HTLV BLOT 2.4
WESTERN BLOT ASSAY

INSTRUCTIONS FOR USE

IVD

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(36 tests kit): 0711088036

NAME AND INTENDED USE

The **MP Diagnostics HTLV Blot 2.4** is a qualitative enzyme immunoassay intended for confirming the presence of and differentiating antibodies to HTLV-I and HTLV-II in human serum and plasma. It is intended for use as a supplemental (additional, more specific) test for human serum and plasma samples with repeatedly reactive results by an FDA licensed HTLV-I/II donor screening test. The MP Diagnostics HTLV Blot 2.4 is intended for use in a manual mode or a semi-automated mode using the MP Diagnostics AutoBlot System 20. This test is not intended for use in medical diagnosis.

INTRODUCTION AND EXPLANATION OF THE TEST

Background

Human T-cell Lymphotropic Viruses (HTLVs) are pathogenic retroviruses that may cause severe hematological and neurological diseases in infected individuals. The HTLV family has two well-studied members: HTLV-I and HTLV-II. HTLV-I is known as the etiological agent of adult T-cell leukemia/lymphoma (ATL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV-associated uveitis. Although less pathogenic than HTLV-I, HTLV-II infection has been associated with leukemia and neurological disease but the causal relationship remains uncertain.

Studies of the geographic distribution of HTLV-I infection reveal that the HTLV-I virus is highly prevalent in Japan, Africa, the Caribbean Islands, and South America. Recent epidemiological studies in the United States and Europe confirm the presence of a mixed prevalence of both HTLV-I and HTLV-II among different high-risk populations, such as intravenous drug users and transfusion recipients. The viruses can be transmitted through sexual contact, through contaminated blood products, and from mother to child via breastfeeding.

Screening tests for HTLV-I/II are available although limited. Repeatedly reactive specimens from screening tests require additional more specific tests to confirm HTLV seropositivity including discrimination of HTLV-I and HTLV-II seropositives. These supplemental assays (i.e. type-specific peptide EIAs, ELISAs, or Western blots) must be capable of identifying antibodies to core (*gag*) and envelope (*env*) proteins of HTLV-I and HTLV-II. Western blot strips incorporating HTLV-I native viral antigens are one such commonly used supplemental test.

Screening of whole blood donations for the presence of antibodies to HTLV-I/II has been required in the United States since 1988. Simple yet specific and sensitive supplemental serological tests are therefore needed to enable rapid confirmation and differentiation of HTLV-I and HTLV-II seropositive samples. A supplemental test is essential to provide additional key information necessary for donor counseling, follow-up testing, and/or treatment.

Virology

HTLV-I and HTLV-II are type C human oncoviruses with single-stranded RNA genomes that are approximately 8,900 base pairs in length. The HTLV-I/II genomes include *gag* and *env* genes which encode structural core proteins p19 and p24, as well as envelope proteins gp46 and p21e,[1] respectively. Like other human retroviruses, the HTLV-I/II *pol* genes encode a reverse transcriptase to allow transcription of the RNA genome into a complementary DNA strand, which is then integrated into the host genome by a *pol* encoded integrase.

Diagnosis

HTLV-I/II infections are generally diagnosed by antibody tests [e.g., Enzyme-linked immunoassay (ELISA), Chemiluminescence assay (ChLIA), Western blot, Immunofluorescence assay (IFA)]. Due to the inclusion of cross-reactive antigens, most assays detect both HTLV-I and HTLV-II antibodies, although sensitivity for HTLV-II may be lower[2]. Natural or recombinant, type-specific, envelope proteins, in IFA or Western blot format permit the differentiation of HTLV-I from HTLV-

II antibodies[3]. The two virus types may also be distinguished by polymerase chain reaction (PCR) or *in-situ* hybridization directed at specific HTLV-I/II proviral DNA or RNA sequences.[4] Quantitative PCR studies have also determined that the proviral DNA load in both HTLV-I and HTLV-II ranges from approximately 10^{-4} to 10^{-1} per peripheral blood mononuclear cell.[5,6]

Epidemiology

HTLV-I is endemic at levels up to five percent (5%) of the general population in central Africa, several Caribbean basin and South American countries, and in southern Japan.[7] Transmission is from mother to child, predominantly by breastfeeding; through sexual intercourse, predominantly in the male-to-female direction: and via parenteral exposure by blood transfusion or needle sharing. In the United States, first time blood donor HTLV-I seroprevalence is about one per ten thousand, and risk factors include maternal or sexual links to HTLV-I-endemic areas. In contrast, HTLV-II seroprevalence is about two per ten thousand, and predominant risk factors are injection drug use and sexual contact with an injection drug user (IDU) [8, 9]. In a 2012 study, the rate of overall HTLV infection (undifferentiated) in all US donors was determined as 1:35,313[76] Researchers have estimated that there may be as many as ten to twenty million persons with HTLV-I infection in the world; a more conservative estimate might be between one to five million[10].

HTLV-II is endemic in certain North, [11] Central[12] and South[13,14] American Indian tribes, with some of the highest seroprevalence values (up to fifty percent) documented in tribes with the least contact with contemporary civilization, such as the Brazilian Kayapo. This led to the hypothesis that HTLV-II was already endemic in these tribes before they migrated across the Bering Land Bridge over ten thousand years ago. A single report of HTLV-II among Mongolians has not been supported by other studies of the same population[15]. However, clusters of HTLV-II infection have been conclusively demonstrated among isolated Pygmy tribes in central Africa[16,17]. Genetic similarities between Pygmy and Native American HTLV-I isolates have not been explained[18-20].

An early study that differentiated HTLV-I from HTLV-II using a competitive HTLV-I/II ELISA technique reported a high seroprevalence of both HTLV-I and HTLV-II among IDU in the New Jersey area[21]. In New Orleans, approximately twenty-five percent (25%) of IDU tested were HTLV-II positive by PCR and another two percent (2%) were infected with HTLV-I[22]. Sixteen percent (16%) of San Francisco IDU are HTLV seropositive, and most of these appear to be infected with HTLV-II[23]. A study of primarily white IDU from the Staten Island, New York area, found PCR-determined prevalence of eleven percent (11%) for HTLV-II and an additional nine percent (9%) for HTLV-I[24]. Finally, measurement of HTLV-I/II antibodies in sera from the CDC-sponsored HIV Sentinel Counties Survey yielded undifferentiated HTLV-I/II prevalence among IDU in methadone treatment centers ranging from 0.4% (Atlanta) to 17.6% (Los Angeles)[25]. Interestingly, there was little concordance in the ranking of cities by HIV prevalence versus HTLV-I/II prevalence.

Based upon the 2000 U.S. Census data, it is estimated that the total number of HTLV-II infected persons in the United States is approximately 197,000. This includes 56,000 in the general population (U.S. population 281,422,000 X 0.02% blood donor prevalence[8]), 100,000 among IDU (1 million IDU X 10% prevalence[25]) and 41,000 among American Indians (4,119,000 Native American/Alaska natives X 1% prevalence[11]).

Disease Associations

HTLV-I causes ATL, a malignancy of mature CD4+ T-lymphocytes that presents most commonly as lymphoma with skin involvement and hypercalcemia.[26] HTLV-I is the causative agent of HAM, a slowly progressive spastic paraparesis that is characterized by weakness in the legs, diffuse hyperreflexia, clonus, loss of vibration sense, and detrusor insufficiency leading to bladder dysfunction. HTLV-I may also be associated with a wider spectrum of neurological manifestations that do not meet diagnostic criteria for HAM. Sensory neuropathy,[27-29] gait abnormalities,[30,31] bladder dysfunction,[27,30-33] erectile dysfunction,[34,35] amyotrophic lateral sclerosis (ALS),[36] mild cognitive deficits[37], and rarely, motor neuropathies[27,29,34,38-40] have all been reported among HTLV-I-infected individuals without HAM. HTLV-I infection has also been implicated in a spectrum of autoimmune conditions such as uveitis, arthritis, and pneumonitis, although there is good epidemiologic evidence of association only with uveitis and arthritis[31, 41, 42].

HTLV-II was initially isolated from two patients with unusual hairy T-cell leukemia. It was subsequently determined that at least one of these patients had a dual disorder: HTLV-II negative B-cell hairy cell leukemia and HTLV-II positive CD8+ lymphoproliferative syndrome[43]. Although the old literature reports suggests HTLV-II is associated with a myelopathic syndrome similar to HTLV-I related HAM is derived from four cases from the HTLV Outcomes Study (HOST) cohort and about a dozen cases of HTLV-II classical HAM, some with virologic evidence of HTLV-II in cerebrospinal fluid[44-50] The role of HTLV-II in neurological diseases is less clear[51].

