HTLV BLOT 2.4

WESTERN BLOT ASSAY

CE
0123

REVISION DATE: 05/05
MAK 0011-ENG-0

Note Changes Highlighted

REF (18 tests kit) : 11080-018
(36 tests kit) : 11080-036

NAME AND INTENDED USE

The **MP Diagnostics (MPD) HTLV BLOT 2.4** is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to HTLV-I and HTLV-II in human serum or plasma. It is intended for use as a more specific supplemental test on human serum or plasma specimens found repeatedly reactive using screening procedures like the Enzyme-Linked Immunosorbent Assays (ELISA).

INTRODUCTION

Recent epidemiology studies in the United States and Europe confirm the presence of a mixed prevelance of both HTLV-I and HTLV-II among different high risk populations such as intravenous drug users. Screening tests for HTLV-I/II are widely available. Repeatedly reactive specimens from screening tests require additional and more specific tests to confirm HTLV-I or HTLV-II seropositivity. Such supplemental tests 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 is one such commonly used supplemental test. However, due to the lack of native envelope antigens on the classical HTLV-I Western Blot, it is often necessary to use radioimmunoprecipitation methods to further confirm for the presence of HTLV-I/II antibodies. Discrimination of HTLV-I and HTLV-II seropositives require supplemental assays (i.e. specific peptide, ELISAs, PCR).

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.

The **MP Diagnostics HTLV Blot 2.4** has improved sensitivity and specificity for both the confirmation and differentiation of HTLV-I and HTLV-II seroreactivities. This is accomplished by incorporating MTA-1, a unique HTLV-I envelope recombinant protein (rgp46-I), K55, a unique HTLV-II envelope recombinant protein (rgp46-II) and GD21, a common yet specific HTLV-I and HTLV-II epitope envelope recombinant protein (rgp21). Each strip also includes an internal sample addition control to minimize the risk of false negatives due to operational errors.

The **MP Diagnostics HTLV Blot 2.4** is intended as a supplemental antibody assay for characterizing samples found repeatedly reactive by initial HTLV-I/II antibody screening methods. The possible serological profiles defined by the **MP Diagnostics HTLV Blot 2.4** include the following: HTLV Seropositive, HTLV-I Seropositive, HTLV-II Seropositive, Seronegative or Indeterminate.

DESCRIPTION OF SYMBOLS USED

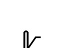
The following are graphical symbols used in or found on **MP Diagnostics** products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the British and European Standard BS EN 980: 2003.

Use by
Synonym for this :
Expiry Date

In vitro diagnostic medical device

Batch Code
Synonyms for this are:
Lot Number
Batch Number

Catalogue Number



Temperature Limitation

Attention.
See Instruction for Use

Manufacturer

Authorised Representative in the European Community

Contains sufficient for <n> tests

Consult instructions for use

Do not reuse

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips are incorporated with HTLV-I viral proteins derived from native inactivated disrupted viral particles and genetically engineered proteins. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and controls. Specific antibodies to HTLV-I/II, if present in the specimen will bind to the HTLV-I/II proteins on the strips. The strips are washed to remove unbound materials while antibodies that bind specifically to the HTLV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate, BCIP/NBT. This method is sensitive enough to detect marginal amounts of HTLV antibodies in serum or plasma.

KIT COMPONENTS

	Component Description	Quantity Provided
ANTIGEN STRIPS	**NITROCELLULOSE STRIPS** Incorporated with HTLV-I viral lysate, recombinant envelope antigens and a serum addition control (anti-human IgG) band. Keep dry and away from light.	Available in 18 or 36 strips
	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)
	STRONG REACTIVE CONTROL I Inactivated human serum with high titered 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)
	STRONG REACTIVE CONTROL II Inactivated human serum with high titered 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 T	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20 and contains thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase.	1 vial (120 µl)
	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 (1g each)
	Incubation Tray, 9 wells each	2 or 4 trays
	Instruction Manual	1 copy
	Forceps	1 piece

Note : Volume of reagents provided are sufficient for 4 runs.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For Professional use only.
3. Please refer to the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection.

