WHO Prequalification of In Vitro Diagnostics Programme PUBLIC REPORT

Product: MP Diagnostics HIV Blot 2.2 Number: PQDx 0198-071-00

Abstract

MP Diagnostics HIV Blot 2.2 with product codes **11030-018** and **11030-036**, manufactured by MP Biomedicals Asia Pacific Pte. Ltd, **CE marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 4 April 2016.

MP Diagnostics HIV Blot 2.2 kit is a qualitative enzyme immunoassay for the in vitro detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. It is intended for use as a supplemental assay on human serum or plasma specimens found repeatedly reactive using EIA. The separated specific HIV-1 viral antigens incorporate 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 specimen addition control to minimize the risk of false negatives due to operations errors and to ensure the addition of specimens.

The nitrocellulose strips are incorporated with separated bound antigenic proteins from partially unpurified inactivated HIV-1 using electrophoretic blotting, plus a specific HIV-2 synthetic peptide on the same strips. Individual nitrocellulose strips are incubated with diluted specimens 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. This method has the sensitivity to detect marginal amounts of HIV specific antibodies in serum or plasma.

Component	18 tests (product code 11030-018)	36 tests (product code 11030-036)
Nitrocellulose Strips : Incorporated with HIV-1 viral lysate, a specific HIV-2 envelope peptide and specimen addition control band.	18 strips	36 strips
Non-reactive control:	1 vial (80µl)	1 vial (80µl)

The test kit contains:

Inactivated normal human serum non-		
reactive for hepatitis B surface antigen		
(HBsAg), antibodies to HIV-1/2, and anti-HCV.		
Contains sodium azide and thimerosal as		
preservatives.		
Strong reactive control:	1 vial (80µl)	1 vial (80µl)
Inactivated normal human serum with high		
titered antibodies to HIV-1 and HIV-2 and		
non-reactive for HBsAg, and anti-HCV.		
Contains sodium azide and thimerosal as		
preservatives.		
Weak reactive control:	1 vial (80µl)	1 vial (80µl)
Inactivated normal human serum with high		
titered antibodies to HIV-1 ONLY and non-		
reactive for HBsAg, anti-HIV-2 and anti-HCV.		
Contains sodium azide and thimerosal as		
preservatives.		
Stock Buffer concentrate (10x):	1 bottle (20ml)	1 bottle (20ml)
Tris buffer with heat inactivated normal goat		
serum. Contains thimerosal as presentative.		
Wash buffer concentrate (20x):	1 bottle (70ml)	1 bottle (70ml)
Tris buffer with Tween-20. Contains		
thimerosal as preservative.		
Conjugate:	1 vial (160µl)	1 vial (160µl)
Goat anti-human IgG conjugated with		
alkaline phosphate. Contains thimerosal as		
preservative.		
Substrate:	1 bottle (100ml)	1 bottle (100ml)
Solution of 5-bromo-4-chloro-3-indoyl-		
phosphate (BCIP) and nitroblue tetrazolium		
(NBT).		
Blotting powder:	10 packets (1g	10 packets (1g
Non-fat dry milk	each)	each)
Incubation tray:	2 trays	4 trays
9 wells each		
Instruction for use:	1 copy	1 сору
	<u> </u>	

Storage:

The test kit should be stored at 2-8 °C.

Shelf-life:

24 months.

WHO special warning:

WHO reviewed the instructions for use that were current at the time of WHO prequalification, and a number of changes were suggested. Most but not all changes were made by the manufacturer (outstanding comments relate to general layout and nomenclature).

Summary of prequalification status for MP Diagnostics HIV Blot 2.2

	Ini	Initial acceptance	
	Date Outcome		
Status on PQ list	4 April 2016	listed	
Dossier assessment	10 October 2014	MR	
Inspection status	05 June 2015	MR	
Laboratory evaluation	N/A	MR	

MR: Meets Requirements NA: Not Applicable

MP Diagnostics HIV Blot 2.2 was accepted for the WHO list of in vitro prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

MP Biomedicals Asia Pacific Pte. Ltd, submitted an application for prequalification of MP Diagnostics HIV Blot 2.2. Based on the established prioritization criteria, MP Diagnostics HIV Blot 2.2 was given priority for prequalification.