Explanation of the Test

The **MP Diagnostics HTLV Blot 2.4** is intended as a supplemental (additional more specific), test to confirm the presence of anti-HTLV-I/II antibodies in blood donor specimens repeatedly reactive on an FDA licensed screening test and to differentiate between HTLV type-I and HTLV type-II infections for donor notification and counseling. The possible serological profiles defined by the HTLV Blot 2.4 include the following: HTLV-I Seropositive, HTLV-II Seropositive, HTLV-I/II Seropositive, Seronegative and Indeterminate.

The **MP Diagnostics HTLV Blot 2.4** uses a combination of HTLV-I/II genetically engineered proteins (i.e., recombinant antigens) and HTLV-I viral proteins derived from native, inactivated viral particles (i.e., viral lysate). The differentiation between HTLV-I and HTLV-II is accomplished through the use of rgp46-I, a unique HTLV-I envelope recombinant protein, and rgp46-II, a unique HTLV-II envelope recombinant protein. Both proteins are derived from the central region of the external glycoprotein, gp46, of HTLV-I and HTLV-II respectively. GD21, a common yet specific HTLV-I and HTLV-II epitope envelope recombinant protein derived from a truncated region of p21e (rgp21), is also used to enhance the specificity of envelope antibody detection: GD21 has demonstrated better specificity over p21e[73], an earlier version of the recombinant antigen. The antigenicity exhibited by these recombinant proteins is either common to HTLV-I and HTLV-II antibodies or type specific to one of the two viral types to allow confirmation and differentiation in a single assay. Additional differentiation between HTLV viral types is effected using *gag* proteins p19 and p24; if p19 is greater than or equal to p24, HTLV-I infection is suggested, and if p24 is greater than p19, HTLV-II infection is suggested[67-71].

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in, or found on, **MP Diagnostics** products and packaging.

Use by
Synonym for this:
Expiration Date

Catalogue Number
Synonyms for this:
Reference Number
Re-order Number

Batch Code
Synonyms for this are:
Lot Number
Batch Number

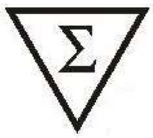
Do not reuse

Temperature Limitation

Caution

Manufacturer

Consult instructions for use



Contents sufficient for <n> tests

CHEMICAL & BIOLOGICAL ASSAY PRINCIPLES

HTLV-I viral proteins, derived from native, inactivated viral particles (viral lysate) and HTLV-I/II genetically engineered proteins, are incorporated into the nitrocellulose strips.

Individual nitrocellulose strips are incubated with diluted serum or plasma specimens; specific antibodies to HTLV-I/II, if present in the specimen, will bind to the HTLV-I/II proteins on the strip. The strips are washed to remove unbound materials, and the remaining antibodies, bound to the HTLV proteins on the strips, are visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate, BCIP/NBT.

Of the proteins applied to the nitrocellulose strips, five are used to confirm the presence of antibodies against HTLV-I/II. These are the following: rgp46-I, rgp46-II, GD21, p19 and p24.

Type-specific recombinant envelope protein rgp46-I is specific for HTLV-I, while rgp46-II is specific for HTLV-II; these antigens are used to differentiate between HTLV-I and HTLV-II infections[74, 75].

GD21, a third recombinant envelope protein, is broadly immunoreactive with sera or plasma from HTLV-I and HTLV-II infected individuals[68, 72, 73].

Two *gag* proteins, p19 and p24, which are reactive to HTLV-I and cross-reactive to HTLV-II, are used to confirm the presence of antibodies. It has been found that reactivity against p19 was greater than, or equal to, reactivity against p24 in subjects who had HTLV-I infection confirmed by PCR. Conversely, p24 bands were stronger than p19 bands in persons who had PCR-confirmed HTLV-II infection[67-71].

KIT COMPONENTS

	Component Description	**Quantity Provided**
ANTIGEN STRIPS		

	NITROCELLULOSE STRIPS Incorporated with HTLV-I viral lysate, HTLV-I and II recombinant envelope antigens, and a sample addition control (anti-human IgG) band. Keep dry and away from light.	Available in 18 or 36 strips
CONTROL –	**NON-REACTIVE CONTROL** Inactivated normal human serum, non-reactive for anti-HCV, anti-HIV-1/2, anti-HTLV-I/II and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 μL)
CONTROL I +	**STRONG REACTIVE CONTROL I** Inactivated human serum with high titer antibodies to HTLV-I and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 μL)
CONTROL II +	**STRONG REACTIVE CONTROL II** Inactivated human serum with high titer antibodies to HTLV-II and non-reactive for anti-HCV, anti-HIV-1/2 and HBsAg. Contains sodium azide and thimerosal as preservatives.	1 vial (80 μL)
BUF LYO. STOCK	**LYOPHILIZED STOCK BUFFER** To be reconstituted in reagent grade water. Tris buffer with heat inactivated animal and non-animal proteins. Contains thimerosal as preservative.	1 or 2 bottles (each to be reconstituted to 100 mL)
BUF WASH 20x	**WASH BUFFER CONCENTRATE (20X)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 mL)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase. Contains sodium azide as preservative.	1 vial (120 μL)

SUBS | BCIP / NBT	**SUBSTRATE** Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).	1 bottle (100 mL)
POWDER | BLOTTING	**BLOTTING POWDER** Non-fat dry milk.	10 packets (1 g each)
	Instructions for Use (IFU)	1 copy
	Protein Finder	1 piece
	Intensity Finder	1 piece
	Forceps	1 pair
	Disposable 9-well incubation tray (manual use only and packed separately from the kit)	2 or 4 trays
	HTLV Blot 2.4 Report Sheet	1 piece

WARNINGS AND PRECAUTIONS

<u>CAUTION</u>: Test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards.

This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection. Follow established laboratory policy and applicable CDC/NIH biosafety and/or OSHA/WISHA hazardous material spill guidelines for appropriate hazardous chemical and/or biological spill response and clean-up.

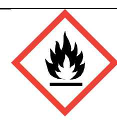
HANDLE ASSAY SPECIMENS, STRONG REACTIVE CONTROL I, STRONG REACTIVE CONTROL II, AND NON-REACTIVE CONTROL AS POTENTIALLY INFECTIOUS AGENTS. It is recommended that the kit components and test specimens be handled with universal precautions as if capable of transmitting infectious disease. Refer to guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* or equivalent, for safe practices in handling specimens. Specimens should be disposed of in accordance with established safety procedures.

The Strong Reactive Control I, Strong Reactive Control II and Non-Reactive Control contain both thimerosal and sodium azide as preservatives; the Lyophilized Stock Buffer and the Wash Buffer Concentrate contain thimerosal and the Conjugate contains sodium azide. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. Build up in piping has led to laboratory explosions. Therefore, dilute and/or flush with copious amounts of water when disposing down the drain. Check with your local, regional, or national ordinances accordingly.

The ingredients present in the kit components are, in their pure form, a dangerous substance. However, their low concentrations, as prepared in these kit components, are not considered a dangerous preparation. Sodium azide ≤ 0.1% w/v is below the regulatory threshold limits according to OSHA standard 29 CFR 1910.1200.

The substrate, BCIP/NBT, can potentially be irritating to the skin and eyes.

Pursuant to EC regulation 1272/2008 (CLP), hazardous components are classified and labelled as follows:

Component:	**Nitrocellulose strips**
Signal Word:	**Danger**
Pictogram:	
Hazard Statements:	H228 Flammable solid
Precautionary Statements:	P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking. P280 Wear protective gloves/protective clothing/eye protection/face protection.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	100% Nitrocellulose

Component:	**WASH BUFFER CONCENTRATE (20x)**
Signal Word:	**Warning**
Pictogram:	
Hazard Statements:	H373 May cause damage to organs through prolonged or repeated exposure

Precautionary Statements:	P260 Do not breathe dust/fume/gas/mist/vapours/spray. P501 Dispose of contents/container in accordance with local/regional/national/international regulations.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	0.1% Thimerosal

General Precautions:

1. Avoid contamination of reagents when opening and removing aliquots from the original vials or bottles.
2. Do not pipette by mouth.
3. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste bags. Wash hands thoroughly afterwards. Disposable clothing is recommended. If reusable clothing is used, refer to procedures under the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for handling potentially infectious laundry.
4. Keep kit materials away from food and drink.
5. In case of accident or contact with eyes, rinse affected area immediately with plenty of water and seek medical advice immediately.
6. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations or other breaks in the skin.
7. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with 1% sodium hypochlorite solution before work is resumed. An alternate decontamination agent or disinfectant (e.g., 70 – 80% ethanol or isopropanol, an iodophor or a phenolic, etc.) may be used. Sodium hypochlorite should not be used on acid containing spills unless the area is first wiped dry with absorbent paper. Materials used, including disposable gloves, should be disposed of as potentially biohazardous material. Do not autoclave material containing sodium hypochlorite.
8. Acceptable methods for decontamination include: Autoclaving of all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal; decontaminating materials in 0.5% sodium hypochlorite solution (a solution with 1:10 dilution (v/v) of household bleach) for 30 to 60 minutes before disposal in biohazard waste bags and hold for professional removal; disinfect with Decon 90 prior to dilution with water or, any other method that complies with local, state or federal regulations.
   (In general laboratory waste is under the special supervision of the authorities (Federal, State and Local). Thus reference to applicable regulations applicable to the territory is recommended.)