HANDLE ASSAY SPECIMENS, STRONG REACTIVE I, STRONG REACTIVE II AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS. It is recommended that the components and test specimens be handled using good laboratory working practices. They should be disposed of in accordance with established safety procedures.

The ***Strong Reactive Control I, Strong Reactive Control II*** and ***Non-Reactive Control*** contain Thimerosal and Sodium azide while Stock Buffer Concentrate and Wash Buffer Concentrate contain Thimerosal and Conjugate contains Sodium azide. Sodium Azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system. The following are the appropriate Risk (R) phrases.

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

The ***Substrate*** contains 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate Risk (R) phrases.

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

1. Avoid Microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.

2. Do not pipette by mouth.

3. Handle test specimens, nitrocellulose strips, Strong Reactive I, Strong Reactive II and Non-Reactive Controls as potentially infectious agents.

4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.

5. It is highly recommended that this assay be performed in a biohazard cabinet.

6. Keep materials away from food and drink.

7. In case of accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.

8. 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.

9. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with 1% sodium hypochlorite solution before work is resumed. Sodium hypochlorite should not be used on acid containing spills unless the area is wiped dry with absorbent paper first. Material used (including disposable gloves) should be disposed off as potentially biohazardous material. Do not autoclave material containing sodium hypochlorite.

10. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.

11. 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.

12. We do not recommend re-use of incubation trays.

ANALYTICAL PRECAUTIONS

1. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction Manual. Deviations from the procedure may lead to aberrant results.

2. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and Western Blot strips are matched for optimal performance. Use only the reagents supplied with the kit.

3. Do not use kit components beyond the expiry date printed on the kit box.

4. Avoid microbial contamination of the reagents,when opening and removing aliquots from the original vials or bottles. As this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.

5. The kit controls should be assayed concurrently with patients' samples for each test run.

6. Use a new pipette tip for each specimen aliquot to prevent cross contamination.

7. For best results dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.

8. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.

9. Use only reagent grade quality, deionised or distilled water to dilute reagents.

10. All reagents must be mixed well before use.

11. Working Conjugate solution, Diluted Wash Buffer and Blotting Buffer should be **prepared fresh prior to use.**

12. The Working Conjugate solution should be prepared using a polypropylene container or beaker.

13. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.

14. The assay should preferably be performed at room temperature (25°C ± 3°C).

15. Make sure that the test strips are laid with the numbers on the strips facing upwards.

16. For Western Blot Assay, it is important to use a rocking platform shaker and not a rotary shaker. Otherwise, performance of the kit will be compromised. The recommended speed and tilt angle of the shaker are 12 to 16 cycles per minute, and 5 to 10 degrees, respectively.

17. Ensure that automated equipment if used is validated before use.

18. Ensure that the specimens are added away from the strip. Tray can be tilted and specimen added where the buffer is collected at lower end. This prevents dark spot formation due to specimen addition on the strip.

19. Avoid the use of self-defrosting freezers for the storage of reagents and samples.

STORAGE INSTRUCTIONS

1. Store MPD HTLV BLOT 2.4 kit and its components at 2-8°C when not in use.
2. All test reagents and strips when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze reagents.

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

B. **Reagents**
 - Store reagents in their original vials or bottles, and they should be closed for storage.
 - Dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
 - Precipitates may form when the Substrate is stored at 2°C to 8°C. This will not affect the performance of the kit.

CAUTION: **Avoid unnecessary exposure of substrate to light.**

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Serum or plama samples collected in EDTA, heparin or sodium citrate may be used. Before storage, ensure that blood clot 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. Clear, non-hemolyzed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

Patients' sera can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:
1. Loosen caps of serum containers.
2. Heat serum at 56°C for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis.