Product dossier assessment

MP Biomedicals Asia Pacific Pte. Ltd, submitted a product dossier for MP Diagnostics HIV Blot 2.2 as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for MP Diagnostics HIV Blot 2.2 for prequalification.

The manufacturer committed to amend and submit additional documentation on the following issues:

- 1. The Manufacturer will investigate the effect of transport conditions on HIV Blot 2.2 shelf life including a higher temperature range (expected completion date Q4 2017).
- 2. The manufacturer will commence testing all material used in the production of positive and negative test kit controls using state-of-the-art methods (i.e. nucleic acid detection).

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (2 Pioneer Place, Singapore) of MP Diagnostics HIV Blot 2.2 in November 2014 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities found at the time of the inspection were accepted 14 July 2015.

The manufacturer committed conduct the following studies which will be reviewed at the next re-inspection: drop and shock testing for HIV Blot 2.2.

Laboratory evaluation

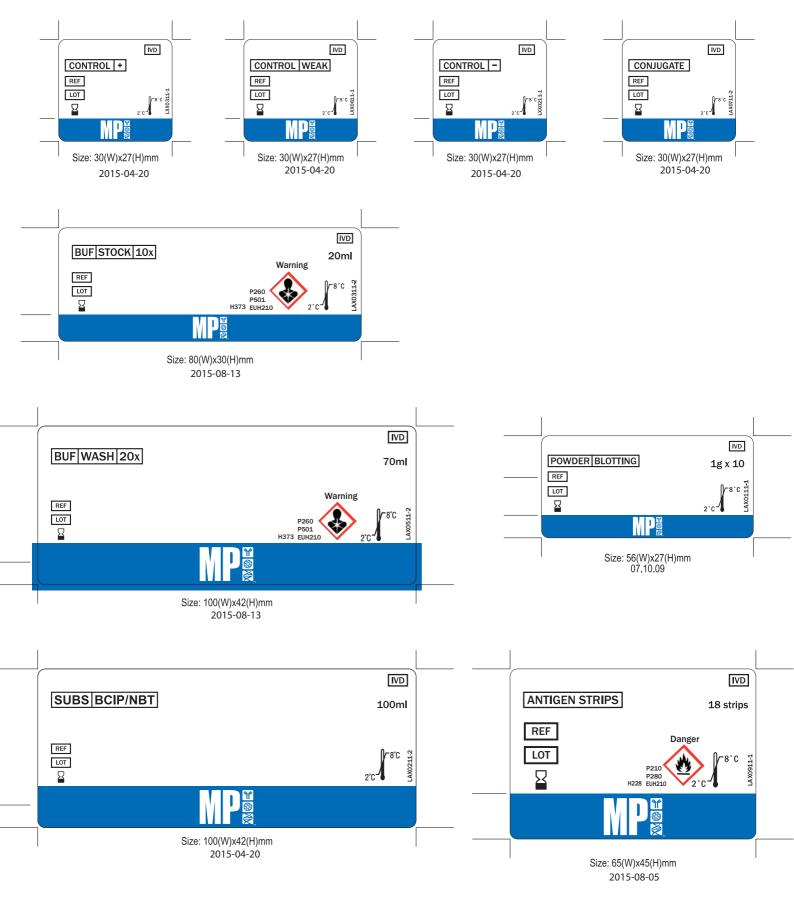
The objective of the performance laboratory evaluation is to assess the performance and operational characteristics of commercially available in-vitro diagnostics for the purpose of advising the governments of WHO Member States on these issues. In particular, suitability for use in resource-limited settings will be assessed.

Based on the risk level associated with the use of the supplemental assays, the known general performance of supplemental assays and role of the supplemental assays in patient care in resource-limited settings, it was decided that WHO will not conduct performance evaluations of these assays as part of the prequalification assessment process.

