HIV BLOT 2.2

WESTERN BLOT ASSAY

Instructions For Use

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Note: Changes Highlighted.

REF (18 tests kit) : 11030-018W
(36 tests kit) : 11030-036W
(WHO Version)

NAME AND INTENDED USE

The **MP Diagnostics HIV BLOT 2.2** is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to human immunodeficiency virus type 1 (HIV- 1) and type 2 (HIV-2) in human serum or plasma. It is intended for use as a more specific supplemental assay on human serum or plasma specimens found repeatedly reactive using screening assays such as the Enzyme-Linked Immunosorbent Assay, Chemiluminescence Assay, and Point of Care Test. This assay is for use by trained professionals in the laboratory setting.

INTRODUCTION

Screening tests are widely available for detecting antibodies to both HIV-1 and HIV-2, the etiologic agents of the Acquired Immunodeficiency Syndrome (AIDS). Such tests can be extremely sensitive but have a potential for being less specific, leading to false positive interpretations. Independent supplemental tests of high specificity are therefore necessary to further confirm the presence of antibodies to HIV-1 and/ or HIV-2.

The **MP Diagnostics HIV BLOT 2.2** kit is intended for use as a more specific supplemental test on human serum or plasma specimens found repeatedly reactive using ELISA. The separated specific HIV-1 viral antigens incorporated onto the strips via electrophoretic and electrotransblot procedures, combined with a specific HIV-2 synthetic peptide on the same strip allow for further delineation of the antibody responses to specific viral proteins. Each strip also includes an internal sample addition control to minimize the risk of false negatives due to operational errors and to ensure the addition of samples.

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. Some of the common symbols are explained in more detail in the European and International Standard EN ISO 15223: 2012.

- Use by / *Synonym for this :* Expiry Date
- Batch Code / *Synonyms for this are:* Lot Number / Batch Number
- Temperature Limitation
- Manufacturer
- Contains sufficient for <n> tests
- Do not reuse
- Contents
- In vitro diagnostic medical device
- Catalogue Number / *Synonyms for this:* Reference number / Re-order number
- Caution
- Authorised Representative in the European Community
- Consult Instructions for Use

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips are incorporated with separated, bound antigenic proteins from partially purified inactivated HIV-1 using electrophoretic blotting, plus a specific HIV-2 synthetic peptide on the same strips. Individual nitrocellulose strips are incubated with diluted serum or plasma and controls. Specific antibodies to HIV-1 and HIV-2 if present in the specimens will bind to the HIV-1 proteins and HIV-2 peptide on the strips. The strips are washed to remove unbound materials. Antibodies that bind specifically to HIV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate BCIP/NBT.

KIT COMPONENTS

	Component Description	Quantity Provided
ANTIGEN STRIPS	**NITROCELLULOSE STRIPS** Incorporated with HIV-1 viral lysate, a specific HIV-2 envelope peptide and a serum addition control band. Keep dry and away from light.	Available in 18 or 36 strips
CONTROL –	**NON-REACTIVE CONTROL** Inactivated normal human serum non-reactive for Hepatitis B surface antigen (HBsAg), antibodies to HIV-1/2, and anti-HCV. Contains 0.1% sodium azide and 0.005% thimerosal as preservatives.	1 vial (80 µl)
CONTROL +	**STRONG REACTIVE CONTROL** Inactivated human serum with high titered antibodies to HIV-1 and HIV-2 and non-reactive for HBsAg & anti-HCV. Contains 0.1% sodium azide and 0.005% thimerosal as preservatives.	1 vial (80 µl)
CONTROL WEAK	**WEAK REACTIVE CONTROL** Inactivated human serum with low titered antibodies to HIV-1 ONLY and non-reactive for HBsAg, anti-HIV-2 and anti-HCV. Contains 0.1%sodium azide and 0.005% thimerosal as preservatives.	1 vial (80 µl)
BUF STOCK 10x	**STOCK BUFFER CONCENTRATE (10x)** Tris buffer with heat inactivated normal goat serum. Contains 0.1% thimerosal as preservative.	1 bottle (20 ml)
BUF WASH 20x	**WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains 0.1% thimerosal as preservative.	1 bottle (70 ml)
CONJUGATE	**CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase. Contains 0.1% sodium azide as preservative.	1 vial (160 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 (1g each)
	Incubation Tray*	
	Instructions For Use	1 copy
	PROTEIN FINDER The protein Finder shows an image of the assayed strip belonging to the same strip number found in this kit and the positions of the specific HIV bands. It helps to locate the HIV bands in the strip.	1 piece
	Forceps	1 pair

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

* Incubation trays provided but packed separately from the kit.

