I and/or HTLV-II in blood and thus is useful as a screen for donated blood to prevent transmission of HTLV-I and/or HTLV-II to recipients of cellular blood components, and as an aid in the clinical diagnosis of HTLV-I or HTLV-II infection and related diseases. It is known that HTLV-I infection acquired by transfusion of infected blood products may result in disease in recipients.[36]

Guidelines[40] 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.

A person whose serum or plasma is found to react in both the ELISA and an additional more specific test is presumed to be infected with the HTLV-I or HTLV-II virus. The medical implications of HTLV-II seropositivity are not known. Appropriate counseling and medical evaluation should be offered, consistent with published guidelines of the Public Health Services.[40] Such an evaluation should be considered an important part of HTLV-I/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. Testing with the Avioq HTLV-I/II Microelisa System alone cannot be used to diagnose these conditions, even if the recommended investigation of reactive specimens confirms the presence of HTLV-I antibodies. A negative test result at any point in the serologic investigation does not preclude the possibility of exposure to or infection with HTLV-I or HTLV-II. Repeat testing using the Avioq HTLV-I/II Microelisa System should be considered where there is clinical suspicion of HTLV-I or HTLV-II infection. Negative results in this assay for 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.

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

EXPECTED RESULTS

The percentage of specimens determined to be repeatedly reactive in a normal donor population differs depending on the prevalence of HTLV-I or HTLV-II antibodies in that geographical area. Areas of high prevalence for HTLV-I include: parts of Africa, Micronesia, Japan, the Hawaiian Islands, and the Caribbean Islands. Areas of high prevalence for HTLV-II include: intravenous drug abuser (IVDA) populations and various Amerindian tribes in North and South America. Experience with blood donors screened in the U.S. is shown in the Specificity section. Experience in testing populations with HTLV-I disease or at high risk for HTLV-I or HTLV-II is summarized in the Sensitivity and Endemic populations sections.

PERFORMANCE CHARACTERISTICS OF THE ASSAY

Reproducibility

Replicates of HTLV-I and HTLV-II antibody-positive specimens with various degrees of reactivity, negative specimens, and kit controls were tested at multiple sites (n=3), using multiple kit lots (n=3) and multiple technicians (n=2) on multiple days (n=4). Total, inter-assay, and intra-assay precision is reported in Table 1 below.

Table 1: Assay Reproducibility

			Total		Inter-assay		Intra-assay	
ID	**N**	**Mean**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
HTLV-I S1	288	2.93	0.346	11.8	0.328	11.2	0.115	3.9
HTLV-I S2	288	1.95	0.246	12.6	0.231	11.8	0.090	4.6
HTLV-I S3	288	1.55	0.228	14.7	0.214	13.8	0.080	5.2
HTLV-I S4	288	1.62	0.206	12.7	0.196	12.1	0.067	4.1
HTLV-I S5	288	0.19	0.024	12.6	0.018	9.5	0.016	8.4
HTLV-II S1	288	3.13	0.348	11.1	0.331	10.6	0.113	3.6
HTLV-II S2	288	2.13	0.270	12.7	0.241	11.3	0.123	5.8
HTLV-II S3	288	2.16	0.248	11.5	0.234	10.8	0.084	3.9
HTLV-II S4	288	1.41	0.201	14.3	0.184	13.0	0.082	5.8
HTLV-II S5	288	0.18	0.029	15.9	0.020	11.1	0.021	11.7
NC	216	0.18	0.027	15.0	0.021	11.7	0.018	10.0
PC HTLV-I	72	2.69	0.338	12.6				

ID Panel Member Identification
N Number of Replicates
MEAN Mean Signal to Cutoff Ratio (SCR)
SD Standard Deviation of SCR
CV Coefficient of Variation of SCR

Specificity

The specificity of this assay was assessed by testing 11415 normal human serum and plasma samples collected at multiple sites. A breakdown of the data, by site and specimen type, is summarized in Table 2 below. Based on an assumed zero prevalence of HTLV-I and HTLV-II antibodies in normal human donors, the overall estimated specificity of this assay was 99.95% (95% confidence limits of 99.89% to 99.98%). Also included for each population are 95% confidence limits for repeat reactive rate and estimated specificity for each site and sample type.