Consequently, laboratory evaluation of MP Diagnostics HIV Blot 2.2 was not conducted.

Labelling

- 1. Labels
- 2. Instructions for use



HIV Blot 2.2





- EN For detection and identification of IgG antibodies to Human Immunodeficiency Virus Type 1(HIV-1) and Type 2 (HIV-2)
- DE
 Für den Nachweis und die Identifizierung von IgG-Antikörpern gegen das Humane Immundefizienz-Virus Typ 1 (HIV-1) und Typ 2 (HIV-2)
- FR
 Pour la détection et l'identification des anticorps IgG du virus d'immunodéficience humaine de type 1 (VIH-1) et de type 2 (VIH-2)
- Image: Per la determinazione e l'identificazione degli anticorpi IgG del virus dell'immunodeficienza umana di tipo 1 (HIV-1) e di tipo 2 (HIV-2)
- ES Para la detección e identificación de anticuerpos IgG del virus de inmunodeficiencia humana tipo 1 (VIH-1) y tipo 2 (VIH-2)
- PT
 Para a detecção e identificação de anticorpos IgG contra o vírus da imunodeficiência humana tipo 1 (HIV-1) e tipo 2 (HIV-2)

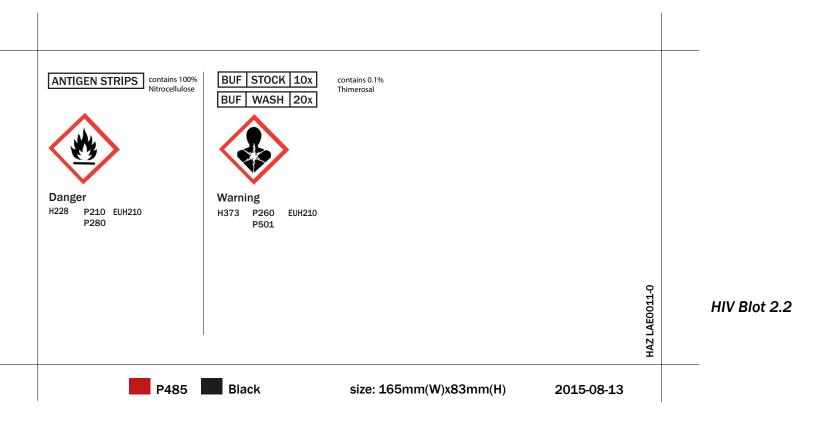
	V 18	$\overline{\Sigma}$
ANTIGEN STRIPS	18 strips	3
CONTROL -	80µl x 1	8
CONTROL +	80µl x 1	8
CONTROL WEAK	80µl x 1	8
BUF STOCK 10x	20ml x 1	2
BUF WASH 20x	70ml x 1	7
CONJUGATE	160µl x 1	1
SUBS BCIP / NBT	100ml x 1	1
POWDER BLOTTING	1g x 10	1

	MP Diagnostics HIV BLOT 2	2		3
3	WESTERN BLOT ASSAY			
trips	• 36 strips	REF		
x 1	80µl x 1	LOT		
x 1	80µl x 1	Я		
x 1	80µl x 1			
x 1	20ml x 1	Σ		
x1	70ml x 1	V		
ul x 1	160µl x 1			[/ −8°0
nl x 1	100ml x 1		 1	
10	1g x 10			20 🛡

EC REP Medical Technology Promedt Consulting GmbH Altenhofstrasse 80, D-66386 St, Ingbert Germany

Tel. No.: +49-68 94-58 1020 Fax. No.: +49-68 94-58 1021 Email: info@mt-procons.com

Colour Code:





HIV BLOT 2.2 WESTERN BLOT ASSAY Instructions For Use

0123	
REVISION DATE 2015-10 MAE0011W-ENG-0	

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

- 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 guality 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.