9. Decontaminate all used chemicals and reagents by adding sufficient volume of sodium hypochlorite to make a final concentration of at least 1%. Leave for 30 minutes to ensure effective decontamination.
10. Do not reuse incubation trays or disposable AutoBlot trays.

ANALYTICAL PRECAUTIONS

1. Optimal assay performance requires **STRICT ADHERENCE** to the Assay Procedure described in this Instructions For Use (IFU) document. Deviations from this procedure may lead to aberrant results.
2. Do not expose reagents to, or perform the test in, an area containing a high level of chemical disinfectant fumes (e.g., hypochlorite fumes). Contact with a high level of chemical disinfectant fumes inhibits color reaction. Also, do not expose reagents to strong light.
3. The assay must be performed at room temperature (22°C to 28°C).
4. Ensure that any automated equipment used is validated before use.
5. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and nitrocellulose strips are matched for optimal performance. Use only the reagents supplied with the kit.
6. Do not use kit components beyond the expiration date printed on the kit box.
7. All reagents contained within the kit must be mixed well before use; mix by inverting the container several times.
8. Avoid contamination of the reagents when opening and removing aliquots from the original vials or bottles. Use a pipette and disposable pipette tips when drawing out aliquots.
9. For best results, dispense all reagents while cold. When running an assay manually, return all reagents to 2°C to 8°C storage immediately after dispensing and during incubation stages. For assays performed using the MP Diagnostics AutoBlot System 20, load all reagents while cold onto the instrument at the beginning of each automated assay run; return remaining reagents to 2°C to 8°C for storage. A study demonstrated the stability of the reagents while onboard the AutoBlot instrument at room temperature for up to 3 consecutive assay runs over a 9 hour period; however, store reagents at refrigerated temperatures when not in use.
10. It is recommended that the glassware used with the reagents be washed with 2M hydrochloric acid and rinsed thoroughly with reagent or deionized water prior to use. Disposable plastic ware may be used in lieu of glassware.
11. The Working Conjugate Solution should be prepared using a polypropylene container or beaker.
12. Use only reagent or deionized water to dilute reagents.
13. Working Conjugate Solution, Diluted Wash Buffer and Blotting Buffer should be **prepared fresh prior to use.**

14. Before, during and after running the assay, always handle the test strips using forceps, holding the strips gently at the tips.
15. Always place the test strips with the numbers (printed on the strips) facing up.
16. The kit controls should be assayed concurrently with samples during each test run.
17. Use a new pipette tip for each specimen/ control aliquot to prevent cross contamination.
18. Add the specimens and controls directly to the buffer at the opposite end of the strip numbers; **DO NOT** add the specimens and controls directly to the strip, as this may cause the formation of dark spots. For the manual procedure, tilt the tray and add the specimen(s) where the buffer is collected at the lower end of each well.
19. The HTLV Blot 2.4 manual assay must be performed using a rocking platform shaker with a speed and tilt angle of 12 to 16 cycles per minute and 5 to 10 degrees respectively. Use of any platform other than that specified may affect the performance of the assay.

STORAGE

1. Store the **HTLV BLOT 2.4** kit and its components at 2°C to 8°C when not in use. Return to refrigerated storage conditions after dispensing and during incubation stages.

2. All test reagents and strips are stable until the expiration date given on the kit under the defined storage conditions only. Do not freeze the reagents.

   A. **Antigen strips**
      - Avoid unnecessary exposure of nitrocellulose antigen strips to light.

   B. **Reagents**
      - Store reagents in their original vials or bottles with the cap tightly closed.
      - Dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.

CAUTION: Avoid unnecessary exposure of substrate to light.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Serum or plasma samples collected in EDTA, PPT, ACD, potassium oxalate, heparin or sodium citrate may be used. Before storing samples, ensure that any blood clots or blood cells have been separated by centrifugation.

Samples should be stored at 2°C to 8°C if the test is to be run within 7 days of collection, or frozen at -20°C or colder if the test is to be delayed for more than 7 days. Grossly lipemic / icteric samples should be avoided.

Frozen specimens should be allowed to thaw completely before processing. Testing of samples subjected to repeated freeze / thaw cycles is acceptable if the samples remain clear. However, avoid testing of specimens subjected to more than 5 freeze / thaw cycles.

If desired, samples may be heat inactivated for 30 minutes at 56 ° C with no loss of reactivity. Inactivate as follows:

1. Loosen caps of sample containers.
2. Heat sample at 56 ° C for 30 minutes in a water bath.
3. Allow sample to cool before retightening cap.
4. Sample can be stored frozen until analysis.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Optimal assay performance requires STRICT ADHERENCE to the assay procedure described below. Deviations in procedure or equipment may produce aberrant results.

Materials

- Clinical laboratory reagent water (CLRW)[77] or deionized water
- Disposable gloves
- Sodium hypochlorite for decontamination
- Handheld sample handling pipettes and disposable tips of appropriate volume

  *"Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Fourth Edition".

Manual Method - Equipment

- Vacuum pump aspirator with sodium hypochlorite waste trap

- Bellco Rocker or equivalent rocking platform (capable of a speed of 12 to 16 oscillations per minute and a tilt angle of 5 to 10 degrees)

Semi-Automated Method – Equipment

- MP Diagnostics AutoBlot System 20.

The integrated protocol to run the MP Diagnostics HTLV Blot 2.4 assay using the MP Diagnostics AutoBlot System 20 is available directly from MP Biomedicals, LLC. Please contact MP Biomedicals' Customer Service.

PREPARATION OF REAGENTS

1. DILUTED WASH BUFFER

(a) DILUTED WASH BUFFER should be **prepared fresh prior to use**.

(b) Dilute 1 volume of WASH BUFFER CONCENTRATE (20x) with 19 volumes of reagent water. Mix well by inverting or stirring.

Wash Buffer Preparation Chart

Number of Strips	Volume of Wash Buffer Concentrate	Volume of Reagent Water
9 – 12	10 mL	190 mL
13 – 18	15 mL	285 mL
19 – 24	18 mL	342 mL
25 – 30	23 mL	437 mL
31 – 36	27 mL	513 mL

2. BLOTTING BUFFER

(a) Reconstitute each bottle of LYOPHILIZED STOCK BUFFER with 100 mL reagent or deionized water. Mix well to dissolve. This RECONSTITUTED STOCK BUFFER is stable for 6 weeks if stored at 2°C to 8°C.

(b) BLOTTING BUFFER **should be prepared fresh prior to use**.
Add 1 g (i.e., packet) of BLOTTING POWDER to every 20 mL of the RECONSTITUTED STOCK BUFFER prepared in step 2(a) above. Mix well by inversion or stirring to ensure powder dissolves completely.

(c) Stir again before dispensing.

Blotting Buffer Preparation Chart

Number of Strips	Packets of Blotting Powder	Volume of Reconstituted Stock Buffer
9	2	40 mL
10 – 12	3	60 mL
13 – 18	4	80 mL
19 – 24	5	100 mL
25 – 30	7	140 mL
31– 36	8	160 mL

3. **WORKING CONJUGATE SOLUTION**

   Note: Prepare solution in polypropylene container / beaker.

   (a) WORKING CONJUGATE SOLUTION should be **prepared fresh prior to use**.

   (b) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE with BLOTTING BUFFER in the ratio of 1:1000 (e.g., 10 μl CONJUGATE to 10 mL BLOTTING BUFFER, etc.).

Working Conjugate Preparation Chart

Number of Strips	Volume of Conjugate	Volume of Blotting Buffer
9	20 µL	20 mL
10 – 12	26 µL	26 mL
13 – 18	38 µL	38 mL
19 – 24	50 µL	50 mL
25 – 30	62 µL	62 mL
31– 36	74 µL	74 mL

4. **SUBSTRATE SOLUTION (ready-to-use)**

   (a) Transfer the required volume from the bottle using a clean pipette. Cap tightly after use.