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, WEAK REACTIVE 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, Weak Reactive Control*** 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.

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:	**STOCK BUFFER CONCENTRATE (10x) 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

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, Reactive, Weak Reactive 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 Instructions For Use. 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 they are freshly taken out from refrigeration and return to 2°C to 8°C storage as soon as possible. This is to preserve the shelf-life of the reagents.
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. The length of the strip must be placed in the same direction as the rocking motion.

17. Ensure that automated equipment if used is validated before use.
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. Tilt the tray slightly and add the specimen(s) where the buffer is collected at the lower end of each well.
19. Avoid the use of self-defrosting freezers for the storage of reagents and samples.
20. We do not recommend the use of diluted or lyophilized samples, as they may give false results. If they form part of quality control procedure, they should be validated by the user prior to use.

STORAGE

1. Store MP Diagnostics HIV BLOT 2.2 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 capped for storage.
- Dispense all reagents while they are freshly taken out from refrigeration and return to 2°C to 8°C storage as soon as possible. Store reagents at refrigerated temperatures when not in use.
- 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 plasma 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.

Samples can be inactivated but this is not a requirement for optimal test performance.

The use of inactivated specimens has not been validated.

Repeated freeze-thawing of sample is not recommended.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water
- Disposable gloves
- Rocking platform (designed with a rocking speed range of 12 to 16 cycles per minute, and which moves through a 5° to 10° tilt to wash membranes evenly)
- Precision Pipettes ranging from 20µl to 1000µl dispensing volume and pipette tips of appropriate volume
- Aspirator with sodium hypochlorite trap
- 56°C water bath (optional)
- Sodium hypochlorite for decontamination
- Paper towel, adhesive tape, worksheet (non-absorbent white paper)

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) BLOTTING BUFFER should be **prepared fresh prior to use.**
(b) Dilute 1 volume of STOCK BUFFER CONCENTRATE (10x) with 9 volumes of reagent grade water. Mix well.
(c) Add 1 g of BLOTTING POWDER to every 20 ml of the diluted STOCK BUFFER prepared in step 2(b) above. Mix well by inversion or stir with a magnetic stirrer to ensure powder dissolves completely. Stir again before dispensing.
(d) 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) For **RAPID ASSAY PROTOCOL**, prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE at 1:500 into BLOTTING BUFFER, for example, 10 µl CONJUGATE to 5ml BLOTTING BUFFER.
(c) For **OVERNIGHT ASSAY PROTOCOL**, prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE at 1:1000 into BLOTTING BUFFER, for example, 5 µl CONJUGATE to 5ml 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.

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED						
	3	6	9	15	20	27	36
Diluted Wash Buffer (ml)	60	100	140	240	300	400	600
Blotting Buffer (ml)	20	40	60	80	100	120	160
Blotting Powder (g)	1	2	3	4	5	6	8
Working Conjugate Solution (ml)	7	13	19	31	41	55	73
Conjugate (µl), Rapid Assay	14	26	38	62	82	110	146
Conjugate (µl), Overnight Assay	7	13	19	31	41	55	73
Substrate (ml)	7	13	19	31	41	55	73