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.				
8	Use by <i>Synonym for this :</i> Expiry Date	IVD	In vitro diagnostic medical device	
LOT	Batch Code Synonyms for this are: Lot Number Batch Number	REF	Catalogue Number Synonyms for this: Reference number Re-order number	
ſ	Temperature Limitation	\triangle	Caution	
	Manufacturer	EC REP	Authorised Representative in the European	
\sum	Contains sufficient for <n> tests</n>		Community	
8	Do not reuse	Ĩ	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.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

· Deionized or distilled water

CONT Contents

- 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.
 - Dilute 1 volume of WASH BUFFER CONCENTRATE (20x) (b) with 19 volumes of reagent grade water. Mix well.

2. BLOTTING BUFFER

- (a) BLOTTING BUFFER should be prepared fresh prior to
- Dilute 1 volume of STOCK BUFFER CONCENTRATE (10x) (b) with 9 volumes of reagent grade water. Mix well. Add 1 g of BLOTTING POWDER to every 20 ml of the (C)
- diluted STOCK BUFFER prepared in step 2(b) above. Mix vell by inversion or stir with a magnetic stirrer to ensure powder dissolves completely. Stir again before dispensing. Stir again before dispensing.

3. WORKING CONJUGATE SOLUTION

- Note : Prepare solution in polypropylene container / beaker. WORKING CONJUGATE SOLUTION should be prepared (a) fresh prior to use.
- For **RAPID ASSAY PROTOCOL**, prepare WORKING (b) 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 (C) 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.

KIT COM	PONENTS		
	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	
	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)	Note : Volu * Incubatio
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)	WARNING 1. For <i>in v</i> 2. For Pro 3. Please hazardo HEALTH A
	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)	VI me vil ST RE AC
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)	sp Th sa The <i>Stron</i> <i>Reactive</i>

BUF WASH 20x WASH BUFFER CONCENTRATE (20x) 1 bottle (70 ml) Tris with Tween-20. Contains 0.1% thimerosal as preservative CONJUGATE CONJUGATE 1 vial Goat anti-human IgG conjugated with alkaline (160 µl) phosphatase. Contains 0.1% sodium azide as preservative. SUBS BCIP / NBT SUBSTRATE 1 bottle

Solution of 5-bromo-4- chloro-3-indolvl-(100 ml) phosphate (BCIP) and nitroblue tetrazolium (NBT). POWDER BLOTTING BLOTTING POWDER 10 packets Non-fat dry milk

(1g each)

AMOUNT OF REAGENTS REQUIRED FOR VARIOUS NUMBER OF STRIPS NUMBER OF STRIPS TO BE USED Reagents 3 6 9 15 20 27 36 60 100 140 240 300 400 600 Diluted Wash Buffer (ml) 20 40 60 80 100 120 160 Blotting Buffer (ml) 1 2 3 4 5 6 8 Blotting Powder (g)
 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 7 | 13 | 19 | 31 | 41 | 55 | 73 Substrate (ml)

ASSAY PROCEDURE - RAPID ASSAY

Incubation Tray*

Note: a) Users can use either the rapid or overnight assay to run the tests. Assaved strips from overnight assav 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 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.
- 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:

- Add 2 ml of DILUTED WASH BUFFER to each well. 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 2 minutes temperature ($25 \pm 3^{\circ}$ 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.)

- 8.
- 9. each well. 10. Cover tray and incubate for <u>1 hour</u> at room temperature **60 minutes**

- platform.
- difficulty in reading.)
- insufficient at this step). 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

2 ml

Instructions For Use	1 сору
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
ume of reagents provided are sufficient for 4 assay	y runs.

