

# HIV-1 BLOT 1.3

## WESTERN BLOT ASSAY

### Instructions For Use

CE 0123

REVISION DATE: 2016-05  
MAJ0011-ENG-4

Note: Changes Highlighted.

**REF** (18 tests kit) : 11010-018  
(36 tests kit) : 11010-036  
(108 tests kit) : 11010-108

#### 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. It is intended for use as a more specific supplemental test on human serum or plasma specimens found repeatedly reactive using screening procedures such as the Enzyme-Linked Immunosorbent Assay (ELISA).

#### 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 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, 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.

#### 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:

- Loosen cap of sample container.
- Heat-inactivate sample at 56°C for 30 minutes in a water bath.
- Allow sample to cool down before retightening caps.
- 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

- DILUTED WASH BUFFER**
  - DILUTED WASH BUFFER should be **prepared fresh prior to use.**
  - Dilute 1 volume of WASH BUFFER CONCENTRATE (20X) with 19 volumes of reagent grade water. Mix well.
- BLOTTING BUFFER**
  - BLOTTING BUFFER should be **prepared fresh prior to use.**
  - Dilute 1 volume of STOCK BUFFER CONCENTRATE (10X) with 9 volumes of reagent grade water. Mix well.
  - 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.
  - Stir again before dispensing.
- WORKING CONJUGATE SOLUTION**

Note : Prepare solution in polypropylene container / beaker.

  - WORKING CONJUGATE SOLUTION should be **prepared fresh prior to use.**
  - 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.
  - 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.

#### DESCRIPTION OF SYMBOLS USED

	Use by <i>Synonym for this :</i> Expiry Date	<b>IVD</b>	In vitro diagnostic medical device
	Batch Code <i>Synonyms for this are:</i> Lot Number Batch Number	<b>REF</b>	Catalogue Number
	Temperature Limitation		Caution
	Manufacturer	<b>EC REP</b>	Authorised Representative in the European Community
	Contains sufficient for <math>n-2</math> tests		Consult Instructions for use
	Do not re-use	<b>CONT</b>	Contents

#### 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	
<b>ANTIGEN STRIPS</b>		
<b>NITROCELLULOSE STRIPS</b>	18 or 36 strips	108 strips
Incorporated with HIV-1 viral lysate and a serum addition control band. Keep dry and away from light.		
<b>CONTROL</b>		
<b>NON-REACTIVE CONTROL</b>	1 vial (80 µl)	3 vials (80 µl)
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.		

#### CONTROL

**STRONG REACTIVE CONTROL** 1 vial (80 µl) 3 vials (80 µl)  
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.

#### CONTROL **WEAK**

**WEAK REACTIVE CONTROL** 1 vial (80 µl) 3 vials (80 µl)  
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.

#### **BUF|STOCK|10x**

**STOCK BUFFER CONCENTRATE (10x)** 1 bottle (20 ml) 3 bottles (20 ml)  
Tris buffer with heat inactivated normal goat serum. Contains thimerosal as preservative.

#### **BUF|WASH|20x**

**WASH BUFFER CONCENTRATE (20x)** 1 bottle (70 ml) 3 bottles (70 ml)  
Tris with Tween-20. Contains thimerosal as preservative.

#### **CONJUGATE**

**CONJUGATE** 1 vial (160 µl) 3 vials (160 µl)  
Goat anti-human IgG conjugated with alkaline phosphatase.

#### **SUBS|BCIP|NBT**

**SUBSTRATE** 1 bottle (100 ml) 3 bottles (100 ml)  
Solution of 5-bromo-4-chloro-3-indolyl- phosphate (BCIP) and nitroblue tetrazolium (NBT).

#### **POWDER|BLOTTING**

**BLOTTING POWDER** 10 packets (1g each) 30 packets (1g each)  
Non-fat dry milk

Instructions For Use 1 copy 1 copy

Forceps 1 pair 1 pair

Incubation Tray\*

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

\* Incubation trays provided but packed separately from the kit.

#### WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For Professional use only
- 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:

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

<b>Component:</b>	<b>STOCK BUFFER CONCENTRATE (10x) WASH BUFFER CONCENTRATE (20x)</b>
<b>Signal Word:</b>	Warning
<b>Pictogram:</b>	
<b>Hazard Statements:</b>	H373 May cause damage to organs through prolonged or repeated exposure

- SUBSTRATE SOLUTION (ready to use)**
  - Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

Reagents	AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS							
	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:-
- Sample should be added only after BLOTTING BUFFER is added.
  - 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.
  - 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:

- Add 2 ml of DILUTED WASH BUFFER to each well. **2 ml**
- 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.
- 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**
- Add 2 ml of BLOTTING BUFFER to each well. **2 ml**