ASSAY PROCEDURE - RAPID ASSAY

Note: a) Users can use either the rapid or overnight assay to run the tests. Assayed strips from overnight assay may appear with additional and darker intensity bands, but the final results interpretation (positive/indeterminate/ negative) is the same.
b) Aspirate all used chemicals and reagents into a trap containing 0.5% Sodium hypochlorite.
c) 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. Add 2 ml of DILUTED WASH BUFFER to each well.	**2 ml**
2. 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.	
3. Incubate the strips for 1 to 2 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration. (Note: Do not allow the strips to dry. Failure may result in watery marks on developed strips for some specimens.)	**2 minutes**
4. Add 2 ml of BLOTTING BUFFER to each well. Tilt the tray slightly and add the specimen(s) where the buffer is collected at the lower end of each well as per next step. Ensure that the strip no. is at the higher end.	**2 ml**
5. Add 20 µl each of specimens to their respective wells, and followed by 20 µl each of Strong Reactive, Weak Reactive and Non-Reactive Controls to their respective wells. The sequence of adding specimens or controls first is flexible.	**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. Add 2 ml of DILUTED WASH BUFFER. Wash each strip 3 times with 5 minutes soak on the rocking platform between each wash. (Note: Each wash cycle consists of dispensing 2 ml of DILUTED WASH BUFFER, soaking time of 5 minutes, and aspiration.)	**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. (Note: The reaction can be stopped before 15 minutes if all the bands are visible for high anti-HIV titer samples to avoid over development of bands and difficulty in reading.)	**15 minutes**
14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step).	**3 x 2 ml**
15. 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.	

ALTERNATIVE PROCEDURE - OVERNIGHT ASSAY

Procedure:

1. Add 2 ml of DILUTED WASH BUFFER to each well.	**2 ml**
2. 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.	
3. Incubate the strips for 1 to 2 minutes at room temperature (25 ± 3°C) on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration. (Note: Do not allow the strips to dry. Failure may result in watery marks on developed strips for some specimens.)	**2 minutes**
4. Add 2 ml of BLOTTING BUFFER to each well. Tilt the tray slightly and add the specimen(s) where the buffer is collected at the lower end of each well as per next step. Ensure that the strip no. is at the higher end.	**2 ml**
5. Add 20 µl each of specimens to their respective wells, and followed by 20 ul each of Strong Reactive, Weak Reactive and Non-Reactive Controls to their respective wells. The sequence of adding specimens or controls first is flexible.	**20 µl**
6. Cover the tray with the cover provided and incubate overnight (16 - 20 hours) at room temperature (25 ± 3°C) on the rocking platform.	**overnight**
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. Add 2ml of DILUTED WASH BUFFER. Wash each strip 3 times with 5 minutes soak on the rocking platform between each wash. (Note: Each wash cycle consists of dispensing 2ml of DILUTED WASH BUFFER, soaking time of 5 minutes, and aspiration.)	**3 x 2 ml**
9. Add 2 ml of WORKING CONJUGATE SOLUTION to each well.	**2 ml**
10. Cover tray and incubate for 30 minutes at room temperature (25 ± 3°C) on the rocking platform.	**30 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. (Note: The reaction can be stopped before 15 minutes if all the bands are visible for high anti-HIV titer samples to avoid over development of bands and difficulty in reading.)	**15 minutes**
14. Aspirate the SUBSTRATE and rinse the strips at least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step).	**3 x 2 ml**
15. 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	Room Temp Rapid Assay	Room Temp Overnight Assay
Nitrocellulose strip	1	-	-
Wash Buffer	2 ml	1-2 mins	1-2 mins
Blotting Buffer	2 ml	-	-
Specimen	20 µl	60 mins	Overnight (16 - 20 hours)
Wash Buffer	3 x 2 ml	3 x 5 mins	3 x 5 mins
Conjugate	2 ml	60 mins	30 mins
Wash Buffer	3 x 2 ml	3 x 5 mins	3 x 5 mins
Substrate (Ready to use)	2 ml	15 mins (or less)	15 mins (or less)
Distilled Water	3 x 2 ml	-	-

We recommend that the Non-Reactive, Strong Reactive and Weak 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 HIV-1 and HIV-2 specific bands should be observed on the Non-Reactive control strips. The band for the serum control should be visible (Fig 1c).

2. STRONG REACTIVE CONTROL
All relevant molecular weight bands must be evident. Figure 1a provides a guide to the relative positioning of bands visualized with the MP Diagnostics HIV BLOT 2.2 and permits identification of bands observed for the STRONG REACTIVE CONTROL. The bands are p17, p24, p31, gp41, p51, p55, p66, gp120/gp160. Other bands associated with core antigens (p39, p42) may also be visible. Be careful not to misinterpret these as gp41. The envelope antigens, gp41, gp120/gp160 appear as diffuse bands as they are typical of glycoproteins; p55 viral band may appear faintly on the actual Strong Reactive Control strip due to low titer of anti-p55 in the Strong Reactive Control provided. The serum control band will be visible. The HIV-2 specific band should also be visible as shown in Figure 1a.