Incubation trays provided but packed separately from the kit.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

. 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:	۲

- 4. Add 2 ml of BLOTTING BUFFER to each well. Tilt the 2 ml 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. 5. Add 20 μ I each of specimens to their respective **20** μΙ 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 6. Cover the tray with the cover provided and incubate for 60 minutes <u>1 hour</u> at room temperature ($25 \pm 3^{\circ}$ C) on the rocking
- platform. 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. Add 2 ml of DILUTED WASH BUFFER. Wash each 3 x 2 ml 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.) Add 2 ml of WORKING CONJUGATE SOLUTION to 2 ml
- $(25 \pm 3^{\circ}C)$ on the rocking platform. 11. Aspirate CONJUGATE from the wells. Wash as in step 3 x 2 ml
- 12. Add 2 ml of SUBSTRATE SOLUTION to each well.
- 13. Cover tray and incubate for 15 minutes on the rocking 15 minutes
- (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
- 14. Aspirate the SUBSTRATE and rinse the strips at 3 x 2 ml least three times with reagent grade water to stop the reaction (A dark background can result if washing is

1. Add 2 ml of DILUTED WASH BUFFER to each well. 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.

2 ml

H228 Flammable solid
P210 Keep away from heat/sparks/ open flames/hot surfaces. – No smoking. P280 Wear protective gloves/protective clothing/eye protection/face protection.
EUH210 Safety Data Sheet is available on request
100% Nitrocellulose
STOCK BUFFER CONCENTRATE (10x) WASH BUFFER CONCENTRATE (20x)
Warning
H373 May cause damage to organs through prolonged or repeated exposure
P260 Do not breathe dust/fume/gas/mist/ vapours/spray. P501 Dispose of contents/container in accordance with local/regional/national/ international regulations.

0.1% Thimerosal Contains:

- 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, 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
- 3. Incubate the strips for 1 to 2 minutes at room 2 minutes temperature ($25 \pm 3^{\circ}$ 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.)

- 4. Add 2 ml of BLOTTING BUFFER to each well. Tilt the 2 ml 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.
- 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.
- 6. Cover the tray with the cover provided and incubate overnight overnight (16 - 20 hours) at room temperature (25 \pm 3°C) on the rocking platform.
- 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 3 x 2 ml 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.)
- 9. Add 2 ml of WORKING CONJUGATE SOLUTION to 2 ml each well.
- 10. Cover tray and incubate for 30 minutes at room 30 minutes temperature ($25 \pm 3^{\circ}$ C) on the rocking platform.
- 11. Aspirate CONJUGATE from the wells. Wash as in step 3 x 2 ml
- 12. Add 2 ml of SUBSTRATE SOLUTION to each well. 2 ml 13. Cover tray and incubate for <u>15 minutes</u> on the rocking **15 minutes** 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.)
- 14. Aspirate the SUBSTRATE and rinse the strips at 3 x 2 ml least three times with reagent grade water to stop the reaction (A dark background can result if washing is insufficient at this step).
- 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.

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 aliguots 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.

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).

- **20** μΙ

3

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).

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 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,	MP Diagnostics HIV Blot 2.2 HIV-1 negative = 187 HIV-1 positive = 0 Indeterminate = 13	HIV-1 Western blot HIV-1 negative = 55 HIV-1 positive = 0 Indeterminate = 145
HCV, and Syphilis by FDA approved tests. (n = 200) 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% Cl (89.14% - 96.49%)	27.50% (55/200); 95% CI (21.44% - 34.24%)

Table 5: Clinical samples (167 samples)

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

- 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
- WHO, CDC and other international criteria. The POL bands p66, p51 and p31 are generally detected simultaneously. However the sensitivity of p66 and p31 are greater 4 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.
- 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 ot be interpreted
- 9. Appearance of p66 alone is not HIV-1 specific, but is most likely a eactivity 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

Lyme Disease Mixed Titer Performance	HIV-1 negative = 8 HIV-1 positive = 0	HIV-1 negative = 1 HIV-1 positive = 0
Panel PTL201 from BBI	Indeterminate = 2	Indeterminate = 9
(n = 10)		
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/	MP Diagnostics	HIV-1
Source	HIV Blot 2.2	Western blot
Rheumatoid Factor samples from BCP Samples with RF values (0-500), (501-999) & (\geq 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

Any viral specific bands present but pattern does not meet criteria for POSITIVE	INDETERMINATE ²
Any viral specific bands present	INDETERMINATE ²
but pattern does not meet	with
criteria for POSITIVE but HIV-2	HIV-2 INDICATED
specific band is visible.	