We recommend that the patients' sera should not undergo repeated freeze-thaw cycles prior to testing.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water
- Disposable gloves
- Rocking platform (desgined with a rocking speed of 12 to 16 oscillations per minute and which moves through a 5° to 10° tilt to wash membranes evenly)
- Pipettors and tips of appropriate volume
- Aspirator with sodium hypochlorite trap
- 56°C water bath (optional)
- Sodium hypochlorite for decontamination

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 grade water. Mix well.

2. BLOTTING BUFFER

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

(b) BLOTTING BUFFER should be **prepared fresh prior to use.** Add 1 g of BLOTTING POWDER to every 20 ml of the RECONSTITUTED STOCK BUFFER prepared in step 2(a) above. Stir to ensure powder dissolves completely.

(c) Stir again before dispensing.

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 1:1000 into BLOTTING BUFFER, for example 10 µl CONJUGATE to 10 ml BLOTTING BUFFER.

4. SUBSTRATE SOLUTION (ready to use)

(a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

ASSAY PROCEDURE

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

b) All incubations are to be carried out on a rocking platform.

Caution:

Some samples cause dark patches on the spot of the strip where they are added. To avoid this problem, one should ensure the following:-

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

ii. Tilt the tray slightly by elevating either the top or bottom end of the tray. The Blotting Buffer will flow to the lower end of the tray. Add the sample where the Blotting Buffer is collected. When all the samples are added, return the tray back to its original flat position. Always ensure that the strips are kept wet during the process.

iii. Alternatively, if tilting the tray is not desired, the samples may be added to the top or bottom end of the well. This way if dark patches showed, the reading of the strip results will not be affected.

Procedure:

1. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive, Weak Reactive and Non-Reactive controls.
2. Add 2 ml of DILUTED WASH BUFFER to each well. — **2 ml**
3. Incubate the strips for at least 5 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 oscillations per minute). Remove buffer by aspiration. — **5 minutes**
4. Add 2 ml of BLOTTING BUFFER to each well. — **2 ml**
5. Add 20 µl each of patients' sera or controls to appropriate wells. — **20 µl**
6. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25 ± 3°C) on the rocking platform. — **60 minutes**
7. Carefully uncover the tray to avoid splashing or mixing of samples . Tilt the tray to aspirate the mixture from the wells. Change aspirator tips between samples to avoid cross-contamination.
8. Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER allowing 5 minutes 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 1 hour at room temperature (25 ± 3°C) on the rocking platform. — **60 minutes**
11. Aspirate CONJUGATE from the wells. Wash as in step 8. — **3 x 2 ml**
12. Add 2 ml of SUBSTRATE SOLUTION to each well. — **2 ml**
13. Cover tray and incubate for 15 minutes on the rocking platform. — **15 minutes**
14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction. — **3 x 2 ml**
15. Using forceps, gently remove strips onto paper towels. Cover with paper towels and dry. Alternatively, allow strips to dry in the wells of the tray.
16. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive tape over the developed bands. Observe the bands (See Interpretation of Results) and grade the results. For storage, keep the strips in the dark.

SUMMARY OF ASSAY PROTOCOLS

Reagents	Qty	Duration
Nitrocellulose strip	1	-
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
Distilled Water	3 x 2 ml	-

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED						
	3	6	9	15	20	27	36
1X Wash Buffer (ml)	60	100	140	240	300	400	520
1X Blotting Buffer (ml)	20	40	60	80	100	120	160
Conjugate (µl)	11	17	23	35	45	59	77
Substrate (ml)	11	17	23	35	45	59	77
Blotting Powder (g)	1	2	3	4	5	6	8

QUALITY CONTROL

We recommend that the Non-Reactive Control and both Strong Reactive Controls be run with every assay regardless of the number of samples tested. 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/II viral specific bands, rgp46-I, rgp46-II or GD21 should be observed on the Non-Reactive control strip. The serum control (anti-human IgG) band should be visible.

2. **STRONG REACTIVE CONTROL I**
   The serum control band and all relevant HTLV-I/II molecular weight bands must be evident . The relevant HTLV-I bands that must be present are p19, p24, gp46, rgp46-I and GD21. Note that the gp46 band is diffused. The serum control (anti-human IgG) band should be visible.