3. WEAK REACTIVE CONTROL
The Weak Reactive control provides a measure of the sensitivity of the kit. Weak bands at p24 and/or gp41 and gp120/gp160 should appear. Some additional weak bands may or may not be present. The serum control band will be visible (Fig 1b).

QUALITY CONTROL

NOTE: Developed strips must be completely dry to avoid misinterpretation.

The presence or absence of antibodies to HIV-1 sample is determined by comparing each nitrocellulose strip to the assay control strips tested with the NON-REACTIVE, STRONG REACTIVE and WEAK REACTIVE controls.

Figure 1a is suggested as an aid to identify the various bands developed on the STRONG REACTIVE Control strip. The Strong Reactive Control as provided in the kit may contain relatively low titer of anti-p55 and anti-p39; as a result, p55 and p39 band for the Strong Reactive Control may appear faintly on the assayed strips. This has no impact on the performance of HIV Blot 2.2 strips in detecting anti-p55 and anti-p39 present in the specimens, as each lot of strip contains sufficient amount of p55 and p39 antigens.

PLEASE NOTE: The numbered end of the strips should be placed at the bottom as shown in the Figure, i.e. the gp120/gp160 bands are the furthest away from the numbered end.

MOLECULAR WEIGHT	GENE	ANTIGEN	DESCRIPTION
gp 160	ENV	Polymeric form of gp41	Broad diffuse glycoprotein
gp 120	ENV	Outermembrane	Diffuse glycoprotein
p66	POL	Reverse Transcriptase	Discreet band
p55	GAG	Precursor protein	Discreet band
p51	POL	Reverse Transcriptase	Discreet band just below p55
p39	GAG	Fragment of p55	Discreet band
gp41	ENV	Transmembrane	Diffuse glycoprotein
p31	POL	Endonuclease	Doublet
p24	GAG	Core protein	Broad band
p17	GAG	Core protein	Broad band

Some of the different antigens mentioned in the Table above are derived from the same precursor protein and may have overlapping epitopes. This should be considered when interpreting the pattern, for example:-

1. It is unlikely to detect gp41 in the absence of gp160 because the gp160 is the polymeric form of gp41 and the concentration of gp160 is higher than gp41 on the MP Diagnostics HIV BLOT 2.2. The gp41 appears as a diffuse band. Any sharp and discreet band at the gp41 region should not be interpreted as gp41 band. Many non-HIV infected and normal specimens are found to be reactive to this non-HIV antigen which is likely to originate from the human cell line used to grow the HIV virus.
2. p55 is the precursor for p24 and p17.
   The p55 band is generally detected when there is strong reactivity to p24 and/or p17, it normally appears as a thin band just above p51 band, sometimes these two bands are indistinguishable and may appear as a single band. The bands seen as p42 and p39 are both GAG fragments and should not be interpreted as gp41 (ENV).

3. p24 protein is abundant in HIV Blot 2.2 strip. For seroconverting specimens, it is well established that anti-p24 is the first to appear on Western Blot assays. Appearance of p24 band in HIV infected patients would fulfil the positive interpretation criteria for gag protein by WHO, CDC and other international criteria.
4. The POL bands p66, p51 and p31 are generally detected simultaneously. However the sensitivity of p66 and p31 are greater than that of p51.
5. HIV-2 cross reactivity is variable but typically shows reactivity with GAG and/or POL antigens. However, there can be cross reactivity with the gp160 band in some cases, but rarely with gp41.
6. There is also a high molecular weight band around 160KD that is presumed to be a GAG-POL precursor protein . This is seen with some high titered HIV-2 or indeterminate (GAG Reactive Only) sera but the band pattern is a sharp discreet band which is different from the diffuse band of ENV gp160.
7. Appearance of single band near p51/p55 is probably an HLA related reactivity (p56), not specific for HIV-1.
8. Appearance of p39 and/or p42 without p24 or p17 should not be interpreted.
9. Appearance of p66 alone is not HIV-1 specific, but is most likely a reactivity with the host cell proteins (p68).