²INTERPRETATION 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

Icteric samples from	HIV-1 negative = 7	HIV-1 negative = 0
BiosPacific	HIV-1 positive = 0	HIV-1 positive = 0
(n = 10)	Indeterminate = 3	Indeterminate = 10
Haemolysed samples	HIV-1 negative = 4	HIV-1 negative = 0
from BiosPacific	HIV-1 positive = 0	HIV-1 positive = 0
(n = 10)	Indeterminate = 6	Indeterminate = 10
Pregnant women	HIV-1 negative = 30	HIV-1 negative = 4
samples from Biochrom,	HIV-1 positive = 0	HIV-1 positive = 0
Germany	Indeterminate = 3	Indeterminate = 29
(n = 33)		
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);	4.94% (4/81);
	95% CI (64.47% -	95% CI (1.36 % -
	84.22%)	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. Retain the kits and the results that were obtained.
- 3. Contact the nearest MP Biomedicals office or your local distributor.

140.

Asia, New Delhi, India.

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.

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%)

Panel Name/	MP Diagnostics	HIV-1
Source	HIV Blot 2.2	Western blot
HIV Surveillance Panels from BCP 2 panels (10 members each) with samples from Ghana and Nigeria (n = 20)		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% Cl (89.43% - 98.47%)	86.92% (93/107); 95% Cl (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 MPD 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
MPD and Chiron have equal	PRB965, PRB966,	10
detection of Positives and	PRB968, PRB969,	
Indeterminates	PRB970, ZMC6243,	
	ZMC6245, ZMC6246,	
	ZMC9019, PRB9032	

BIBLIOGRAPHY

- 1. V.C.W.Tsang, K. Hancock, M. Wilson. D.F. Palmer, S. Whaley, J.S. Mc Dougal, and S. Kennedy. March 1985. Developmental Procedure Enzyme-linked Immunoelectro-transfer Blot technique for HTLV-III/ LAV antibodies; CDC, Altanta.
- 2. H. Towbin, T. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and some applications. Proc. Natl. Acad. Sci.. USA 76: 4350-4354.
- 3. J. Schupbach, M. Popovic, R. V. Gilden. M.A. Gonda, M. G. Sarngadharan and R. C. Gallo. 1984. Serological Analysis of subgroup of Human T-Lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 224, 503-505.
- M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schupbach and R. C. Gallo. 1984. Antibodies reactive with human T-Lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 224, 506-608
- CDC. 1985. "Provisional public health service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing Acquired Immune Deficiency Syndrome" - United States Morbidity and Mortality Weekly Report 34 (1) :1-5.
- Proposed World Health Organization 1990 criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-I/ HTLV-II, Weekly Epidemiological Record 65(37), 281-283.
- F. Clavel, D. Guetard., F. Brun-Vezinet, et al. 1986 Isolation of a new human retrovirus from West African patients with AIDS. Science; 233:343-346
- 8. F. Clavel., 1987. HIV-2, the West African AIDS virus. AIDS 1:135-
- R.S. Tedder, A. Hughes, T. Corrah et al 1988. Envelope crossreactivity in Western Blot for HIV-1 and HIV-2 may not indicate dual nfection. Lancet 11:927-930.
- 10. Bottiger B., A. Karlsson, F. Andreasson et al. 1990. Envelope crossreactivity between Human Immunodeficiency Virus Type 1 and Type 2 detected by different serological methods: Correlation between cross-neutralization and reactivity against the main neutralizing site. J. Virol. 64(7):3492-3499.
- 11. Centers for Disease Control. 2001. Revised Guidelines for HIV Counseling, Testing, and Referral and Revised Recommendations for HIV Screening of Pregnant Women - United States, Morbid. Mortal. Weekly Rep. 50: RR-19.
- 12. Fiebig, E. W., D. J. Wright, B. D. Rawal, P. E. Garrett, R. T. Schumacher, L. Peddada, C. Heldebrant, R. Smith, A. Conrad, S. H. Kleinman, and M. P. Busch. 2003. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. 17:1871-1879. 13. Ly, T. D., C. Edlinger, and A. Vabret. 2000. Contribution of combined detection assays of p24 antigen and anti-human immunodeficiency virus (HIV) antibodies in diagnosis of primary HIV infection by routine testing. J Clin Microbiol. 38:2459-2461.
- 14. Sethoe, S. Y., A. E. Ling, E. H. Sng, E. H. Monteiro, R. K. Chan. 1995. PCR as a confirmatory test for human immunodeficiency virus type 1 infection in individuals with indeterminate western blot (immunoblot) profiles. J Clin Microbiol. 33:3034-3036.
- 15. Constantine, N. T. and H. Zink. 2005. HIV testing technologies after two decades of evolution. Indian J Med Res. 121:519-538 16. World Health Organization. 2004. Guidelines for HIV Diagnosis and monitoring of antiretroviral therapy. Regional Office for South-East