Table 2: Estimated Specificity in Whole Blood and Plasma Random Donor and Source Plasma Populations

	Number Tested	Initial Non-reactive	Initial Reactive	Repeat Reactive	Repeat Reactive (%)	Repeat Reactive 95% Confidence Limits** (%)		Supplemental Test Positive***	*Estimated Specificity (%)	Specificity 95% Confidence Limits** (%)	
Serum Site 1	1315	1314	1	1	0.08	0.004	0.334	0	99.92	99.58	99.99
Serum Site 2	3754	3753	1	1	0.03	0.002	0.117	0	99.97	99.85	99.99
Plasma Site 1	1255	1255	0	0	0.00	0.000	0.153	0	100.00	99.71	100.00
Plasma Site 2	3812	3809	3	3	0.08	0.020	0.204	0	99.92	99.77	99.98
Source Plasma Site	1279	1276	3	1	0.08	0.004	0.344	0	99.92	99.57	99.99

* = $\frac{\text{(Number Screened – Number Repeat Reactive) X 100}}{\text{(Number Screened – Number Confirmed Positive)}}$

** = Confidence limits for specificity were calculated using the exact method.

*** = A positive result in these studies was defined by the presence of antibodies to two gene products (gag, p19 and/or p24 and env, gp46 and/or 61/68) using Western Blot and/or RIPA.

Additional supplemental tests and HTLV-I and HTLV-II type differentiation were performed using the following research use assays: reactivity to the recombinant or native gp46-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, HTLV-I and HTLV-II IFA, and/or PCR (using specific primers to the *tax* and *pol* regions).

Reactivity with potentially interfering medical conditions

Samples from individuals with medical conditions that may cause nonspecific assay reactivity were tested with this assay. Specimens tested are shown in Table 3 below. All specimens were non-reactive.

Table 3: Reactivity in Specimens from Individuals with Medical Conditions Unrelated to HTLV-I or HTLV-II Infection

Specimen Category	Number of Specimens Tested	Number of Specimens Initially Reactive
Cytomegalovirus antibodies	10	0
Epstein-Barr virus antibodies	10	0
Herpes simplex virus antibodies	10	0
HIV-1 antibodies	10	0
HIV-2 antibodies	10	0
Hepatitis B virus surface antigen	10	0
Syphilis antibodies	10	0
Antinuclear antibodies	10	0
Multiple transfusions	10	0
Multiparous females	10	0
Rheumatoid factor	10	0
HCV antibodies	10	0
Hypergammaglobulinemia IgG	10	0
Hypergammaglobulinemia IgM	10	0
Toxoplasmosis antibodies	10	0
Influenza vaccine recipients	36	0

Reactivity in specimens with potentially interfering substances

Specimens which were lipemic (n=10), hemolyzed (n=10) or contained elevated bilirubin (n=10), were tested for non-specific reactivity in this assay. All specimens were nonreactive. In addition, since this assay incorporates a recombinant protein produced in *E. coli*, a series of twenty-two (22) samples previously determined to be positive for the presence of antibodies to *E. coli* was tested to assess the potential for cross-reactivity in the test system. All specimens were non-reactive.

Sensitivity

The sensitivity of this assay was assessed by evaluating samples found to be seropositive by research use supplemental tests (Western blot, RIPA, IFA, and, in some cases, PCR). These samples were from populations with HTLV associated diseases, intravenous drug abuser (IVDA) populations, and HTLV infected blood donors. A listing of the results is provided in Table 4 below. This assay was reactive with all 636 of the research use supplemental test positive samples. This assay has an estimated sensitivity of 100% (interval of 99.97% to 100%) for 636 research use supplemental test positive specimens by the binomial distribution at 95% confidence.

Table 4: Reactivity with Supplemental Test HTLV-I, HTLV-II, and HTLV-I/II Antibody Positive Specimens

Group	Supplemental Test Result[a]	No. Tested	No. Repeatedly Reactive with Licensed HTLV-I Tests	No. Repeatedly Reactive with Avioq HTLV-I/II
Adult T-Cell Leukemia	HTLV-I	47	47	47
Tropical Spastic Paraparesis	HTLV-I	43	43	43
Nasopharyngeal Lymphoma	HTLV-I	1	1	1
Intravenous Drug Abusers	HTLV-I HTLV-II	5 95	5 94[c]	5 95
Hospital Patients[b]	HTLV-I HTLV-II	107 38	107 38	107 38
Blood Donors	HTLV-I HTLV-II HTLV-I/II	146 138 16	146 138 16	146 138 16
TOTAL		**636**	**635**	**636**

[a] A positive result in these studies was defined by the presence of antibodies to two gene products (gag, p19 and/or p24 and env, gp46 and/or 61/68) using Western Blot and/or RIPA.