ASSAY PROCEDURE

NOTE: This section describes the **Manual Assay Procedure**. The automated assay procedure using the MP Diagnostics AutoBlot System 20 is provided as an addendum. To receive a copy, please contact MP Biomedicals, LLC.

Note: a) Aspirate all used chemicals and reagents into waste trap containing sodium hypochlorite.

b) Add samples and controls carefully to avoid mixing up the order of the addition of samples and controls.

c) All incubation steps are to be carried out on a rocking platform.

d) New disposable trays should be used for each assay; do not reuse trays.

Caution: Adding sample or control directly to the strip may result in the formation of dark patches on the strip in the location where the sample/control was added. To ensure the proper addition of sample or control:

i. Nitrocellulose strips should be added with the numbered end of the strip facing up and located at the top of the tray well (the side furthest away from the operator). It is strongly recommended that the strip numbers be placed in ascending order and the tray wells be numbered.

ii. Sample should be added only after BLOTTING BUFFER is added.

iii. Tilt the tray slightly by elevating the top of the tray. Add the sample at the bottom of the tray well where the Blotting Buffer has collected. When all the samples have been added, return the tray back to its original flat position. Always ensure that the strips are kept wet during the process.

Procedure:

1.	Add 2 mL of DILUTED WASH BUFFER to each well.	**2 mL**
2.	Using forceps, carefully remove a nitrocellulose strip from the tube and place numbered side up into the first well in the tray. The number should be placed at the top of the tray well. Repeat this process until the correct number of strips has been added to the tray. Include strips for Strong Reactive Control I, Strong Reactive Control II and Non-Reactive Control.	
3.	Incubate the strips for <u>5 minutes</u> at room temperature (25°C ± 3°C) on a rocking platform with a speed of 12 to 16 oscillations per minute and a 5° - 10° tilt. Remove buffer using a vacuum pump aspirator with sodium hypochlorite waste trap.	**5 minutes** **25°C ± 3°C**
4.	Add 2 mL of BLOTTING BUFFER to each well.	**2 mL**

5.	Add 20 µL each of test sample or control to appropriate wells.	**20 µL**
6.	Cover the tray with the cover provided and incubate for <u>1 hour</u> at room temperature (25 °C ± 3 °C) on a rocking platform with a speed of 12 to 16 oscillations per minute and a 5° - 10° tilt.	**60 minutes** **25 °C ± 3 °C**
7.	Carefully uncover the tray to avoid splashing or mixing of samples. Tilt the tray to aspirate the mixture from the wells. Change the manual aspirator tips (if possible) between samples to avoid cross-contamination.	
8.	Wash each strip 3 times with 2mL of DILUTED WASH BUFFER allowing <u>5 minutes</u> to soak on the rocking platform between each wash.	**3 x 2 mL**
9.	Add 2 mL of WORKING CONJUGATE SOLUTION to each well.	**2 mL**
10.	Cover tray and incubate for <u>1 hour</u> at room temperature (25 °C ± 3 °C) on the rocking platform.	**60 minutes** **25 °C ± 3 °C**
11.	Aspirate WORKING CONJUGATE SOLUTION from the wells. Wash. (See Step 8.)	**3 x 2 mL**
12.	Add 2 mL of SUBSTRATE SOLUTION to each well.	**2mL**
13.	Cover tray and incubate for <u>15 minutes</u> on the rocking platform.	**15 minutes** **25 °C ± 3 °C**
14.	Aspirate the SUBSTRATE and rinse strips 3 times with reagent or deionized water to stop the reaction. Rinse the strips by adding 2 mL of reagent water, manually rocking the tray gently to ensure that the strips are fully covered with water, and immediately aspirating. Repeat this procedure an additional 2 times.	**3 x 2 mL**
15.	Using forceps, gently remove strips and place on laboratory paper towels or Office Printer paper. Allow to dry for 30 minutes. Alternatively, allow the strips to dry in the wells of the tray for 3 hours.	

16. Mount strips on the Report Sheet or equivalent worksheet (non-absorbent white paper). If using adhesive tape for mounting, do not apply over the developed bands. Observe the bands (See Interpretation of Results) and grade the results within 24 hours of drying. For storage, keep the strips in the dark.

SUMMARY OF ASSAY PROTOCOLS		
Reagents	**Qty**	**Duration**
Nitrocellulose strip	1 strip	-
Wash Buffer	2 mL	5 mins
Blotting Buffer	2 mL	-
Specimen	20 µL	60 mins
Wash Buffer	3 x 2 mL	3 x 5 mins
Conjugate	2 mL	60 mins
Wash Buffer	3 x 2 mL	3 x 5 mins
Substrate (Ready to use)	2 mL	15 mins
Reagent or Deionized Water	3 x 2 mL	-

QUALITY CONTROL

The Non-Reactive Control, Strong Reactive Control I, and Strong Reactive Control II must be run with the assay regardless of the number of samples tested. **Figure 1** shows the appearance of these control strips.

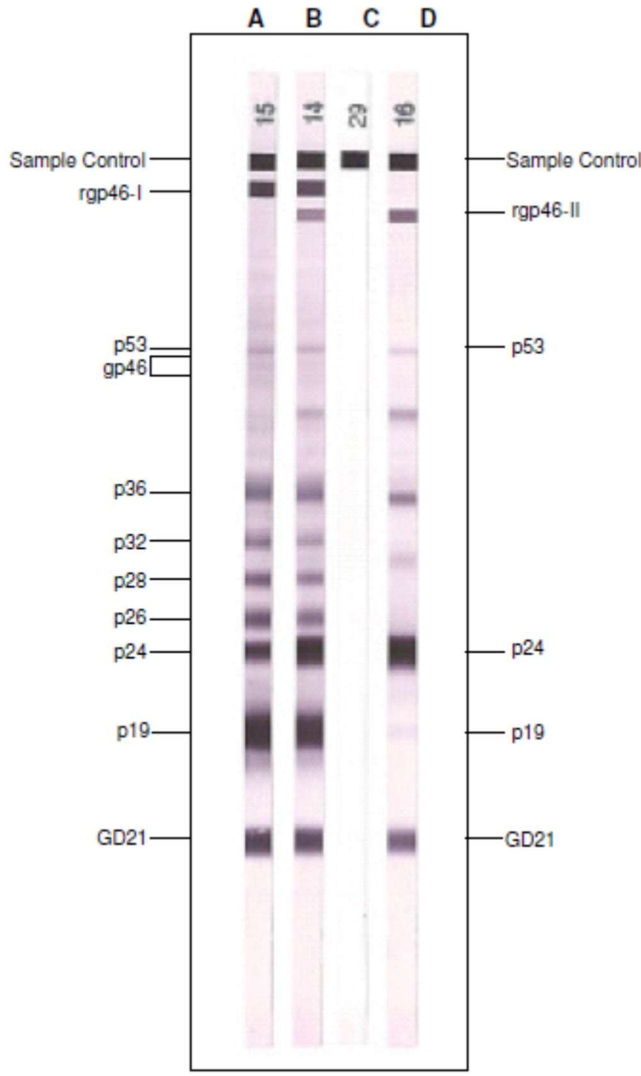


Figure 1: Examples of control strips

A. Strong Reactive Control I. (Reactive for HTLV-I only)
B. HTLV-I/II serum
C. Non-reactive Control
D. Strong Reactive Control II (Reactive for HTLV-II only)

In order for the results obtained from any assay to be considered valid, the following conditions must be met:

1. NON-REACTIVE CONTROL

No HTLV-I viral specific bands, rgp46-I, rgp46-II or GD21 should be observed on the Non-Reactive control strip. The sample control (anti-human IgG) band should be visible.

2. STRONG REACTIVE CONTROL I

The relevant HTLV bands that must be present are p19, p24, rgp46-I and GD21. The sample control (anti-human IgG) band should be visible.

Note: Although uncommon, a gp46 viral band may be present. If present, it appears as a diffuse band. Because of the rarity of gp46 and misreading of viral bands in this molecular weight range, viral gp46 is not used as part of the assay's interpretative criteria.

3. STRONG REACTIVE CONTROL II

The relevant HTLV bands that must be present are p24, GD21 and rgp46-II. The sample control (anti-human IgG) band should be visible.

INTERPRETATION OF RESULTS

The HTLV Blot 2.4 assay should be performed and interpreted by qualified and trained operators to ensure the reliability of test results. For information regarding training, please contact MP Biomedicals' Customer Service or your local representative.

The sample control band (anti-human IgG) serves as an indicator of sample addition for each strip **(Figure 1).** The absence of this band indicates that no test sample, conjugate or substrate has been dispensed onto, or reacted with, the test strip. Operational errors can also be indicated by the absence of the sample control band. Any test strip that does not show reactivity to the sample control band is considered invalid and must be repeated; only valid test strips may be interpreted. Refer to the guide included in the back of this insert to troubleshoot this or any assay problems.