The interpretation process involves the following:-

1. Validate that the serum control band is visible. If the control is negative, the results should be considered invalid as this indicates a technical error such as not adding sample, conjugate or substrate.
2. Identify the molecular weight of each band of the test strip using the STRONG and/or WEAK REACTIVE Control strips as a guide.
3. Interpretation of the test strip is then based on the detection of specific band patterns as recommended by the appropriate authorities (i.e. Health Ministry, World Health Organization, etc.)

Specific guidelines for interpretation may differ depending on the local policies. MP Diagnostics recommends following the accepted policy to be in accordance with local regulations.

We recommended the following guidelines for the interpretation of the MP Diagnostics HIV BLOT 2.2. Results should be recorded for each band detected, result should be interpreted as NEGATIVE, POSITIVE or INDETERMINATE.

PATTERN	INTERPRETATION
No viral specific bands present	NEGATIVE
Detection of p17 antibodies **ONLY**, no other bands	NEGATIVE
Detection of 2 ENV (gp160/gp41and gp120) and 1 GAG (p17, p24, p55) **or** 1 POL (p31, p51, p66)	HIV-1 POSITIVE
Detection of 2 ENV (gp160/gp41 and gp120) and 1 GAG (p17, p24, p55) **or** 1 POL (p31, p51, p66) and HIV-2 specific band is visible	HIV-1 POSITIVE with HIV-2 INDICATED
Any viral specific bands present but pattern **does not** meet criteria for POSITIVE	INDETERMINATE[2]
Any viral specific bands present but pattern **does not** meet criteria for POSITIVE but HIV-2 specific band is visible.	INDETERMINATE[2] with HIV-2 INDICATED

[2]INTERPRETATION OF RESULTS FOR INDETERMINATE

INDETERMINATE results should not be used as the basis for diagnosis of HIV-1 infection. Based on the fact that most persons with an initial INDETERMINATE result who are infected with HIV-1 will develop detectable HIV antibodies within 1 month, US CDC (2001) recommended such persons be re-tested for HIV-1 infection ≥1 month later. Persons with continued INDETERMINATE results after 1 month are unlikely to be HIV-infected unless recent HIV exposure is suspected.

Based on a recent study of Fiebig *et al* (2003), although the window period for Western Blot in the case of a primary HIV-1 infection could be as long as 22 days, the progression from an INDETERMINATE blot to a full POSITIVE profile took no longer than 8 days. In addition, this laboratory stage of having Western Blot INDETERMINATE was always accompanied with detectable RNA of HIV-1 with cases of true infection. Conversely, no seroconversion was evident in follow-up studies of individuals having screened positive and Western Blot INDETERMINATE results, once confirmed as negative by PCR methods (Sethoe *et al*, 1995). Therefore, it is reasonable to consider persons having Western Blot INDETERMINATE results but additionally tested negative by a RNA test as unlikely to be HIV-infected, especially when the tested individuals are known as not having any risk factor associated with exposure.

In particular, persons having Western Blot INDETERMINATE results derived from a test algorithm using fourth generation ELISAs as the primary screen test should additionally be tested for viral RNA using a molecular-base test such as RT-PCR with primer sets covering HIV-1/2/O. If necessary, a follow-up should be conducted with an additional supplemental assay on a second specimen collected 1 month later. The unique design of fourth generation ELISAs is for a simultaneous detection of both antigen and antibody. Consequently specimens identified as positive by a fourth generation ELISA should contain either antibody or antigen or both. Although more than 95% of those cases of true positive identified by a fourth generation ELISA were anti-HIV related and verifiable (confirmed) by Western Blot (Ly *et al.*, 2000), a supplemental test using RT-PCR for viral RNA detection appeared unavoidable for the small portion of reactivity relating to p24 antigen. Again, persons without any risk of exposure are unlikely HIV-infected, if identified as positive by a fourth generation ELISA accompanied by a Western Blot INDETERMINATE but the findings could not be further supported by a POSITIVE result using a RNA test with primer sets covering HIV-1/2/O.

However, nucleic acid tests (NAT) for HIV DNA or RNA were not approved for diagnostic purpose by the relevant authorities (US CDC, 2001; Constantine & Zink, 2005) until very recently. To date, only one RNA qualitative assay has been approved by the US FDA for diagnosis of primary and acute infection of HIV-1. Therefore, test algorithms recommended by the US CDC (2001) and WHO (2004) are yet to be updated, and NAT are yet to be included as methods for resolving INDETERMINATE Western Blot results. Nevertheless, US CDC (2001) acknowledged that when in consultation with clinical and infection status among persons with an initial INDETERMINATE Western Blot.