Additional supplemental tests and HTLV-I and HTLV-II type differentiation were performed using the following research use assays: reactivity to the recombinant or native gp46-I or gp46-II peptides on a Western Blot, HTLV-I and HTLV-II peptide EIAs, HTLV-I and HTLV-II IFA, and/or PCR (using specific primers to the *tax* and *pol* regions).

[b] Asymptomatic and some symptoms indicative of HTLV disease.

[c] The licensed HTLV-I test missed one IVDA (signal/cutoff values 0.8, 0.9, 0.9) that was indeterminate by Western Blot (p21 only) and typed as HTLV-II by PCR.

Populations in HTLV-I and HTLV-II Endemic Areas

Performance of this assay was evaluated with specimens from one population in an HTLV-I endemic area and from two populations in HTLV-II endemic areas. For the population from the HTLV-I endemic area, 532 specimens from a high prevalence Pacific Islands population were evaluated. There were 20 specimens from this population that were found to be HTLV-I antibody positive by research use supplemental methods (Western blot, IFA, or RIPA). All 20 of the HTLV-I antibody positive specimens were repeatedly reactive by this assay and by a licensed HTLV-I assay. An additional 3 specimens which were repeatedly reactive by the licensed HTLV-I test were non-reactive in this assay; none of these 3 specimens was found to be HTLV-I antibody positive in the supplemental tests used. Results of this study are summarized in the table below.

In the studies of the HTLV-II endemic areas, a total of 525 specimens were obtained from a Native American population in New Mexico (361) and from an Amazonian tribe, Kayapo, in Brazil (164). From both populations, 60 specimens were found to be HTLV-II positive by research use supplemental tests (Western blot, IFA, RIPA, or, in some cases, PCR). Of the specimens, 58 were repeatedly reactive with this assay. The two specimens reported non-reactive by this assay were from the Kayapo Indian population. All HTLV-II specimens positive by supplemental testing from the Native American population were repeatedly reactive in this assay. One specimen from the Native American population was repeatedly reactive in this assay, but was not supplemental test (Western blot) positive for HTLV-II antibody. Results of this study are summarized in Table 5 below.

Table 5: Reactivity with Specimens from Populations in HTLV-I and HTLV-II Endemic Areas

Endemic Area	No. Tested	No. Positive by Supplemental Tests[a]	No. Repeatedly Reactive	No. (Percent) of Supplemental Test Positive that were EIA Repeatedly Reactive
Pacific Islands (HTLV-I)	532	20[b]	20	20 (100%)
New Mexico (HTLV-II)	361	7[c]	8	7 (100%)
Brazil (HTLV-II)	164	53[c]	51[d]	51 (96.2%)

[a] A positive result in these studies was defined by the presence of antibodies to two gene products (gag, p19 and/or p24 and env, gp46 and/or 61/68) using Western Blot and/or RIPA.

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

[b] The number of supplemental test positive specimens was based on HTLV-I/HTLV-II Western blot, IFA and RIPA research test results of any specimen that was repeatedly reactive or was initially reactive within a 20% negative gray zone by ELISA.

[c] The number of supplemental test positive specimens was based on HTLV-I/HTLV-II Western blot, IFA and RIPA research test results of all specimens (361 from New Mexico and 164 from Brazil).

[d] The two samples were Western Blot indeterminate, positive for HTLV antibodies by RIPA, positive for HTLV-II antibodies by IFA, and positive for HTLV DNA by PCR.

PERFORMANCE CHARACTERISTICS OF ASSAY MANUFACTURED AT NEW SITE

The Avioq HTLV-I/II Microelisa System is identical to the Vironostika® HTLV-I/II Microelisa System previously manufactured by bioMerieux, Inc., with a change in the manufacturing location. Studies conducted to evaluate the performance of the Avioq HTLV-I/II Microelisa System manufactured at the new site demonstrated that the assay manufactured at the new site has reproducibility, specificity and sensitivity that are comparable to test kits manufactured at the original site.

Reproducibility

In order to demonstrate that the reproducibility of the Avioq HTLV-I/II Microelisa System manufactured at the new site, a panel consisting of HTLV-I and HTLV-II antibody-positive specimens with varying degrees of reactivity (four HTLV-I and four HTLV-II) and two negative specimens were tested with each of the three kit lots over a four day period using two analysts using the manual test method. Each sample was tested in quadruplicate on each of the four days. The total CV for the positive specimens using the three validation lots ranged from 8.9 – 19.7% (n=96) compared to the total CV range for the positive specimens of 11.1 – 15.9% for the assay manufactured at the previous location (n=288, 3 sites, 3 lots, 2 operators, 4 days and testing performed in quadruplicate) .