The reading and interpretation of results are summarized as follows:

1. Mounting of the control strips and/or sample test strips on the Report Sheet or non-absorbent white paper using adhesive tape (two-sided or regular) or glue stick. (Do not apply adhesive tape over the developed bands);
2. Identification of the bands on the two control strips with the Protein Finder;
3. Identification of the bands on the sample strips with the control strips;
4. Scoring of the band intensity with the Intensity Finder or by presence or absence of the band;
5. Interpretation of the strip results.

The **Report Sheet** provided is used for storing, reading and interpreting the strips following processing; a blank Report Sheet is included with each kit. For extra report sheets, the blank Report sheet can be photocopied or downloaded from www.mpbio.com.

IDENTIFICATION OF BANDS

The **Protein Finder (Figure 2)** provided in the kit is used to locate and identify bands on the strips run with Strong Reactive Control I and Strong Reactive Control II. These control strips are then used to identify bands present on strips used with test specimens. Each Protein Finder is lot specific; only the Protein Finder that comes with the kit should be used to locate and identify bands.

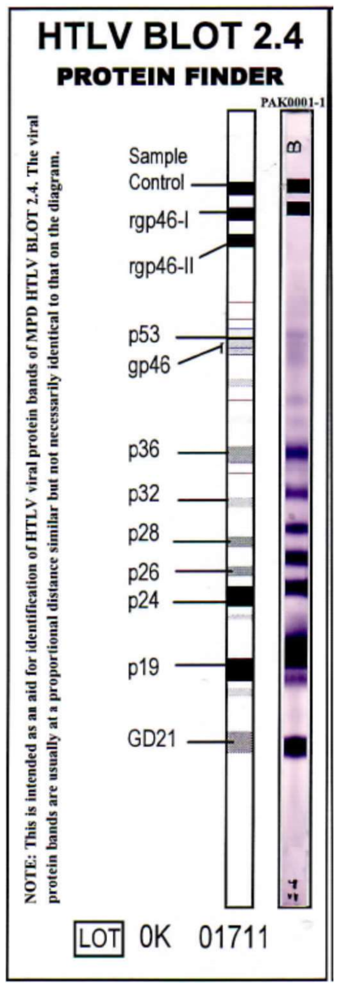


Figure 2: Example of a Protein Finder

To use the **Protein Finder**, line up the Strong Reactive Control I (SRCI) strip with the HTLV Blot strip on the Protein Finder so that the sample control bands are aligned. Compare any bands present on the SRCI strip to that of the Protein Finder Strip and verify the band positions on the SRCI strip.

Next, repeat the process by lining up the HTLV Blot strip on the Protein Finder with the Strong Reactive Control II (SRCII) strip so that the sample control bands are aligned. Compare any bands present on the SRCII strip to that of the Protein Finder Strip and verify the band positions on the SRCII strip.

If the control strips meet the quality control criteria, proceed with reading and interpreting the sample test strips. Interpretation is done by comparison of the bands of interest on the sample strips to those of the control strips.

If only the control strips are pasted onto the Report Sheet, the sample test strips can be manually aligned to the appropriate control strip (prior to mounting) to determine the target band's presence and position.

If both the control and sample test strips are pasted onto the report sheet, a ruler can be used to measure the distance between the target band and a fixed band (e.g. Control band) on the sample strip. This measurement can then be compared to the measurement of the same bands on the control strip to determine the presence of the target band on the sample test strip.

Strips with uneven background color development that obscures the reading of significant portions of the strip should not be interpreted, unless the readable portions of the strip result in an accurate interpretation of HTLV-I Seropositive, HTLV-II Seropositive, or HTLV-I/II Seropositive. The most significant portion of the strip for interpretation of results is defined as the reading frame from the sample control band to the GD21 band, and the significant HTLV bands to look up for are rgp-46-I, rgp46-II, p24, p19, and GD21, as shown in **Figure 2**. Strips with dark, even background along the entirety of the strip should not be interpreted.

If a result cannot be interpreted due to background color development, the test is considered invalid and a fresh sample should be obtained for repeat HTLV antibody testing.

SCORING OF BANDS

Bands can be scored qualitatively based on the visual presence or absence of a band, or semi-quantitatively based on the reactivity of a band. The **Intensity Finder (Figure 3)** provided in the kit should be used to semi-quantitatively determine the reactivity of any band.

Note: A band of ± is considered present by the criteria of this assay.
In addition, if the intensity of p19 and p24 bands is similar, the broadness of these bands is used to grade the relative reactivity of the p19 and p24 bands to differentiate between HTLV-I and HTLV-II.

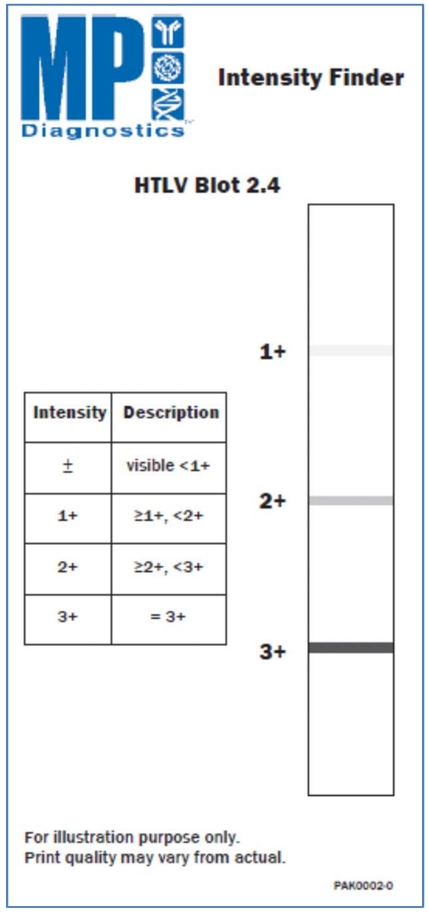


Figure 3: Example of an Intensity Finder

The major HTLV-I/II gene products that have been identified are listed in Table 1.

Table 1: Listing of major HTLV-I/II Gene Products

Band	**Gene Product**	**HTLV-I/II**
rgp46-I	Recombinant *env* glycoprotein	I
rgp46-II	Recombinant *env* glycoprotein	II
p53	Precursor of *gag* protein	I
gp46	Outer ENV glycoprotein	I
p36	*gag* protein intermediate	I
p32	*gag* protein intermediate	I

p28	*gag* protein intermediate	I
p26	*gag* protein intermediate	I
p24	Major *gag* capsid protein	I
p19	Major *gag* matrix protein	I
GD21	Recombinant transmembrane ENV protein	I/II

The above bands are the only bands that should be read and considered in the interpretation of test samples.

Use the following guidelines to determine the interpretation of test samples:

SERONEGATIVE INTERPRETATION:

- No reactivity to HTLV specific proteins; or
- Any combination of *gag* proteins excluding p24 (p19, p26, p28, p32, p36, p53)[b]; or
- Any single *gag* protein other than p19 or p24 (p26, p28, p32, p36, p53).

	GD21 Recombinant *env* Protein	**p19 Major *gag* Matrix**	**p24 Major *gag* Capsid**	**rgp46-II Recombinant *env* Protein**	**rgp46-I Recombinant *env* Protein**	**Non-major *gag* Proteins* (p26, p28, p32, p36, p53)**
Seronegative						
		X				**X**
						X
***presence of one or more**						

HTLV-I SEROPOSITIVE:

(Note: The non-major gag proteins (p26, p28, p32, p36, p53) may or may not be present and are not utilized in determining HTLV-I seropositivity)

- Reactivity to p19, GD21 ***and*** rgp46-I: or
- Reactivity to p19, p24 ***and*** GD21, with reactivity to p19 greater than or equal to p24[c]

	GD21 Recombinant *env* Protein	**p19 Major *gag* Matrix**	**p24 Major *gag* Capsid**	**rgp46-II Recombinant *env* Protein**	**rgp46-I Recombinant *env* Protein**
HTLV-I Seropositive	**X**	**X**		**X****	**X**
	X	**X**			**X**
	X	**X**	**X**		**X**
	X	**X***	**X**		
reactivity to p19 ≥ p24** * low level reactivity**					

HTLV-II SEROPOSITIVE:

(Note: The non-major gag proteins (p26, p28, p32, p36, p53) may or may not be present and are not utilized in determining HTLV-II seropositivity)