LIMITATION OF THE METHOD

Detection of antibodies to HIV-1 does not constitute a diagnosis of Acquired Immune Deficiency Syndrome (AIDS). A NEGATIVE BLOT is not a guarantee that the causative agent for AIDS is not present. Although a HIV-1 positive test result by Western Blot indicates infection with the virus, a diagnosis of AIDS can only be made clinically if a person meets the case definition of AIDS established by the Center for Disease Control (USA), the World Health Organization or other relevant authorities.

It is known that persons who have recently seroconverted may display incomplete pattern but increase reactivity (both number and intensity of bands) occurs when followed for a period of two to six months. Most blots with POSITIVE results will have other viral specific bands present.

INDETERMINATE results should not be used as the basis for diagnosis of HIV-1 infection. It is recommended that all INDETERMINATE blots be repeated using the original specimen and sequential samples. Blood donors with an INDETERMINATE blot should be re-tested using a fresh specimen after one month (US CDC, 2001). In addition, antibodies to p24 and p31 are known to decrease during the course of AIDS leading to a shift in blot interpretation from POSITIVE to INDETERMINATE. Interpretation of results should then be based on subsequent blot testing and clinical evaluations in such situations.

Due to its highly specific nature , NON-REACTIVITY of samples with HIV-2 specific envelope peptide on an Indeterminate viral blot, does not exclude the possibility of infection with other strains of HIV-2.

Samples that are indicated as HIV-2 infections should be further tested with specific HIV-2 supplemental assays.

SPECIFIC PERFORMANCE CHARACTERISTICS

SENSITIVITY

HIV POSITIVE SAMPLES

The sensitivity of MP Diagnostics HIV Blot 2.2 was evaluated using 209 HIV-1 & 108 HIV-2 positive samples which were well characterized and commercially available. The HIV Blot 2.2 performance was evaluated and compared with established Western blots for HIV-1 and HIV-2.

Table 1: HIV-1 positive samples (209 samples)

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-1 Western blot
HIV Surveillance Panels from BioClinical Partners, Inc., USA (BCP) 5 panels (10 members each) and 1 panel (9 members) with samples from USA, China, Venezuela, Thailand, Cameroon and India. (n = 59)	HIV-1 positive = 58 Indeterminate = 1	HIV-1 positive = 58 Indeterminate = 1
Panel HIV SFTS 94 from Sanguine Nationale Transfusion Societes (SFTS), France 15 members which were HIV-1 Western blot positive as indicated on the SFTS data sheet were used. (n = 15)	HIV-1 positive = 15 Indeterminate = 0	HIV-1 positive = 9 Indeterminate = 6
HIV-1 positive samples from Boston Biomedical Inc., USA (BBI) and Serologicals Inc., USA (n = 38)	HIV-1 positive = 38 Indeterminate = 0	HIV-1 positive = 38 Indeterminate = 0
HIV-1 positive plasma from BBI (n = 50)	HIV-1 positive = 50 Indeterminate = 0	HIV-1 positive = 50 Indeterminate = 0
HIV-1 positive plasma from LifeBiotech AG (n = 47)	HIV-1 positive = 47 Indeterminate = 0	HIV-1 positive = 47 Indeterminate = 0
Total no.	209	209
No. of true positives	208	202
No. of false negatives	0	0
No. of indeterminates	1	7
Sensitivity	99.52% (208/209); 95% CI (97.36% - 99.99%)	96.65% (202/209); 95% CI (93.22% - 98.64%)

Table 2: HIV-2 positive samples (107 samples)