Table 6: Summary of Reproducibility Study for the Avioq HTLV-I/II Microelisa System (Manual Method)

				Total				Inter-Assay		Intra-Assay	
Panel ID	**Status**	**N**	**Mean S/C**	**SD**	**%CV**	**S/C Lower 95% CI**	**S/C Upper 95% CI**	**SD**	**%CV**	**SD**	**%CV**
HTLV-I S1	Pos	96	3.10	0.276	8.9	3.04	3.15	0.244	7.9	0.136	4.4
HTLV-I S2	Pos	96	2.92	0.300	10.3	2.85	2.98	0.246	8.4	0.176	6.0
HTLV-I S3	Pos	96	2.50	0.312	12.5	2.44	2.56	0.259	10.4	0.180	7.2
HTLV-I S4	Pos	96	2.18	0.226	10.4	2.13	2.22	0.190	8.7	0.126	5.8
HTLV-I S5[a]	Neg	96	0.25	0.023	9.2	0.24	0.25	0.020	8.0	0.012	4.8
HTLV-II S1	Pos	96	3.29	0.561	17.1	3.17	3.40	0.482	14.7	0.298	9.1
HTLV-II S2	Pos	96	3.15	0.569	18.1	3.03	3.26	0.537	17.1	0.210	6.7
HTLV-II S3	Pos	96	2.46	0.486	19.7	2.36	2.56	0.462	18.8	0.170	6.9
HTLV-II S4	Pos	96	2.22	0.364	16.4	2.14	2.29	0.300	13.5	0.214	9.6
HTLV-II S5[a]	Neg	96	0.23	0.020	8.7	0.22	0.23	0.016	7.2	0.012	5.4

[a] Specimen negative for antibodies to HTLV-I and antibodies to HTLV-II

Specificity

In order to demonstrate that the clinical specificity of the Avioq HTLV-I/II Microelisa System manufactured at the new site was comparable to that of the originally licensed test, serum (n = 1000) and plasma (n = 1000) samples of unknown status from low risk populations (blood donors) were tested with three kit lots using the manual test method. Each lot was used to test similar numbers of specimens. Of the 2000 specimens tested, two were repeatedly reactive (see Table 7). Both specimens were negative by a research use HTLV-I and HTLV-II IFA and Western blot. Therefore, the estimated specificity of the Avioq assay observed in this study was 1998/2000 = 99.90% (95% CI 99.44 – 100%), compared to 11409/11415 = 99.95 % (95% CI of 99.89 – 99.98%) for the assay manufactured at the previous location.

Table 7: Estimated Specificity of the Avioq HTLV-I/II Microelisa System in Random Blood Donors

	Number Tested	Non-reactive	Repeatedly Reactive	Supplemental Test Positive	Estimated Specificity (%)	Specificity 95% Confidence Limits (%)	
Serum	1000	999	1	0	99.90	99.44	100.00
Plasma	1000	999	1	0	99.90	99.44	100.00

Sensitivity

In order to evaluate whether the clinical sensitivity of the Avioq HTLV-I/II Microelisa System manufactured at the new site was comparable to that of the originally licensed test, a panel of 200 seropositive serum or plasma repository samples (100 HTLV-I and 100 HTLV-II) was tested on three kit lots using the manual test method (Table 8). All specimens were previously found to be repeatedly reactive on an FDA licensed HTLV-I/II donor screening test and confirmed positive for HTLV-I/II antibodies with a research use supplemental test (WB, IFA, and/or RIPA). Eighty-seven percent of these specimens were from US blood donors and none were previously tested using the original licensed Vironostika assay. The estimated sensitivity of the assay observed in this study was 200/200 = 100% (95% CI 98.17 – 100%) compared to 636/636 = 100% (95% CI of 99.97 – 100%) %) for the assay manufactured at the previous location.

Table 8: Reactivity of the Avioq HTLV-I/II Microelisa System with HTLV-I/II Seropositive Repository Samples

Number Tested	Number Repeatedly Reactive	NumberNon-reactive	EstimatedSensitivity (%)	Sensitivity95% Confidence Limits (%)	
200	200	0	100.00	98.17	100.00

PERFORMANCE CHARACTERISTICS OF THE ORTHO® SUMMIT SYSTEM (OSS) WITH ORTHO® SUMMIT SAMPLE HANDLING SYSTEM (SUMMIT PIPETTER)

Reproducibility

The reproducibility of the Avioq HTLV-I/II Microelisa System on the OSS instrument was performed using a panel of 10 specimens tested in duplicate (four HTLV-I positives, four HTLV-II positives, and two negatives). The study was conducted at two sites on a total of three instruments twice per day for four days using one validation lot of the assay kit and compared to testing this panel using the manual method using the same validation lot. The results of this study are summarized in Table 9.