- Reactivity to p24, GD21 ***and*** rgp46-II: or

- Reactivity to p19, p24 ***and*** GD21, with reactivity to p24 greater than p19[c,d]

	GD21 Recombinant *env* Protein	p19 Major *gag* Matrix	p24 Major *gag* Capsid	rgp46-II Recombinant *env* Protein	rgp46-I Recombinant *env* Protein
HTLV-II Seropositive	X		X	X	
	X		X	X	X**
	X	X	X	X	
	X	X	X*		
reactivity to p24 > p19** * low level reactivity**					

HTLV-I/II SEROPOSITIVE:

(Note: The non-major gag proteins (p26, p28, p32, p36, p53) may or may not be present and are not utilized in determining HTLV-I/II seropositivity)

- Reactivity to GD21, p19, p24, rgp46-II ***and*** rgp46-I

	GD21 Recombinant *env* Protein	p19 Major *gag* Matrix	p24 Major *gag* Capsid	rgp46-II Recombinant *env* Protein	rgp46-I Recombinant *env* Protein
HTLV-I/II Seropositive	X	X	X	X	X

INDETERMINATE[e]:

- Reactivity to HTLV specific bands that do not meet the criteria for HTLV-I seropositive, HTLV-II seropositive, HTLV-I/II seropositive or seronegative. The list below includes some, but not all, of the indeterminate band pattern patterns:

Common Indeterminate Band Patterns

p19 only	p24 only	GD21 only
p19, p24	GD21, p19	rgp46-I only
rgp46-I, GD21	rgp46-I, rgp46-II	GD21, p19, rgp46-II
GD21, p24, rgp46-I	GD21, p19, rgp46-II	p19, p24, p26, p28, p32, p53

[a]The p36 band is not associated with HTLV-II infection. A band with similar molecular weight may appear with HTLV-II samples and should be disregarded.

[b]HTLV-I *gag* Indeterminate Western Blot patterns (HGIP) refer to the presence of p19, p26, p28, p32, p36, p53 (in various combinations) but absence of p24 and any ENV proteins. While HGIP would be interpreted as HTLV seroindeterminate based on 1990 guideline[52], various studies suggested that HGIP should be interpreted as seronegative especially with healthy blood donors.[53-66].

[c]Comparison of reactivity is based on intensity and broadness of band. If intensity of bands is similar, the reactivity is determined by comparing broadness of band.

[d]The p24 *gag* protein from the HTLV-I viral lysates cross-reacts with HTLV-II antibodies and is therefore used as an HTLV-II diagnostic marker.[67-71]

[e]If an indeterminate result occurs, a fresh sample should be obtained for repeat HTLV antibody testing.

LIMITATIONS OF THE PROCEDURE

1. The MP Diagnostics HTLV Blot 2.4 "PREPARATION OF REAGENTS", "ASSAY PROCEDURE', and "INTERPRETATION OF RESULTS" must be followed closely when confirming and differentiating the presence of antibodies to HTLV-I or HTLV-II in donor plasma or serum; failure to follow procedures as described may produce aberrant results. This assay was designed and validated for use with human serum or plasma specimens from individual donor specimens; performance has not been established using cadaveric specimens or body fluids such as urine, saliva, pleural fluid, amniotic fluid, or semen.
2. A seronegative result using the HTLV Blot 2.4 may be due to levels of anti-HTLV below the limit of detection in this assay; levels of anti-HTLV may be undetectable in early infection. Reactivity to any of the HTLV critical antigens in the strip (i.e., p19, p24, GD21, rgp46-II, and rgp46-I) is possible evidence of infection with HTLV; therefore, all seroindeterminate results should be followed to ascertain whether increased reactivity is present. A specimen that is reactive by a licensed HTLV screening test and seronegative by the HTLV Blot 2.4 does not exclude the possibility of infection with HTLV.
3. A specimen from an individual with a higher level of hemoglobin was shown to cross react with the HTLV Blot 2.4, producing erroneous results; reactivity was at a ± intensity score. Samples from potentially interfering medical conditions, such as HIV, hemophilia and Sjogren's disease, have also been shown to cross react with the HTLV Blot 2.4 to produce low level bands. Donor specimens seropositive by the criteria of the HTLV Blot 2.4 using ± bands only should be retested using a fresh sample to confirm infection.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. MP Diagnostics HTLV Blot 2.4 Performance Characteristics in Known Positive Population and Normal Blood Donors

The performance of the MP Diagnostics HTLV Blot 2.4 was evaluated in clinical studies on blood donor populations by comparison of HTLV Blot 2.4 results with those obtained from matched plasma specimens tested using the California Department of Public Health (CDPHL) HTLV Supplemental Algorithm.[1] Sensitivity was evaluated using a known positive population, characterized as archival specimens from deferred blood donors who had tested repeatedly

[1] A licensed, HTLV supplemental assay was not available at the time of testing. The CDPHL HTLV Supplemental Algorithm consists of a series of in-house developed, HTLV supplemental assays. The HTLV Algorithm includes the following assays in sequence: ELISA: IFA: Western Blot; & RIPA. The number of assays that a sample will be tested with is dependent upon the sample results within the HTLV Algorithm.

reactive by at least one licensed HTLV screening assay and were confirmed positive through additional, research use supplemental assays, including, IFA, Western blot and RIPA. Specificity was evaluated using archival specimens from normal volunteer blood donors that had tested HTLV non-reactive by a licensed screening assay. The MP Diagnostics HTLV Blot 2.4 testing was performed at three, geographically distinct clinical testing sites.

1.1 Sensitivity in Known Positive Population

A total of 200 repository specimens from a well-characterized, known positive population were evaluated at three geographically distinct clinical testing sites. These specimens were from deferred donors that had previously tested repeatedly reactive using a licensed screening assay in conjunction with research use HTLV supplemental testing, including ELISA, IFA, Western blot and RIPA. The summary results from testing the known positive population are shown in Table 1.

Table 1: MP Diagnostics HTLV Blot 2.4 and CDPHL HTLV Supplemental Algorithm Results for 200 Known Positive Specimens

		CDPHL Algorithm				
		HTLV-I POS	*HTLV-II POS*	*IND*	*NEG*	Total
MP Diagnostics HTLV Blot 2.4	*HTLV-I POS*	79	1	1	0	81
	HTLV-II POS	0	100	0	4	104
	HTLV-I/II POS	9	1	0	0	10
	IND	1	2	0	1	4
	NEG	0	0	0	1[a]	1
	Total	89	104	1	6	200

[a] One sample was negative by both the MP Diagnostics HTLV Blot 2.4 and the CDPHL HTLV Algorithm.

A greater number of known positive specimens were identified as positive by the MP Diagnostics HTLV Blot 2.4 than by the CDPHL HTLV Algorithm (195 versus 193, respectively). Additionally, the MP Diagnostics HTLV Blot 2.4 identified more samples as reactive (i.e., positive or indeterminate) than the CDPHL HTLV Algorithm (199 versus 194, respectively). Of the 195 specimens identified as Positive by the HTLV Blot, 185 (81 + 104) were interpreted as HTLV-I Positive or HTLV-II Positive, and 10 (9 + 1) were HTLV-I/II Positive. Additionally, the HTLV Blot 2.4 identified more samples as reactive (i.e., Positive or Indeterminate) than the CDPHL Algorithm

(199 versus 194, respectively). Of the six samples identified as Negative by the CDPHL Algorithm, four were identified as HTLV-II Positive by the MP Diagnostics HTLV Blot 2.4.

Although these 200 specimens were previously identified as Positive for HTLV antibodies using the CDPHL algorithm, six specimens were Negative and one was Indeterminate on retesting by the CDPHL Algorithm. This Indeterminate specimen was determined to be HTLV-I Positive by the MP Diagnostics HTLV Blot 2.4. One sample was negative by both the HTLV Blot 2.4 and the CDPHL Algorithm.

In this study, the sensitivity of the MP Diagnostics HTLV Blot 2.4 was 97.5%[2] (195/200) with a 95% CI of 94.26 - 99.18%. The indeterminate rate for this study was 2% (4/200).

1.2 Specificity in Normal Blood Donors Testing HTLV Non-reactive by a Licensed Screening Assay

A total of 200 repository specimens from a normal blood donor population were evaluated at three geographically distinct clinical testing sites. These specimens were from blood donors that had previously tested HTLV non-reactive using a licensed HTLV screening assay. The summary results from testing the HTLV screening assay negative population are shown in Table 2.