- 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**
- Cover the tray with the cover provided and incubate for **1 hour** at room temperature (25 ± 3°C) on the rocking platform. **60 minutes**
- 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.
- 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**
- Add 2 ml of WORKING CONJUGATE SOLUTION to each well. **2 ml**
- Cover tray and incubate for **1 hour** at room temperature (25 ± 3°C) on the rocking platform. **60 minutes**
- Aspirate CONJUGATE from the wells. Wash as in step 8. **3 x 2 ml**
- Add 2 ml of SUBSTRATE SOLUTION to each well. **2 ml**
- 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**
- 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**
- 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.
- 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:**
- Add 2 ml of DILUTED WASH BUFFER to each well. **2 ml**
  - 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.
  - 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**

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

- Avoid Microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
- Do not pipette by mouth.
- Handle test specimens, nitrocellulose strips, Strong Reactive, Weak Reactive and Non-Reactive Controls as potentially infectious agents.
- Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.
- It is highly recommended that this assay be performed in a biohazard cabinet.
- Keep materials away from food and drink.
- In case of accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
- 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.
- 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.
- 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.
- 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.
- We do not recommend re-use of incubation trays.

#### ANALYTICAL PRECAUTIONS

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

#### 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:

- 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)).
- 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 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.
- 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 RESULTS

**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 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	Outer membrane	Diffuse glycoprotein
p66	POL	Reverse Transcriptase	Discreet band
p55	GAG	Precursor protein	Discreet band
p51	POL	Reverse Transcriptase	Discreet band just below p55

- The kit controls should be assayed concurrently with patients' samples for each test run.
- Use a new pipette tip for each specimen aliquot to prevent cross contamination.
- For best results dispense all reagents while cold and return to 2°C to 8°C storage as soon as possible.
- 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.
- Use only reagent grade quality, deionized or distilled water to dilute reagents.
- All reagents must be mixed well before use.
- Working Conjugate solution, Diluted Wash Buffer and Blotting Buffer should be **prepared fresh prior to use.**
- The Working Conjugate solution should be prepared using a polypropylene container or beaker.
- 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.
- The assay should preferably be performed at room temperature (25°C ± 3°C).
- Make sure that the test strips are laid with the numbers on the strips facing upwards.
- 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.

- Ensure that automated equipment if used is validated before use.
- 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.
- Avoid the use of self-defrosting freezers for the storage of reagents and samples.
- 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

- Store **MP Diagnostics HIV-1 BLOT 1.3** kit and its components at 2°C to 8°C when not in use.
- 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.

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

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:-

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

The interpretation process involves the following:-

- 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.
- Identify the molecular weight of each band of the test strip using the STRONG and/or WEAK REACTIVE Control strips as a guide.
- 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 Biomedicals** recommends following the accepted policy to be in accordance with local regulations. Listed below are some of the criteria guidelines recommended by different international organizations.

ORGANIZATION	CRITERIA FOR POSITIVE INTERPRETATION OF WESTERN BLOT TESTS
Association of State and Territorial Public Health Laboratory Directors / Centers for Disease Control (ASTPHLD/ CDC), 1989 USA	Any two of p24, gp41, gp120/gp160 bands
Center Nationale Transfusion Sanguine	Two ENV bands with GAG or POL
World Health Organization (WHO), 1990	Two ENV bands with or without GAG or POL
Consortium for Retrovirus Serology Standardization (CRSS), 1988 USA	One ENV band with p24 or p31
American Red Cross (ARC), 1988 USA	One band each of GAG, POL and ENV
Chinese Center for Disease Control and Prevention (CCDCP), 2004 PRC	Two ENV bands OR one ENV with p24 band
National and State Reference Laboratories (NRL) 1987, Australia	One ENV band with any three of GAG or POL bands
German Association for Control of Viral Diseases (DVV)	One ENV with at least one GAG or POL band, see also DIN 58 969, part 41

<sup>1</sup>ASTPHLD has been renamed Association of Public Health Laboratories (APHL) in 1998.

We recommend the following guidelines for the interpretation of the **MP Diagnostics HIV-1 BLOT 1.3**. Results should be recorded for each band detected, result should be interpreted as **NEGATIVE, POSITIVE or INDETERMINATE**.