Table 9: Reproducibility Study for the Avioq HTLV-I/II Microelisa System on OSS (Automated Method)

				Total				Inter-Center		Intra-Center	
Panel ID	**Status**	**N**	**Mean S/C**	**SD**	**%CV**	**S/C Lower 95% CI**	**S/C Upper 95% CI**	**SD**	**%CV**	**SD**	**%CV**
HTLV-I S1	Pos	96	4.54	0.375	8.3	4.47	4.62	0.305	6.7	0.226	5.0
HTLV-I S2	Pos	96	4.03	0.366	9.1	3.95	4.10	0.288	7.1	0.232	5.8
HTLV-I S3	Pos	96	3.63	0.325	8.9	3.57	3.70	0.253	7.0	0.208	5.7
HTLV-I S4	Pos	96	2.84	0.283	10.0	2.78	2.89	0.218	7.7	0.184	6.5
HTLV-I S5[a]	Neg	96	0.26	0.052	20.3	0.25	0.27	0.045	17.5	0.028	10.8
HTLV-II S1	Pos	96	5.86	0.421	7.2	5.77	5.94	0.323	5.5	0.276	4.7
HTLV-II S2	Pos	96	5.75	0.347	6.0	5.68	5.82	0.270	4.7	0.224	3.9
HTLV-II S3	Pos	96	4.95	0.463	9.4	4.85	5.04	0.385	7.8	0.268	5.4
HTLV-II S4	Pos	96	4.22	0.332	7.9	4.16	4.29	0.277	6.6	0.189	4.5
HTLV-II S5[a]	Neg	96	0.13	0.028	20.7	0.13	0.14	0.023	17.0	0.016	12.1

[a] Specimen negative for antibodies to HTLV-I and HTLV-II

The reproducibility study demonstrated that the total variability for the positive specimens ranged from 6% to 10% using the OSS method compared to 8.9 to 19.7% for the positive specimens using the manual method (see Table 6).

Analytical Sensitivity

To demonstrate that the analytical sensitivity of the Avioq HTLV-I/II Microelisa System on the OSS instrument is comparable to the manual method, eight dilution panels (two-fold serial dilutions of four HTLV-I and four HTLV-II antibody-positive samples down to the assay cutoff value) were tested using both the manual and automated methods. To evaluate the correlation of the two methods, Deming's regression analysis was performed. This analysis showed that there was a high correlation for the S/CO ratio for both formats, with a correlation coefficient of 0.94. To demonstrate the equivalence of the S/CO ratio between the two methods, a paired t-test was performed. The overall mean S/CO for the manual method was 3.143 (n=459) compared to an overall mean of 4.198 (n=459) on the OSS instrument. Although this difference is statistically significant, the sensitivity by end-point dilutions was comparable by the two methods (*i.e.*, the assay on the OSS instrument did not have diminished sensitivity).

Sensitivity

A panel of 100 blood donor specimens that were repeatedly reactive on an FDA licensed assay was tested using the Avioq HTLV-I/II Microelisa System using both methods (manual method and automated method on the OSS instrument). Ninety-five (95) of the specimens were repeatedly reactive (100% concordance) using both assay methods (manual and automated). Five (5) of the 100 specimens in the panel were non-reactive using the Avioq assay (both manual and automated). Additional confirmatory testing on these five specimens showed that none were positive on a supplemental test. It should be noted that these specimens were previously subjected to five freeze/thaw cycles prior to testing; data obtained by Avioq indicates that samples may be frozen and thawed once with no loss of reactivity.

The S/CO ratio of the 95 specimens detected by the Avioq assay ranged from 1.058 – 8.400 for the manual method and 1.280 – 7.403 for the automated method on OSS; the correlation coefficient for the S/CO ratio was 0.94. Twenty-nine (29) of these specimens were positive for antibodies to HTLV-I and 46 were positive for antibodies to HTLV-II on a research use supplemental test, and 20 were positive for antibodies to HTLV-I/II (not typed).

Clinical Specificity

A study was performed at two sites to assess the specificity of the assay when used with the automated ORTHO® Summit System (OSS). A total of 16,339 serum and plasma samples randomly collected from volunteer blood donors were tested. The results of this testing are shown in Table 10.