- 17. Ming Guan, Frequency, causes and new challenges of indeterminate results in Western Blot Confirmatory Testing for Antibodies to Human Immunodeficiency Virus. Clinical and Vaccine Immunology, June 2007, Vol.14, No.6, p649-659.
- 18. Reed DL, et al. HIV-1 Western Blot Standardization. European Ligand Assay Society Sept 1995; 43-47.



MP Biomedicals Asia Pacific Pte Ltd. 2 Pioneer Place



Fax. No. : + 65 6774 6146 Email : enquiry_ap@mpbio.com Medical Technology Promedt EC REP Consulting GmbH

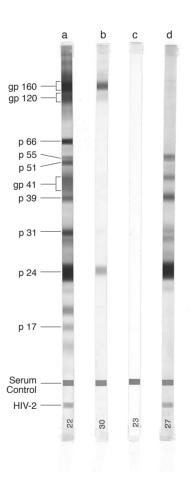
> Altenhofstrasse 80 D-66386 St. Ingbert Germany Tel. No. : + 49 68 94 58 1020 Fax No. : + 49 68 94 58 1021 Email : info@mt-procons.com

Regional Office:

MP Biomedicals SAS Parc d'Innovation, BP 50067 67402 Illkirch Cedex France Tel No. : (33) 388 67 4607 Fax No. : (33) 388 67 5420

Email: custserv.eur@mpbio.com

* U.S. Patent 5,721,095



- a. Strong Reactive Control (Reactive for HIV-1 and HIV-2)
- h Weak Reactive Control (Reactive for HIV-1 only). c. Non-Reactive Control.
- d. A typical HIV-2 seropositive serum.
- 12

