HIV-1 BLOT 1.3

Instructions For Use

FOR RESEARCH USE ONLY
NOT FOR USE IN
DIAGNOSTIC PROCEDURES

Note Changes Highlighted

REVISION DATE: 2016-05
MAJ 0012-ENG-2

REF (18 tests kit) : 11012-018
(36 tests kit) : 11012-036

TRADE NAME AND INTENDED USE

The **MP Diagnostics HIV-1 BLOT 1.3** is a qualitative enzyme immunoassay for the *in-vitro* detection of antibodies to HIV-1 in human serum or plasma. This kit is supplied for research purposes only.

It is not intended for use in the diagnosis or prognosis of diseases. In particular, this test cannot be used to evaluate blood specimens for the purpose of donor screening, or as a confirmatory diagnostic.

INTRODUCTION

Screening tests are widely available for detecting antibodies to HIV-1, 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.

The **MP Diagnostics HIV-1 BLOT 1.3** kit is an informational research test on serum or plasma specimens. The separated specific HIV-1 viral antigens incorporated onto the strips via electrophoretic and electrotransblot procedures, will 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 European Standard EN 980:2008 and International Standard ISO 15223-1:2007.


Use by
Synonym for this:
Expiry Date


Catalogue
Number


Batch Code
Synonym for this are:
Lot Number
Batch Number


Caution


Temperature Limitation


Manufacturer


Consult
Instructions for Use


Contains sufficient
for <n> tests


Contents


Do not reuse

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. Individual nitrocellulose strips are incubated with diluted serum or plasma and controls. Specific antibodies to HIV-1 if present in the specimens, will bind to the HIV-1 proteins on the strips. The strips are washed to remove unbound materials. Antibodies that bind specifically to HIV-1 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 has the sensitivity to detect marginal amounts of HIV-1 specific antibodies in serum or plasma.

KIT COMPONENTS

Component Description	Quantity Provided	
ANTIGEN STRIPS **NITROCELLULOSE STRIPS** Incorporated with HIV-1 viral lysate and a serum addition control band. Keep dry and away from light.	18 or 36 strips	108 strips
CONTROL –  **NON-REACTIVE CONTROL** Inactivated normal human serum non-reactive for Hepatitis B surface antigen (HBsAg), antibodies to HIV-1/2 and HCV. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)	3 vials (80 µl)
CONTROL +  **STRONG REACTIVE CONTROL** Inactivated human serum with high titered antibodies to HIV-1 and non-reactive for HBsAg and anti-HCV. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)	3 vials (80 µl)
CONTROL WEAK  **WEAK REACTIVE CONTROL** Inactivated human serum with low titered antibodies to HIV-1 and non-reactive for HBsAg and antibodies to HCV. Contains sodium azide and thimerosal as preservatives.	1 vial (80 µl)	3 vials (80 µl)
BUF STOCK 10x **STOCK BUFFER CONCENTRATE (10x)** Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.	1 bottle (20 ml)	3 bottles (20 ml)
BUF WASH 20x **WASH BUFFER CONCENTRATE (20x)** Tris with Tween-20. Contains thimerosal as preservative.	1 bottle (70 ml)	3 bottles (70 ml)
CONJUGATE **CONJUGATE** Goat anti-human IgG conjugated with alkaline phosphatase.	1 vial (160 µl)	3 vials (160 µl)
SUBS BCIP / NBT  **SUBSTRATE** Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT).	1 bottle (100 ml)	3 bottles (100 ml)
POWDER BLOTTING **BLOTTING POWDER** Non-fat dry milk	10 packets (1g each)	30 packets (1g each)
Incubation Tray, 9 wells each	2 or 4 trays	12 trays
Instructions For Use	1 copy	1 copy
Forceps	1 pair	1 pair

Note : Volumes of reagents provided are sufficient for 4 runs (18/36 tests) and 12 runs (108 tests).

WARNINGS AND PRECAUTIONS

1. For *Research Use Only*. It is not intended for use in diagnostic purposes.
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, Strong 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 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.

20. We do not recommend the use of diluted or lyophilized samples, as they may give false results. If they form part or a whole QC panel, they should be validated.