Table 10: Summary of Clinical Specificity Results for the Avioq HTLV-I/II Microelisa System on OSS (Automated Method)

	Site 1			Site 2			All Sites/Lots Combined
	Lot 10003	Lot 10004	Lot 10005	Lot 10003	Lot 10004	Lot 10005	
Number Tested	1366	1366	1358	4160	4080	4009	16,339
Non-Reactive	1364	1366	1358	4157	4078	4007	16,330
Initially Reactive	2	0	1	3	3	2	11
Repeatedly Reactive	2	0	0	3	2	2	9
Confirmed Positives	0	N/A	N/A	0	0	0	0
Total False Positives	2			7			9
Total Tested	4,090			12,249			16,339
Estimated Specificity	**99.95%**			**99.94%**			**99.94%**
95 % CI	**99.82%-99.99%**			**99.88%-99.98%**			**99.90%-99.97%**
Overall Estimated Specificity	**99.95%**						
95% CI	**99.89% to 99.98%**						

Of the 16,339 blood donor specimens tested, nine were repeatedly reactive. All nine were classified as false positive based on the results of testing using a research use Western blot (Site 1) or IFA (site 2). The confidence intervals for the estimated specificity for the Avioq assay for all three lots at each site and for all sites and lots combined overlap with that for the Vironostika assay (95% CI: 99.89 – 99.98%). The overall estimated specificity of the Avioq HTLV-I/II assay on OSS was 16,330/16,339 = 99.94% (95% CI: 99.90 - 99.97%) compared to 11,409/11,415 = 99.95% (95% CI: 99.89 - 99.98%) using the manual method.

These studies demonstrated that the performance of the Avioq HTLV-I/II Microelisa System on the OSS instrument is comparable to that using the manual testing method.

PERFORMANCE CHARACTERISTICS OF THE ORTHO® SUMMIT SYSTEM (OSS) WITH THE ORTHO VERSEIA® PIPETTER

Reproducibility

The reproducibility of the Avioq HTLV-I/II Microelisa System on OSS with the ORTHO VERSEIA® pipetter was performed using a panel consisting of three HTLV-I positive, two HTLV-II positive and one negative samples. The study was conducted in three sites on a total of five VERSEIA® and, for comparison, SUMMIT pipetters, each of which tested six replicates in two runs per day over a period of five non consecutive days. The results of this study are summarized in Table 11. The reproducibility study demonstrated that the total variability of the two instrument types were comparable.

Table 11: Reproducibility Study for the Avioq HTLV-I/II Microelisa System on OSS

Panel ID	SUMMIT Pipetter						VERSEIA® Pipetter					
	N	Mean	SD	%CV	S/C 95% CI		N	Mean	SD	%CV	S/C 95% CI	
					Lower	Upper					Lower	Upper
A (HTLV-I)	150	2.549	0.515	20.2	2.468	2.631	150	2.602	0.503	19.3	2.524	2.680
B (HTLV-I)	150	2.623	0.480	18.3	2.548	2.699	150	2.708	0.491	18.1	2.632	2.785
C (HTLV-I)	150	1.718	0.416	24.2	1.653	1.783	149	1.869	0.448	24.0	1.798	1.939
D (HTLV-II)	150	2.802	0.562	20.1	2.713	2.891	150	2.823	0.601	21.3	2.728	2.918
E (HTLV-II)	150	1.985	0.365	18.4	1.928	2.042	150	2.114	0.421	19.9	2.049	2.180
F (Negative)	150	0.061	0.016	26.0	0.059	0.064	149	0.060	0.016	26.6	0.058	0.063

Analytical Sensitivity

To demonstrate that the analytical sensitivity of the Avioq HTLV-I/II Microelisa System on OSS with the VERSEIA® Pipetter is comparable to that with the SUMMIT Pipetter, ten dilution panels (four dilutions with target S/C ranging from 0.5 to 3.0 of five HTLV-I and five HTLV-II antibody-positive samples through the assay cutoff value) were tested using both instruments. To evaluate the correlation of the two methods, Deming's regression analysis was performed. This analysis showed that there was a high correlation for the S/CO ratio for both instruments, with a slope of 1.00, intercept of 0.01 and Pearson correlation coefficient (r) of 1.00.