3. **STRONG REACTIVE CONTROL II**
   The serum control band and all relevant HTLV-I/II molecular weight bands must be evident . The relevant HTLV II bands that must be present are p24, GD21 and rgp46-II. The serum control (anti-human IgG) band should be visible.

INTERPRETATION OF RESULTS

The serum control band serves as a check for serum addition in the assay. Absence of this band indicates that no test serum or conjugate or substrate has been dispensed onto the test strip or other operational errors.

Locate and identify bands on the strips run with Strong Reactive Controls. These strips are then used to identify bands present on strips used with test specimens.

PATTERN	INTERPRETATION
1. No reactivity to HTLV specific proteins	SERONEGATIVE
2. Reactivity to GAG (p19 with or without p24) and two ENV (GD21 and rgp46-I)	HTLV-I SEROPOSITIVE
3. Reactivity to GAG (p24 with or without p19) and two ENV (GD21 and rgp46-II)	HTLV-II SEROPOSITIVE
4. Reactivity to GAG (p19 and p24) and ENV (GD21) *-HTLV-I SEROPOSITIVE indicated if p19 ≥ p24* *-HTLV-II SEROPOSITIVE indicated if p19 < p24*	HTLV SEROPOSITIVE*
5. HTLV specific bands detected but does not meet criteria for HTLV-I, HTLV-II or HTLV Seropositive. However, the following indeterminate banding patterns can be interpreted as *SERONEGATIVE:* - *HTLV-I GAG Indeterminate Western blot patterns (HGIP) Presence of p19, p26, p28, p32, p36, p53 but absence of p24 and any ENV proteins* - *Any combination of GAG proteins (p19, p26, p28, p32, p36, p53) but absence of p24 and any ENV proteins* - *Any single GAG proteins (p19, p24, p26, p28, p32, p36, p53)*	INDETERMINATE**

* Non-typeable HTLV seropositive can be further resolved by using *Wiktor et al's* algorithm in the absence of rgp46-I and rgp46-II. This algorithm which uses the relative reactivity of p19 and p24 is shown to be effective in differentiating the two serotypes.[7,11,12,13,14]

** Indeterminate interpretation is based on WHO's 1990 guideline.[15] However, various studies suggested that certain indeterminate banding patterns (as listed) can be interpreted as seronegative especially with healthy blood donors.[16-25] For example, a study involving 37,724 healthy blood donors, confirmed that HGIP can be safely interpreted as seronegative.[26] However, caution should be exercised when indeterminate patterns are obtained with IVD users or blood donors from endemic areas, and neurological disease patients.[26,27]

Serum from individuals with dual infections although rare, may occur and can also be differentiated based on the above criteria. Banding patterns of such specimens will indicate HTLV-I and HTLV-II positive. Available data demonstrates that the seroreactivities to rgp46-I and rgp46-II are specific for HTLV-I and HTLV-II respectively. Therefore sera reactive to rgp46-I, rgp46-II, GD21, p19 and p24 will provide a dual infection classification.

LIMITATIONS OF THE METHOD

Optimal assay performance requires the strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

A NEGATIVE result does not exclude the possibility of exposure to or infection with HTLV-I or HTLV-II. INDETERMINATE blots should not be used as the basis for diagnosis of HTLV-I/II infection.

It is also reported that both p19 or p24 seroreactivities have been observed in uninfected low risk populations although p24 indeterminants are relatively rare.

The sensitivity of rgp46-I has been reported to be 95% in France, 100% of PCR confirmed samples in Jamaica and the United States, and 98% of HTLV-I seropositive blood donors. The sensitivity of rgp46-II has been shown to be more than 98% among PCR confirmed samples from United States.

The overall sensitivity of each type specification, rgp46-I and rgp46-II, is estimated to be greater than 97%. The small percentage of HTLV-I and HTLV-II samples that are not reactive with either rgp46-I or rgp46-II are reactive with at least GD21 and one or more GAG bands, p19, or p24 meeting the criteria of either HTLV seropositive (Pattern 4) or Indeterminate (Pattern 5). There were no false negative interpretations reported.