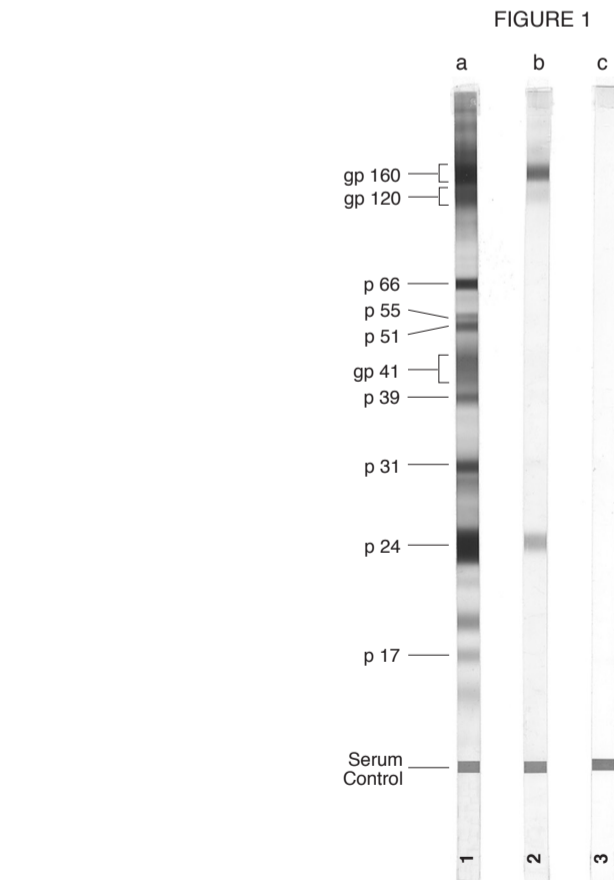
PATTERN	INTERPRETATION
No viral specific bands present	NEGATIVE
Detection of p17 antibodies <b>ONLY</b> , no other bands	NEGATIVE
Detection of 2 ENV (gp160/gp41 and gp120) and GAG (p17, p24, p55) or POL (p31, p51, p66)	HIV-1 POSITIVE
Any viral specific bands present but pattern <b>does not meet</b> criteria for POSITIVE	INDETERMINATE <sup>2</sup>

#### <sup>3</sup>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  $\geq$  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.

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Viral specific bands as visualized with:

- Strong Reactive Control
- Weak Reactive Control
- Non-Reactive Control

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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 considered with any supplemental test 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 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. Never theless, US CDC (2001) acknowledged that when in consultation with clinical and infection status among persons with an initial INDETERMINATE Western Blot.

Western Blot results. Never theless, US CDC (2001) acknowledged that when in consultation with clinical and infection status among persons with an initial INDETERMINATE Western Blot.

#### LIMITATIONS OF THE PROCEDURE

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 blot POSITIVE for antibodies to HIV-1 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 blots 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 retested using a fresh specimen after two to six months. It is also known that 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.

As HIV-1 and HIV-2 strains are cross-reactive, a HIV-2 specimen may appear as INDETERMINATE on **MP Diagnostics HIV-1 BLOT 1.3**. If a HIV-2 specimen is suspected, a more specific

HIV-2 test, such as **MP Diagnostics HIV-2 Western Blot 1.2**, should be used to confirm HIV-2 infection. The **MP Diagnostics HIV-1 BLOT 1.3** is not intended for the detection of HIV-2.

#### SPECIFIC PERFORMANCE CHARACTERISTICS

The **MP Diagnostics HIV-1 BLOT 1.3** is similar to the **MP Diagnostics HIV BLOT 2.2**. Technically and biologically they have similar :  

- conditions of use;
- specifications / properties;
- design;
- deployment methods;
- principles of operation; and
- same materials in contact with the same body fluids

The only difference between the two products / assays is the absence of the HIV-2 peptide band on the **MP Diagnostics HIV-1 BLOT 1.3**.

Consequently the sensitivity and specificity performance of the **MP Diagnostics HIV-1 BLOT 1.3** for the detection of antibodies to HIV-1 is based on data obtained for the **MP Diagnostics HIV BLOT 2.2**. The performance characteristics obtained are indicated as follows:

#### Sensitivity & Specificity

Table 1 : Sensitivity study of HIV-1 viral antigen reactivity with HIV-1 seropositive samples. (Number of samples = 201)

SEROLOGICAL PROFILE	HIV BLOT 2.2	DUPONT/ORTHO HIV-1 WB
GAG, POL and ENV	97.5%	95.4%
p24, p31, gp41 and/or gp120/gp160	94.9%	90.9%
ENV and GAG or POL	100.0%	100.0%

Table 2: Specificity study of HIV-1 viral antigen reactivity with normal donor samples and sera with other viral infections.

SAMPLE TYPE	NUMBER TESTED	POSITIVE	HIV-1 REACTIVITY	
			INDETERMINATE <sup>1</sup>	NEGATIVE
Normal Donors	208	0	11	197
HTLV-1	5	0	0	5
CMV	5	0	1	4
EBV (IgM)	5	0	1	4
Vzoster (IgG)	5	0	1	4
Measles	6	0	2	4
Rubella	5	0	1	4
Mumps	4	0	1	3
Adenovirus	5	0	2	3
Herpes simplex	5	0	0	5
Dengue	5	0	1	4
<b>Total</b>	<b>258</b>	<b>0</b>	<b>21</b>	<b>237</b>

<sup>1</sup>All showed as a p24 or p17 band only.