Sensitivity

A sensitivity panel consisting of 104 frozen HTLV antibody positive specimens (52 HTLV-I and 52 HTLV-II antibody positive specimens) was used in this study. These samples were generated by dilution of frozen

positive samples to create a combination of samples that are low to moderate reactive. Thus, it is expected that some of these samples with signal to cutoff values approaching 1.000 will vary from above (positive) to below (negative) the cutoff.

Samples were tested in triplicate in each of three sites using the SUMMIT and VERSEIA® Pipetters. A total of 935 valid results were obtained and used for data analysis for each instrument. Of the 935 observations, 98.1% (917/935) of Summit results were reactive whereas 98.5% (921/935) of VERSEIA® results were reactive. McNemar's test for discordant results shows no significant difference between the two pipetters with a *p*-value of 0.3438, indicating that the Avioq HTLV-I/II Microelisa System is compatible with the VERSEIA® Pipetter.

Clinical Specificity

A total of 3,146 random donor serum and plasma samples were tested in singleton using both instruments types (SUMMIT and VERSEIA®) in three sites, each of which tested similar numbers of samples. Of the 3,146 samples tested, 3,140 were non-reactive with either instrument type. Of the remaining 6 samples, 5 were reactive with both pipetters while one showed discordant test results between the two pipetter types (reactive with the VERSEIA® but non-reactive with the Summit); all six samples were indeterminate by Western blot analysis. Assuming all samples were negative for HTLV antibody, the specificity of the assay was 99.81% (95% CI: 99.59% - 99.93%) and 99.84% (95% CI: 99.63% - 99.95%) when used with the VERSEIA and Summit instruments, respectively (Table 12). Statistical analysis showed that there was no significant difference in specificity for the two instrument types (99.81% vs. 99.84%).

Table 12: Summary of Clinical Specificity Results

		SUMMIT Pipetter		
		Positive	Negative	Total
VERSEIA® Pipetter	Positive	5	1	6 (0.19%)
	Negative	0	3140	3140 (99.81%)
	Total	5(0.16%)	3141 (99.84%)	3146 (100.00%)

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Reproducibility was evaluated using 20 non-reactive cadaveric samples (10 serum/10 plasma) and 20 non-reactive living donor samples (10 serum/10 plasma). From each donor source (cadaveric and living) 5 serum and 5 plasma samples were spiked with either anti-HTLV-I or anti-HTLV-II source material to a target reactivity of 2.0 signal to cutoff ratio (S/CO). Each spiked sample was tested once on six different days in each of three kit lots at one site. Percent CVs were comparable between cadaveric and living donor samples.

Table 13 – Assay Reproducibility in Cadaveric and Living Donor Plasma Samples

							Total		Inter-Assay		Intra-Assay	
Pre / Post	**Type**	**Matrix**	**N**	**Mean**	**Lower 95%**	**Upper 95%**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
POST	HTLV-I	Plasma	90	2.13	2.05472	2.21510	0.383	17.9	0.324	15.2	0.215	10.1
		Serum	90	2.31	2.20140	2.41140	0.501	21.7	0.430	18.7	0.273	11.8
	HTLV-II	Plasma	90	2.09	2.00987	2.17973	0.405	19.4	0.348	16.6	0.221	10.6
		Serum	90	1.94	1.86472	2.02168	0.375	19.3	0.288	14.8	0.248	12.8
PRE	HTLV-I	Plasma	90	1.89	1.81144	1.96652	0.370	19.6	0.290	15.4	0.238	12.6
		Serum	90	1.97	1.89267	2.05320	0.383	19.4	0.322	16.3	0.219	11.1

							Total		Inter-Assay		Intra-Assay	
Pre / Post	**Type**	**Matrix**	**N**	**Mean**	**Lower 95%**	**Upper 95%**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
	HTLV-II	Plasma	90	2.22	2.11903	2.32092	0.482	21.7	0.373	16.8	0.315	14.2
		Serum	90	1.96	1.88779	2.03181	0.344	17.5	0.280	14.3	0.208	10.6

Sensitivity

Samples tested included approximately equal numbers of serum and plasma samples from both post-mortem (C) samples (n=91; 46 serum, 45 plasma) and normal donor (N) samples (n=91; 45 serum, 46 plasma). Samples were previously tested for HTLV-I/II and found to be non-reactive. The samples were used to prepare HTLV-I and HTLV-II spiked panels. Each sample was split in two and spiked with a predetermined amount of HTLV-I or HTLV-II positive antibody sera. The sensitivity was assessed by testing both the post-mortem and normal donor HTLV-I and HTLV-II seropositive samples with three HTLV-I/II kit lots at two testing sites. Negative test results by the ELISA test were considered false negative. Sensitivity and 95% confidence intervals were calculated for both post-mortem and normal donor samples spiked with HTLV-I and HTLV-II positive antibody as shown in Table 14 below. The Avioq HTLV-I/II Microelisa System has an overall estimated sensitivity in spiked cadaveric specimens of 100% with a 95% confidence interval of 92.29%-100.00% for serum, and 92.13-100% for plasma.