Table 2: MP Diagnostics HTLV Blot 2.4 and CDPHL HTLV Supplemental Algorithm Results on HTLV Screening Assay Negative Population

		CDPHL Algorithm			
		POS	*IND*	*NEG*	Total
MP Diagnostics HTLV Blot 2.4	*POS*	0	0	0	0
	IND	0	0	43	43
	NEG	0	0	157	157
	Total	0	0	200	200

The MP Diagnostics HTLV Blot 2.4 identified 157 as negative and 43 as indeterminate; there were no positive samples identified in this population. Of these 200 specimens tested by the CDPHL HTLV Algorithm, 15 were repeatedly reactive by ELISA. The majority of these repeatedly reactive samples were resolved at the Western blot stage of the CDPHL HTLV Algorithm, based on non-

[2] Sensitivity was calculated as follows: TP/(TP+FN) x 100% where TP = true positives, that is, the number of specimens positive by MP Diagnostics HTLV Blot 2.4; and FN = false negatives, that is, the number of specimens indeterminate or negative by MP Diagnostics HTLV Blot 2.4.

reactivity from both the IFA and Western blot. Two of these 15 samples, however, showed reactivity with the p21e protein on the Western blot and were subjected to additional testing using RIPA. A non-reactive result on the RIPA for these 2 specimens resulted in an overall call of negative by the CDPHL Algorithm. All but one of these 15 specimens was negative by a single MP Diagnostics HTLV Blot 2.4 assay.

In this study the indeterminate rate of MP Diagnostics HTLV Blot 2,4 for licensed HTLV-I/II ELISA negative specimens was 21.5% (43/200)

2. Comparative Testing of Repeatedly Reactive Specimens Identified by Specific Licensed HTLV-I/II Screening Tests

A total of 200 repeatedly reactive samples were evaluated at three geographically distinct clinical testing sites. These specimens were from blood donors that had previously tested repeatedly reactive using the Abbott PRISM HTLV-I/II ChLIA. Of these 200 samples, the MP Diagnostics HTLV Blot 2.4 identified 3 as positive, 88 as negative and 109 as indeterminate or equivocal (Table 3). Comparatively, the CDPHL HTLV Algorithm identified 3 as inconclusive and 197 as negative. Follow-up testing that was available on one donor confirmed that the MP Diagnostics HTLV Blot 2.4 had correctly identified that specimen as positive. Additionally, three inconclusive CDPHL HTLV Algorithm samples that were identified as negative by the MP Diagnostics HTLV Blot 2.4 were confirmed as negative during donor follow-up; the CDPHL HTLV Algorithm result of inconclusive was due to a false positive western blot that used the less specific p21e recombinant.
In this study the indeterminate rate of MP Diagnostics HTLV Blot 2,4 for Abbott PRISM HTLV-I/II false positive specimens was 55% (109/197)

Table 3: Performance of MP Diagnostics HTLV Blot 2.4 against the CDPHL algorithm with samples that are RR on Abbott PRISM HTLV-I/II screening test

		CDPHL Algorithm			
		POS	*IND*	*NEG*	Total
MP Diagnostics HTLV Blot 2.4	*POS*	0	0	3	3
	IND	0	0	109	109
	NEG	0	3	85	88
	Total	0	3	197	200

A total of 105 preselected repository samples that were repeatedly reactive using the Avioq HTLV-I/HTLV-II Microelisa System were evaluated at one clinical testing site as well as in-house at MP Biomedicals, LLC. Of these 105 samples, the MP Diagnostics HTLV Blot 2.4 identified 50 as positive, 18 as indeterminate and 37 as negative (Table 4). Comparatively, the CDPHL HTLV Algorithm identified 50 as positive, 51 as negative and 4 as inconclusive or equivocal. The percent positive agreement of the MP Diagnostics HTLV Blot 2.4 with the CDPHL HTLV Algorithm was 100% and the overall percent agreement was 82.18% (95% CI of 73.30 to 89.08%). The four CDPHL HTLV Algorithm inconclusive results were due to the presence of p21e; all sample results were resolved as negative by the MP Diagnostics HTLV Blot 2.4 due to the inclusion of GD21, a more specific envelope recombinant.

In this study the indeterminate rate of MP Diagnostics HTLV Blot 2,4 for Avioq HTLV-I/II Microelisa System false positive was 35% (18/51)

Table 4: Performance of MP Diagnostics HTLV Blot 2.4 against the CDPHL algorithm with samples that are RR on Avioq HTLV-I/II Microelisa System

		CDPHL Algorithm			
		POS	*IND*	*NEG*	Total
MP Diagnostics HTLV Blot 2.4	*POS*	50	0	0	50
	IND	0	0	18	18
	NEG	0	4	33	37
	Total	50	4	51	105

Among the 50 positive specimens, the HTLV Blot 2.4 identified 18 as HTLV-I and 29 as HTLV-II, and three specimens as HTLV-I/II Undifferentiated (see Table 5). In comparison, the CDPHL Algorithm identified 15 as HTLV-I, 29 as HTLV-II, and eight as HTLV-I/II Undifferentiated. These data indicated overall agreement between the HTLV Blot 2.4 and the CDPHL algorithm to differentiate HTLV-I and HTLV-II infections with concordant differentiation by the HTLV Blot 2.4 of 13/15 specimens categorized as HTLV-I by the CDPHL algorithm and 27/27 specimens categorized as HTLV-II by the CDPHL algorithm.

Table 5: Differentiation of positive specimens against the CDPHL algorithm for those RR using the Avioq HTLV-I/II Microelisa System

		CDPHL HTLV Algorithm			
		HTLV-I POS	*HTLV-II POS*	*HTLV-I/II POS Undifferentiated*	Total
MP Diagnostics HTLV Blot 2.4	*HTLV-I POS*	13	0	5	18
	HTLV-II POS	0	27	2	29
	HTLV-I/II POS Undifferentiated	2	0	1	3
	Total	15	27	8	50

3. Comparative Testing of Repeat Reactive Specimens Identified by initial HTLV-I/II Screening Test I and Non-Reactive to the second HTLV Screening.

The Dual Algorithm Post-Market Clinical Study was conducted to further evaluate the effectiveness of the dual testing algorithm in identifying US donors that are uninfected with HTLV. Specimens were obtained from American Red Cross (ARC) blood donors who screened repeat reactive on the Abbott Prism HTLV assay and non-reactive on the Avioq HTLV ELISA assay. 100 plasma specimens which screened repeat reactive on the Abbott Prism HTLV assay and non-reactive on the Avioq HTLV ELISA were enrolled and assessed using the HTLV Blot 2.4 between May 03-May 31, 2017. Using three operators over two lots of HTLV Blot 2.4 test kits, the following HTLV Blot 2.4 results were obtained:

- Seronegative 79/100 (79.0%)
- Indeterminate 21/100 (21.0%)
- Seropositive 0/100 (0.0%)

Of the 21 indeterminate results, 14/21 (66.7%) demonstrated an isolated p19 (13) or p24 (1) band, which has been reported in previous HTLV Blot 2.4 clinical studies. Results demonstrate the consistent sensitivity and specificity of the western blot assay as outlined in the current product approved labeling.

4. Reproducibility

The reproducibility of the MP Diagnostics HTLV Blot 2.4 assay was established in a study that assessed assay reproducibility within operator, within site, within lot, and between lots. This study tested two replicates of a three-member panel at three clinical sites with each of three product lots over multiple days by three operators. The three-member panel consisted of one HTLV-I antibody specimen, one HTLV-II antibody specimen, and one specimen non-reactive to antibodies for HTLV-I/II. For each of the three kit lots, there were a total of 54 HTLV Blot 2.4 strips tested with each panel member. Reproducibility was calculated as percent agreement of positive results / negative results.

In this study, no strips were incorrectly interpreted. These data demonstrate that the MP Diagnostics HTLV Blot 2.4 assay is reproducible across multiple sites, operators and lots.

4.1 Reproducibility of Test Strip Interpretation at 24 Hours post assay

Between June 16, 2013 to December 10, 2015, 950 unique HTLV RR plasma specimens were evaluated with the HTLV Blot 2.4. Of the 945/950 (99.5%) per-protocol specimens evaluated, 491/945 (52.0%) were HTLV seropositive, 271/945 (28.7%) seronegative, 180/945 (19.0%) indeterminate and 3/945 (0.31%) invalid. HTLV Blot 2.4 test result interpretation agreement within 3 & 24 hours post assay is summarized below:

HTLV Blot 2.4 Test Result	Result Interpretation within 3 Hrs. n (%)	Result Interpretation Agreement at 24 Hrs. n (%)	% Agreement
Seronegative	271 (28.7)	271 (28.7)	(100.0)
HTLV I	201 (21.3)	201 (21.3)	(100.0)
HTLV II	245 (25.9)	245 (25.9)	(100.0)
HTLV I / II	45 (4.8)	45 (4.8)	(100.0)
Indeterminate	180 (19.0)	179 (18.9)	(99.44)
Invalid	3 (0.3)	3 (0.3)	(100.0)
Total	**945**	**944**	**(99.89)**

Data generated from this expanded access sub-study confirm the concordant HTLV Blot 2.4 assay results interpretation within three and at 24 hours following assay completion.