#### Seroconversion

A total of 15 commercial HIV-1 seroconversion panels were tested with **MP Diagnostics HIV-1 BLOT 1.3** and results showed that the **MP Diagnostics HIV-1 BLOT 1.3** was able to detect antibody to HIV earlier or in the same sample in all the panels.

#### LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no 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, 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.

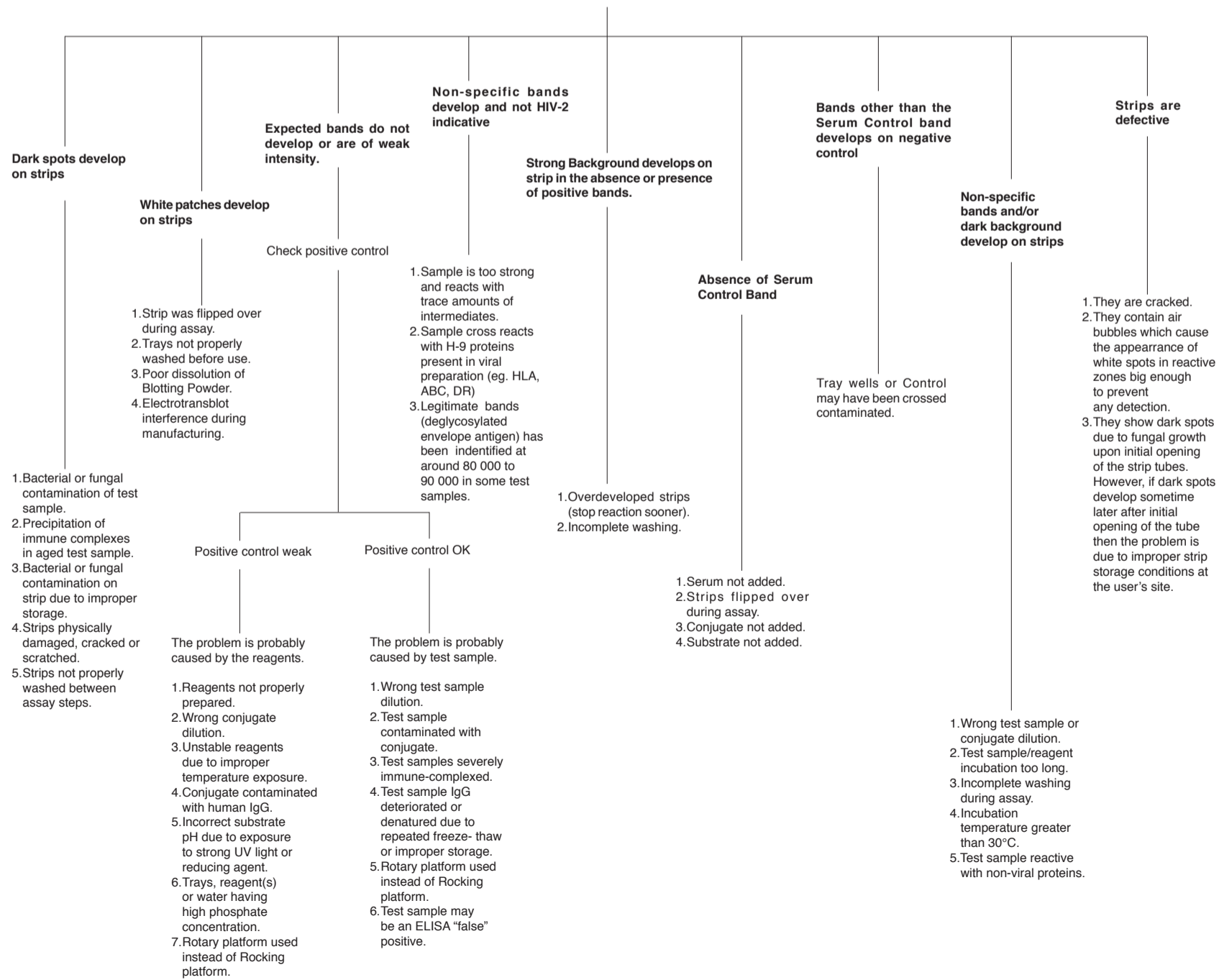
#### TECHNICAL PROBLEMS / COMPLAINTS TECHNICAL PROBLEMS / COMPLAINTS

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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## TROUBLE SHOOTING CHART



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\* U.S. Patent 5,721,095

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