STORAGE INSTRUCTIONS

1. Store MP Diagnostics HIV-1 BLOT 1.3 kit and its components at 2°C to 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 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 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.

Inactivate as follows:
1. Loosen cap of sample container.
2. Heat-inactivate sample at 56°C for 30 minutes in a water bath.
3. Allow sample to cool down before retightening caps.
4. Sample can be stored frozen until analysis.

Repeated freeze-thawing of sample is not recommended.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Reagent grade , 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)
- 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) 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. Stir to ensure powder dissolves completely.
   (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
1x Wash Buffer (ml)	60	100	140	240	300	400	600
1x Blotting Buffer (ml)	20	40	60	80	100	120	160
Blotting Powder (g)	1	2	3	4	5	6	8
Working Conjugate (ml)	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. HIV bands are more developed and more bands may appear with the overnight assay, but the overall performance of the two assays is the same.

b) Aspirate all used chemicals and reagents into a trap containing 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.	**2 ml**
5.	Add 20 µl each of patients' sera or controls to appropriate wells. Care should be taken to ensure specimens are not added directly on the strips.	**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. (Note: The reaction can be stopped before 15 minutes if all the bands are visible.)	**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.	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.

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.	**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 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.	Wash each strip 3 times with 2ml 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 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.)	**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.	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	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	-	-

QUALITY CONTROL

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 specific bands should be observed on the Non-Reactive control strips. The band for the serum control should be visible (Fig 1, Strip (c)).

2. **STRONG REACTIVE CONTROL**
   All relevant molecular weight bands must be evident. Figure 1, Strip (a) provides a guide to the relative positioning of bands visualized with the MP Diagnostics HIV-1 Blot 1.3 and permits identification of bands observed for the STRONG REACTIVE CONTROL. The bands are p17, p24, p31, gp41, p51, p55, p66 and 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 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.

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 gp120/160 should appear. Some additional weak bands may or may not be present. The serum control band will be visible (Fig 1, Strip (b)).

INTERPRETATION OF BANDS

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

The presence or absence of antibodies to HIV-1 in a 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 1, Strip (a) is suggested as an aid to identify the various bands which develop on the strip with the STRONG REACTIVE control. 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 1.3 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

MOLECULAR WEIGHT	GENE	ANTIGEN	DESCRIPTION
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-1 BLOT 1.3. 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. The bands seen as p42 and p39 are both GAG fragments and should not be interpreted as gp41 (ENV).

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.

3. The POL bands p66, p51 and p31 are generally detected simultaneously. However the sensitivity of p66 and p31 are greater than that of p51.

4. 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.

5. 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.

LIMITATIONS OF THE PROCEDURE

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

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no warranty other than that the test kit will function as a *Research Use Only* 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, express or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any 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 howsoever caused by the product in the use or in the application thereof.

TECHNICAL PROBLEM / COMPLAINT

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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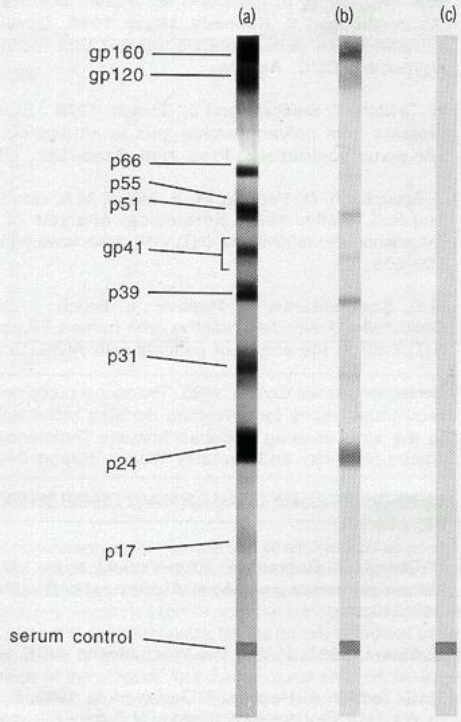
* U.S. Patent 5,721,095

FIGURE 1



Viral specific bands as visualized with:

a) Strong Reactive Control
b) Weak Reactive Control
c) Non-Reactive Control

TROUBLE SHOOTING CHART