5. Effect of Potentially Interfering Substances

Specimens with potentially interfering substances were obtained from well characterized repositories and tested for non-specific reactivity using the HTLV Blot 2.4. Specimen types consisted of the following: elevated bilirubin (n = 20); elevated triglyceride (n = 20); bacterially contaminated samples (n = 20); hemolyzed samples (n = 20); icteric samples (n = 20); lipemic samples (n = 20).

A total of one hundred twenty (120) samples were assessed. The impact of different potentially interfering substances on the performance of the MP Diagnostics HTLV Blot 2.4 was assessed using the six (6) different populations as both unspiked samples and spiked to a low level of reactivity at a dilution of 1:80. Of the 120 samples, only one sample was resulted as positive; samples with elevated hemoglobin levels may produce erroneous results due to non-specific reactivity. The results are presented in Table 6. Potentially interfering substances in HTLV-positive samples did not impact the sensitivity of the HTLV Blot 2.4. A high strip background level obscured the reading of bands in 7 out of the 120 samples (5.8%). As both spiked and unspiked samples were affected, it was determined that the higher background was mostly likely due to the presence of an interferent.

Table 6: Effect of Potentially Interfering Substances In Unspiked Samples

Potentially Interfering Condition	Level of Interferent	Number of Specimens Tested	Number of Positive Results
Bilirubin	25.1 to 44 mg/dL	20	0
Triglyceride	859 to 1883 mg/dL	20	0
Bacterial Contamination	As determined by gram stain	20	0
Hemoglobin	25 to 200 mg/dL	20	1
Icteric samples	Icteric samples with bilirubin levels between 23.32 to 39.47 mg/dL	20	0
Lipemic sample	Triglyceride levels between 798 to 2418 mg/dL	20	0

6. Effect of Anti-Coagulants

The effects of anti-coagulants on the performance of MP Diagnostics HTLV Blot 2.4 was evaluated using matched sets consisting of seven (7) types of anti-coagulant plasma (ACD, CPD, Sodium Citrate, K-Oxalate, K2 EDTA, Sodium Heparin, and PPT) and a serum specimen for reference. Each sample was tested both unspiked and spiked with a positive HTLV-I or HTLV-II specimen as compared to the reference sample. The presence or type of anticoagulant did not impact the performance of the HTLV Blot 2.4 in either spiked or unspiked samples.

7. Effect of Dilution

The effect of serial dilution on HTLV-positive samples (5 HTLV-I and 5 HTLV-II positives) was assessed by testing neat samples and at the following dilutions: 1:40; 1:120: 1:360; and 1:1080. The end-point dilution for HTLV-I samples with critical bands of GD21, p19, p24, and rgp46-I is 1:1080. The end-point dilution for HTLV-II samples with critical bands of GD21, p24, and rgp46-II is 1:120. This study showed that there was no impact from serial dilution of samples up to 1:120 on performance of the HTLV Blot 2.4. In addition, the study indicated that band intensity of the critical bands and the amount of antibodies present in the samples were correlated.

8. Effect of Unrelated Medical Conditions

The effect of unrelated medical conditions on the performance of the HTLV Blot was evaluated using 200 specimens from individuals with various medical conditions, including HIV (n = 20), HCV (n = 20), HBV (n = 20), EBV (n = 20), CMV (n = 20), patients vaccinated with the influenza vaccine (n = 10), hemophiliacs (n = 20), dialysis patients (n = 20), multiparous women (n = 10), high rheumatoid factor (n = 20), Hashimoto's disease (n = 10), and Sjogren's disease (n = 10). Specimens were tested both unspiked and spiked with an HTLV-I or HTLV-II positive specimen. Specimen results are shown in Table 7. Of the 200 spiked samples, all but one of the samples remained positive; one sample from the EBV population was resulted as indeterminate. Of the 200 unspiked samples tested, 4 were resulted as positive; one each from the HIV, dialysis, hemophiliac, and Sjogren's populations. The specimen from the dialysis population was determined to be a true positive based on subsequent testing, and was further excluded from calculations.

Table 7: Effect of Unrelated Medical Conditions

Potentially Interfering Medical Condition	Number of Specimens Tested	Number of Specimens Positive in Unspiked Population	Number of Specimens Negative in Spiked Population
HIV	20	1	0
HCV	20	0	0
HBV	20	0	0
EBV	20	0	0
CMV	20	0	0
Influenza Vaccine	10	0	0
Hemophiliac	20	1	0
Dialysis	19	0	0
Multiparous Women	10	0	0
Elevated Rheumatoid Factor	20	0	0
Hashimoto's disease	10	0	0
Sjogren's disease	10	1	0

The high strip background obscured the reading of bands in 7 out of 200 cross-reactive samples (3.5%). This affected both the unspiked samples and spiked HTLV-positive samples. The principal observation was the appearance of randomly occurring critical bands with many of the unspiked samples. This occurred across all twelve populations, and it caused many unspiked samples to be interpreted as indeterminate, rather than the expected negative interpretation. From this data, it is reasonable to conclude that the presence of the potential cross reactants increases the frequency of indeterminate results and may produce erroneous results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no expressed warranty other than that the test kit will function as an in-vitro diagnostic assay within the specifications and limitations described in the Instructions for Use (IFU) when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss however caused by the product in the use or in the application thereof.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be a technical problem / complaint, please do the following:

1. Note the kit lot, strip lot number and the expiration date.
2. Retain the kits and the results that were obtained.
3. Contact MP Biomedicals' Customer Service.

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* US Patent	5,066,579; 5,614,366; 5,763,572 ; 5,814,441 ; 5,871,933 ; 5,643,714: 6.110.662
* Australian Patent:	613350 ; 667189 ; 690540
* Canadian Patent:	1337799
* European Patent:	0395634
* Japanese Patent:	2559482

TROUBLE SHOOTING CHART

Dark spots develop on strips

1. Bacterial or fungal contamination of test sample.
2. Precipitation of immune complexes in aged test sample.
3. Bacterial or fungal contamination on strip due to improper storage.
4. Strips physically damaged, cracked or scratched.
5. Strips not properly washed between assay steps.
6. Sample added directly on top of strip.

White patches develop on strips

1. Strip was flipped over during assay.
2. Tray may have been re-used
3. Poor dissolution of Blotting Powder.
4. Electrotransblot interference during manufacturing

Expected bands do not develop or are of weak intensity

Check positive control

Positive control weak

The problem is probably caused by the reagents.

1. Reagents not properly prepared.
2. Wrong conjugate dilution.
3. Unstable reagents due to improper temperature exposure.
4. Conjugate contaminated with human IgG.
5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.
6. Trays, reagent(s) or water having high phosphate concentration.
7. Rotary platform used instead of Rocking platform

Positive control OK

The problem is probably caused by test sample.

1. Wrong test sample dilution.
2. Test sample contaminated with conjugates.
3. Test samples severely immune-complexed.
4. Test sample IgG deteriorated or denatured due to repeated freeze-thaw or improper storage.
5. Rotary platform used instead of Rocking platform
6. Test sample may be an ELISA “false” positive

Strong Background develops on strip in the absence or presence of positive bands

1. Overdeveloped strips (stop reaction sooner).
2. Incomplete washing.
3. Sample overloaded.

Absence of Sample Control Band

1. Sample not added.
2. Strips flipped over during assay.
3. Conjugate not added.
4. Substrate not added.

Bands other than the Sample Control Band develops on negative control

Tray wells or Control may have been crossed contaminated.

Non-specific bands and/or dark background develop on strips

1. Wrong test sample or conjugate dilution.
2. Test sample/reagent incubation too long.
3. Incomplete washing during assay.
4. Incubation temperature greater than 30ºC.
5. Test sample reactive with non-viral proteins.
6. Sample overloaded.

Strips are defective

1. They are cracked.
2. They contain air bubbles which cause the appearance of white spots in reactive zones big enough to prevent any detection.
3. They show dark spots due to fungal growth upon initial opening of the strip tubes. However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user’s site

HTLV-specific bands detected on negative control and/or known negative samples

1. Trays contaminated with positive test sample or positive control.
2. Negative control/test sample contaminated with positive control/ test sample.
3. Same pipette tip used for delivery and/or removal of, test samples/control.
4. Test sample may be an ELISA “false” negative.

Watery marks on developed strips

1. Strips left to dry after pre-soaking step prior to adding Blotting Buffer