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-2 Western blot
HIV Surveillance Panels from BCP 2 panels (10 members each) with samples from Ghana and Nigeria (n = 20)	HIV-2 indicative = 17 Indeterminate = 3	HIV-2 positive = 12 Indeterminate = 8
Panel HIV SFTS 94 from SFTS, France. 7 members which were HIV-2 Western blot positive as indicated on the SFTS data sheet were used. One of the 7 members is a 1/10 diluted sample. (n = 7)	HIV-2 indicative = 6 Indeterminate = 1	HIV-2 positive = 7 Indeterminate = 0
HIV-2 positive samples from Dr. Oliveiro Varnier, Laboratory of Human Retrovirology, Genova, Italy (n = 45)	HIV-2 indicative = 44 Indeterminate = 1	HIV-2 positive = 43 Indeterminate = 2
HIV-2 positive samples from BBI, BCP and Serologicals (n = 10)	HIV-2 indicative = 10 Indeterminate = 0	HIV-2 positive = 10 Indeterminate = 0
HIV-2 Performance Panel PRF 201 (15 samples) and PRZ 202 (10 samples) from BBI (n = 25)	HIV-2 indicative = 25 Indeterminate = 0	HIV-2 positive = 21 Indeterminate = 4
Total no.	107	107
No. of true positives	102	93
No. of false negatives	0	0
No. of indeterminates	5	14
Sensitivity	95.33% (102/107); 95% CI (89.43% - 98.47%)	86.92% (93/107); 95% CI (79.02% - 92.66%)

SEROCONVERSION

This study was conducted by a third party institution, using a total of 15 commercial seroconversion panels (SeraCare & Zeptometrix) which were qualified according to common technical specifications for IVD medical devices (2009/886/EC). The seroconversion sensitivity of MP Diagnostics HIV Blot 2.2 and Chiron RIBA HIV-1/HIV-2 SIA are comparable and both assays reacted similarly in the same panel follow up samples. See Table 3.

Table 3: Performance of Kit based on Positives and/or Indeterminates detected in seroconversion panels

Performance	Panels	No of Panels
MP Diagnostics and Chiron have equal detection of Positives and Indeterminates	PRB965, PRB966, PRB968, PRB969, PRB970, ZMC6243, ZMC6245, ZMC6246, ZMC9019, PRB9032	10
MP Diagnostics detected Positives or Indeterminates earlier	PRB967, PRB972, ZMC6240, ZMC12008	4
Chiron detected Positives or Indeterminates earlier	PRB971	1

SPECIFICITY

The specificity of MP Diagnostics HIV Blot 2.2 was evaluated using 200 negative blood donor samples, 81 clinical samples, and 167 potentially interfering samples. The HIV Blot 2.2 performance was evaluated and compared with established Western blot for HIV-1.

Table 4: Normal blood donors (200 samples)

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-1 Western blot
Normal human donor plasma (Lot VP8104) from Biomedical Resources (BMR) Lot VP8104 has 356 individual units of blood donations, 200 of which were randomly selected for testing. All the samples were tested negative for HBsAg, HIV-1 antigen and antibodies for HIV-1/2, HCV, and Syphilis by FDA approved tests. (n = 200)	HIV-1 negative = 187 HIV-1 positive = 0 Indeterminate = 13	HIV-1 negative = 55 HIV-1 positive = 0 Indeterminate = 145
Total no.	200	200
No. of true negatives	187	55
No. of false positives	0	0
No. of indeterminates	13	145
Specificity	93.50% (187/200); 95% CI (89.14% - 96.49%)	27.50% (55/200); 95% CI (21.44% - 34.24%)

Table 5: Clinical samples (167 samples)

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-1 Western blot
Chagas Panel (Panel TC-6215) from BCP (n = 25)	HIV-1 negative = 16 HIV-1 positive = 0 Indeterminate = 9	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 25
Cross Reactivity Panel (from BCP) comprising of HSV-1, HSV-2, Toxoplasma, Rheumatoid Arthritis, SLE, Osteoporosis, Sjogren's Syndrome, UCTD/MCTD, Scleroderma, VZV, Polycystic Ovary samples (n = 74)	HIV-1 negative = 63 HIV-1 positive = 0 Indeterminate = 10 True HIV-1 positive = 1 *(Positive on both devices and was not included in the calculations)*	HIV-1 negative = 16 HIV-1 positive = 0 Indeterminate = 57 True HIV-1 positive = 1