Supplemental test such as PCR (HTLV-I and HTLV-II) may be useful in the discrimination of HTLV seropositive samples that cannot be identified as either HTLV-I or HTLV-II with the MPD HTLV Blot 2.4 (i.e Pattern 4).

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of MPD HTLV Blot 2.4 for the detection of antibodies to HTLV-I and HTLV-II was evaluated using HTLV-I/II seropositive and seronegative specimens and was compared with two line immunoassays which incorporate HTLV I and HTLV II antigens (recombinant proteins or peptides).

Sensitivity

Specimens which are established to be positive for HTLV I or / and HTLV II antibodies by commercial ELISAs were used to determine the sensitivity of MPD HTLV Blot 2.4.

A. Comparison to Line Immunoassay 1
Blot result comparing to MPD HTLV Blot 2.4 and Line Immunoassay 1 (LI 1) for positive samples purchased from Boston Biomedica, Inc., USA (BBI), and ProMedDx were as follows:

Method		Line Immunoassay 1		Total
		NEG / IND	POS	
MPD HTLV BLOT 2.4	NEG / IND	3*	0	3
	POS	0	102	102
	Total	3	102	105

* MPD HTLV Blot 2.4 gave 2 indeterminate and 1 negative result which was also detected negative by Line Immunoassay 1. Line Immunoassay 1 gave 3 negative results.

The two blots gave the following discriminations for the 102 HTLV positive samples:

Method	Interpretation				Total
	HTLV I	HTLV II	HTLV I & HTLV II**	Non-typeable***	
MPD HTLV Blot 2.4	45	53	4	0	102
LI 1	48	51	0	3	102

** Both HTLV I and HTLV II specific markers appeared, indicating co-infection.
*** Unable to type HTLV strains because of the absence of specific markers.

Both MPD HTLV Blot 2.4 and LI 1 gave similar results. The few discordant results are due to different antigens immobilized on the blots and the different methods used.

MPD HTLV Blot 2.4 showed a sensitivity of 97.1% which was equivalent to that obtained with Line Immunoassay 1.

B. Comparison to Line Immunoassay 2
The French Society of Blood Transfusion Anti-HTLV-I and II Performance Panel, SFTS-94 consisting of 26 HTLV-1 and 6 HTLV-II samples were studied. Results of MPD HTLV Blot 2.4 on this panel were compared to Line Immunoassay 2 (LI 2) as follows:

Method	Interpretation				Total
	HTLV I	HTLV II	Non-typeable	False NEG	
MPD HTLV Blot 2.4	26	6	0	0	32
LI 2	21	6	4	1	32

MPD HTLV Blot 2.4 correctly identifies HTLV positive samples, giving a sensitivity of >99.9% in this panel. Using the same panel, the comparative kit (LI 2) gave a sensitivity of 96.9%.

Specificity

A total of 200 blood donor samples were tested resulting in a specificity of 92.5%. 15 samples were indeterminate and there were no false positive results.

If 150 clinical specimens, 50 pregnancy specimens, 50 potentially interfering specimens (10each of icteric, haemolysed, triglyceride, lipemic, total proteinspecimens), and 73 potentially cross-reactive specimens (TB, *Helicobactor pylori,* HEV, Dengue, HBV, HCV, HIV-1, HIV-2), are included, the overall specificity was 89.2% (461/517). 56 samples were indeterminate and there were no false positive results. 6 samples were true positives confirmed by another confirmatory test.

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 product Instruction Manual 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 number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.