Table 14 – Reactivity Against Spiked Post-Mortem and Normal Donor Samples

Site	Lot Number	Sample Type (Serum)	Number Tested	Initial Non-Reactive	Initial Reactive	Percent Reactive	95% Confidence Limit
1	1	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
	2	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
	3	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
2	1	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
	2	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
	3	C	46	0	46	100%	92.29 – 100.00
		N	45	0	45	100%	92.13 – 100.00
Site	Lot Number	Sample Type (Plasma)	Number Tested	Initial Non-Reactive	Initial Reactive	Percent Reactive	95% Confidence Limit
1	1	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00
	2	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00
	3	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00
2	1	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00
	2	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00
	3	C	45	0	45	100%	92.13 – 100.00
		N	46	0	46	100%	92.29 – 100.00

Specificity

Plasma samples from both post-mortem (C) donors (n=45) and normal (N) living donors (n=45; collected from a low risk population (blood bank)) were each tested, as per Avioq HTLV-I/II Microelisa System package insert, with three HTLV-I/II kit lots at two test sites. Specificity and 95% confidence intervals were calculated for both post-mortem and normal donor samples as shown in Table 15 below. The Avioq HTLV-I/II Microelisa System has an overall estimated specificity in plasma cadaveric specimens of 100% with a 95% confidence interval of 92.13-100 %.

Table 15 – Estimated Specificity in Post-Mortem and Random Plasma Samples

Site	Lot	Sample Type (Plasma)	Number Tested	Non-Reactive	Repeat Reactive	Estimated Specificity (%)*	Specificity 95% Confidence Limits (%)**
Site 1	Lot 1	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0
	Lot 2	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0
	Lot 3	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0
Site 2	Lot 1	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0
	Lot 2	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0
	Lot 3	Cadaveric	45	45	0	100	92.13-100.0
		Normal	45	45	0	100	92.13-100.0

* = $\frac{\text{(Number Screened – Number Repeat Reactive)}}{\text{(Number Screened – Number Confirmed Positive)}} \times 100$

** = Confidence limits for specificity were calculated using the exact method.

Serum samples from 218 individual post-mortem donors were tested as per Avioq HTLV-I/II Microelisa System package insert with one of three HTLV-I/II kit lots (approx. 1/3 of samples per lot) at a single site. Specificity and 95% confidence intervals were calculated as shown in Table 16 below. The Avioq HTLV-I/II Microelisa System has an overall estimated specificity in cadaveric specimens of 100% with a 95% confidence interval of 98.32-100 %.

Table 16 – Estimated Specificity in Post-Mortem Serum Samples

Lot	Sample Type(Serum)	Number Tested***	Initially Non-Reactive	Repeat Reactive	Estimated Specificity (%)*	Specificity 95% Confidence Limits (%)**
Lot 1	Cadaveric	73	73	0	100	95.07-100.0
Lot 2	Cadaveric	73	73	0	100	95.07-100.0
Lot 3	Cadaveric	72	71	0	100	95.01-100.0
Total		218	217	0	100	98.32-100.0

* = (Number Screened – Number Repeat Reactive) X 100
(Number Screened – Number Confirmed Positive)

** = Confidence limits for specificity were calculated using the exact method.

*** = In a separate study, 46 cadaveric and 46 normal living donor serum samples were each tested using three different HTLV-I/II kit lots at two sites. One cadaveric sample was repeatedly reactive across all three kit lots at both sites. A second cadaveric sample was repeatedly reactive on all three kit lots at one site and on two kit lots at the other site.

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AVAILABILITY

Avioq Inc.

Avioq HTLV-I/II Microelisa System

192 Test kit	REF	Product number 500192
576 Test kit	REF	Product number 500576
9600 Test Kit	REF	Product number 509600
Wash Buffer 100 ml bottle	REF	Product number 559879
Wash Buffer 4 X 100 ml bottles	REF	Product number 559880

For technical assistance in the U.S.A., contact Avioq Customer Service at 1-919- 314- 5535.

For technical assistance outside the U.S.A. contact Emergo Europe (31)(0)70345-8570.

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