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-1 Western blot
Lyme Disease Mixed Titer Performance Panel PTL201 from BBI (n = 10)	HIV-1 negative = 8 HIV-1 positive = 0 Indeterminate = 2	HIV-1 negative = 1 HIV-1 positive = 0 Indeterminate = 9
Tuberculosis samples from BCP (n = 9)	HIV-1 negative = 3 HIV-1 positive = 0 Indeterminate = 6	HIV-1 negative = 1 HIV-1 positive = 0 Indeterminate = 8
Anti-HCV Mixed Titer Performance Panel (PHV 202) samples from BBI (n = 10)	HIV-1 negative = 4 HIV-1 positive = 0 Indeterminate = 6	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 10
SFTS 94, HTLV I/II positive panel from SFTS, France (n = 10)	HIV-1 negative = 9 HIV-1 positive = 0 Indeterminate = 1	HIV-1 negative = 2 HIV-1 positive = 0 Indeterminate = 8
HEV positive sera from Armed Forces Research Institute of Medical Sciences, Thailand (n = 9)	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 9	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 9
H. pylori positive samples from Dr Roost Laboratory (n = 10)	HIV-1 negative = 4 HIV-1 positive = 0 Indeterminate = 6	HIV-1 negative = 1 HIV-1 positive = 0 Indeterminate = 9
Dengue positive samples from Singapore General Hospital (n = 10)	HIV-1 negative = 10 HIV-1 positive = 0 Indeterminate = 0	HIV-1 negative = 10 HIV-1 positive = 0 Indeterminate = 0
Total no.	167	167
No. of true negatives	117	31
No. of false positives	0	0
No. of indeterminates	49	135
No. of true positives	1	1
Specificity	70.48% (117/166); 95% CI (62.92 % - 77.3 %)	18.67% (31/166); 95% CI (13.06 % - 25.45 %)

Table 6: Potential interfering and pregnant women samples (81 samples)

Panel Name/ Source	MP Diagnostics HIV Blot 2.2	HIV-1 Western blot
Rheumatoid Factor samples from BCP Samples with RF values (0-500), (501-999) & (≥1000) are 2, 2 & 14, respectively. (n = 18)	HIV-1 negative = 15 HIV-1 positive = 0 Indeterminate = 3	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 18
Lipemic samples from BiosPacific (n = 10)	HIV-1 negative = 5 HIV-1 positive = 0 Indeterminate = 5	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 10
Icteric samples from BiosPacific (n = 10)	HIV-1 negative = 7 HIV-1 positive = 0 Indeterminate = 3	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 10
Haemolysed samples from BiosPacific (n = 10)	HIV-1 negative = 4 HIV-1 positive = 0 Indeterminate = 6	HIV-1 negative = 0 HIV-1 positive = 0 Indeterminate = 10
Pregnant women samples from Biochrom, Germany (n = 33)	HIV-1 negative = 30 HIV-1 positive = 0 Indeterminate = 3	HIV-1 negative = 4 HIV-1 positive = 0 Indeterminate = 29
Total no.	81	81
No. of true negatives	61	4
No. of false positives	0	0
No. of indeterminates	20	77
Specificity	75.31% (61/81); 95% CI (64.47% - 84.22%)	4.94% (4/81); 95% CI (1.36 % - 12.16%)

REPRODUCIBILITY

Using a HIV-1 Strong Positive sample (Accurun 24), Strong Reactive Control, Weak Reactive Control and Non-Reactive Control, the assay reproducibility of the MP Diagnostics HIV Blot 2.2 assay was demonstrated with three technicians performing the assays (operator variability), on different days (environmental variability), on three different strip lots (lot to lot variability) produced on two different production days (batch variability). The various combinations and variations show that the MP Diagnostics HIV Blot 2.2 is highly reproducible within a lot, across lots, batches and operators.

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 Instructions For Use 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 purpose. 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 howsoever 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, the expiry date and the strip lot number.
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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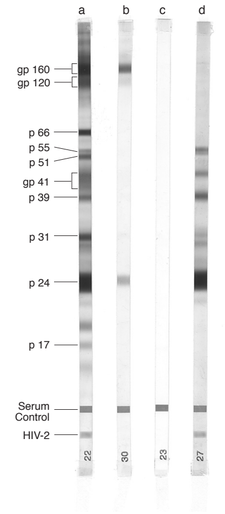
* U.S. Patent 5,721,095

FIGURE 1



a. Strong Reactive Control (Reactive for HIV-1 and HIV-2)
b. Weak Reactive Control (Reactive for HIV-1 only).
c. Non-Reactive Control.
d. A typical HIV-2 seropositive serum.

TROUBLE SHOOTING CHART