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

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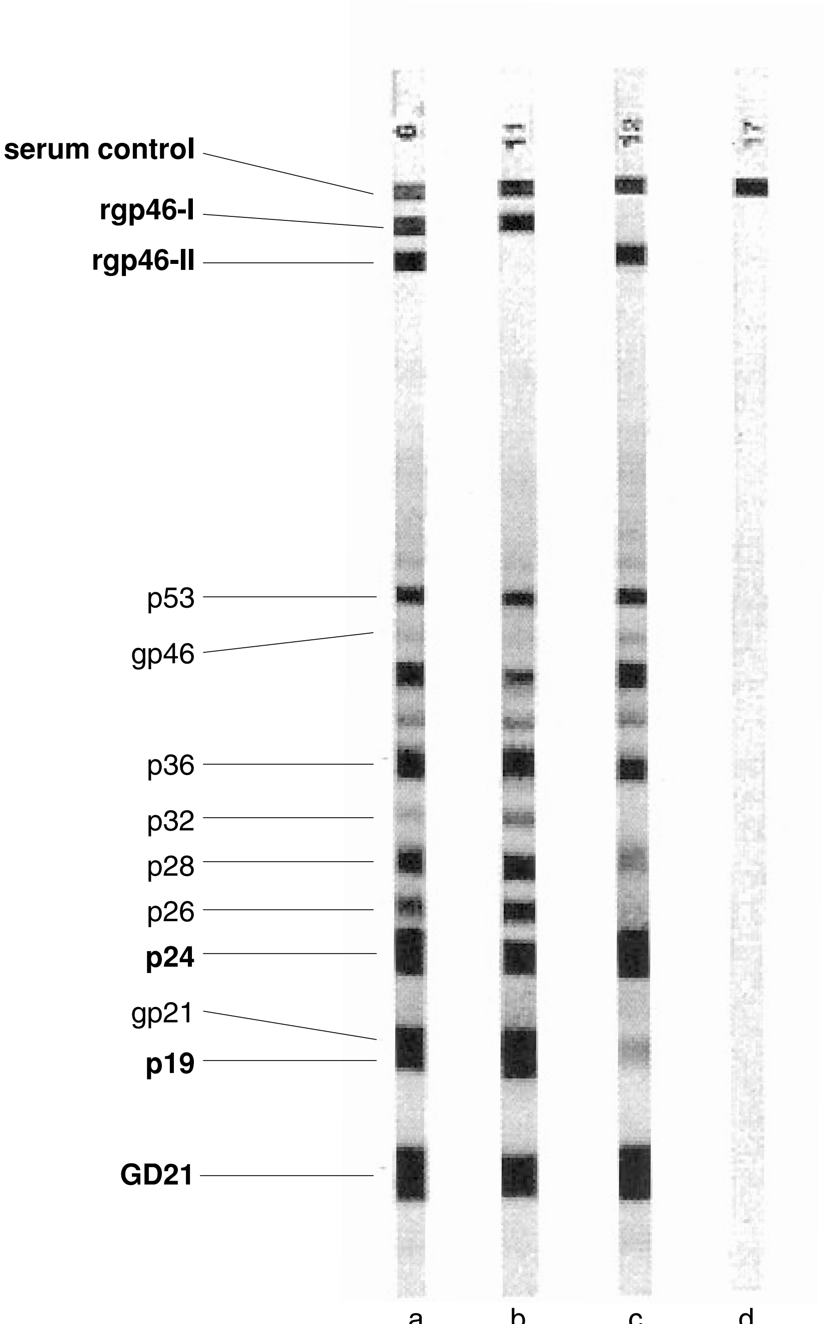
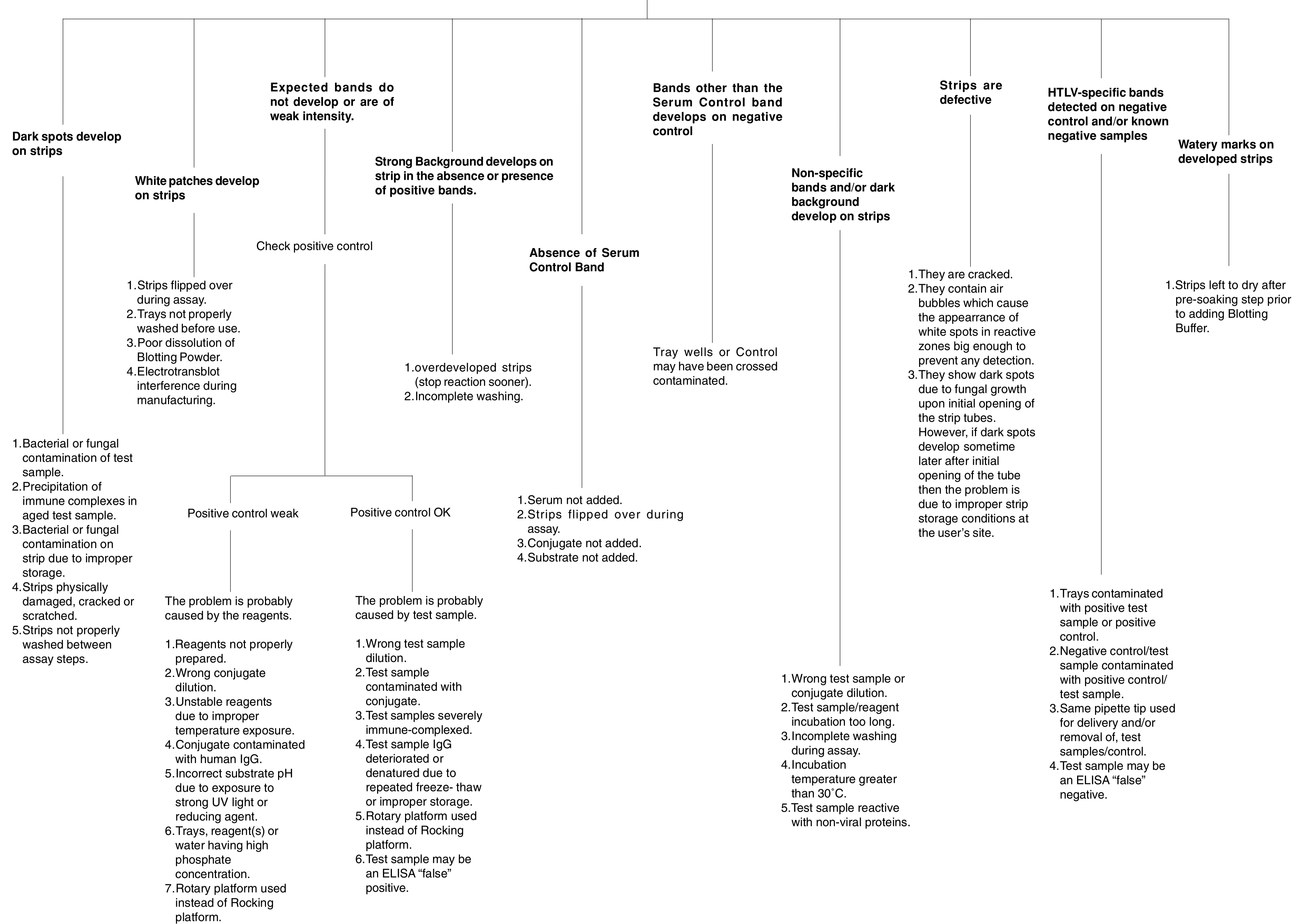
FIGURE 1



Viral specific bands as visualized with:

a. A HTLV-I/II dual infection serum.
b. Strong Reactive Control I. (Reactive for HTLV-I only)
c. Strong Reactive Control II. (Reactive for HTLV-II only)
d. Non-reactive Control.



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.
White patches develop on strips
1. Strips flipped over during assay.
2. Trays not properly washed before use.
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 conjugate.
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.
Absence of Serum Control Band
1. Serum not added.
2. Strips flipped over during assay.
3. Conjugate not added.
4. Substrate not added.
Bands other than the Serum 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 30˚C.
5. Test sample reactive with non-viral proteins.
Strips are defective
1. They are cracked.
2. They contain air bubbles which cause the appearrance 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.