FIGURE 1

TROUBLE SHOOTING CHART

Dark spots develop on strips not develop or are of weak intensity. develops on strip in the absence or presence of positive band. Non-spacific advelop on strips Strips are detective detective advelop on strips Wore detective advelop on strips Wore advelop on strips 1. Bacterial or tungal contamination or strip asample. 3. For advelop exective advelop scatched sample. Positive control (VK 1. Overdeveloped strips (diop reaction scone) 1. Wore contex advelop scatched sorage contaminated with conjugate 1. Wore gets sample contaminated with conjugate 1. Wore gets sample contadvelop scatched contaminated with conjugate			ite patches elop on strips	develop a HIV-2 indi	cative	around	the Seru	ther than m Control /elops on		Sharp, dise		
Dark spots develop on strips of weak intensity. absence or presence of positive bands. Non-specific bands and/or derive por strips are defective and background develop on strips Water develop an strips 1. Strips was lipped over during assay. 1. Strips are strips are during assay. 1. Overloading of negative sample or ennoeus higher conjugate concentration or erroneous blocking buffer or over incubation. Absence of serum Control Band 1. They are cracked. 1. Strips are cracked					develops on strip in the		negative control		band at gp41 region			
develop on strips positive bands. Non-specific detective develops and single develop and single develops and single develops	Dark spot	s									<u></u>	
Check positive control Overloading of negative sample or erroneous higher origidate concentration or erroneous blocking buffer or over during assay. Absence of serum Control Band Non-specific dark background develop on strips 1. Strips was flipped over during assay. Trays not properly washed before use. Note: This is not caused by hook effect because hook effect will result in take negative result. Absence of serum Control Band 1. They are cracked. 1. Strips 2. Strips was flipped over during assay. 2. Sample cross reacts with H-9 proteins present in viral proparation (eff. HLA, ABC, DF) Tray wells or contamination of test sample. Tray wells or the apparation (eff. HLA, ABC, DF) 1. Deverdeveloped strips 1. Bacterial or fungal contramination of test sample. Positive control OK 1. Overdeveloped strips Tray wells or contamination of test samples. 1. Noving test sample indentified at around 80-90k0 in some test samples. 1. Serum not added. 1. The problem is probability creation socond. 3. Bacterial or fungal contamination of test sample. 1. Noving test sample indentified at around 80-90k0 in some test samples. 1. Overdeveloped strips (toor prevent any detection. 3. They show dark spots develop sometime iatar after initial opening of the tube then the problem is properly prepared. 1. Wrong test sample or conjugate dilution. 1. Serum not added. 1. Wrong test sample or conjugate dilution. 1. This is not gp41 as gp41 is spossibly a cell ine protein, p42. 3. Incomplete washing cracked or scratched. 3. Incomplete washing conjugate dilut			of weak in						Strip	s are	Watery m	
 1. Overloading of negative sample or erroneous higher conjugate concentration or during assay. 2. Trays not properly washed before use. 3. Poor dissolution of Bioting Powder. 4. Electortransblot interference during manufacturing. 1. Bacterial or fungal contamination of test sample. 3. Bacterial or fungal contamination of test sample. 4. Electortransblot interference during manufacturing. 4. Electortransblot interference during manufacturing. 4. Bacterial or fungal contamination of test sample. 5. Respine control Weak 4. Bacterial or fungal contamination of test sample. 5. Respine control Weak 6. Respine control OK 6. Conjugate control OK 7. Notice sample. 7. The problem is probably caused by the reagents. 8. Bacterial or fungal contamination of test sample. 8. Bacterial or fungal contamination of test sample. 8. Bacterial or fungal contamination of test sample. 9. Bacterial or fungal contamination of test to improper strage. 1. The problem is probably caused by test sample fulfution. 1. The problem is probably caused by test sample ind due to improper strage. 1. The problem is probably caused by test sample ind due to improper strage. 1. The problem is probably caused by test sample ind due to improper strage. 2. Toron indepret sample ind due to improper strage. 3. Interminet example or conjugate dilution. 4. Test sample ind due to repret many			Check posit	ive control					cific defe		develope	u suips
 sample of erroneous inglere over during assay. Strips was flipped over during assay. Tray snot properly washed before use. Poor dissolution of Bioting Powdor. Electrotransbiot interference during manufacturing. Bacterial or fungal contamination of test sample. Poolitive control weak assay steps. Agatemia or fungal contaminated with eragents use of the reagents. Proporting addition. The problem is probably caused by the tagents. Proporting addition of test sample. Strips not properly washed before use. Contog agent. Reagents such as Blotting of the reagents. Strips not properly washed by the reagents. Conjugate control washing. Reagents such as Blotting or creducing agent. Reagents such as Blotting or readule agent. Reagents and gravitation. The problem is probably caused by the tagents. Norm gest stample. Strips not properly washed before use stropy. Strips not properly mashed by the reagents. Conjugate control washing. The problem is probably caused by the tagents ample. Nerving conjugate dilution. The problem is probably caused by the tagents and the reagents. Conjugate control washing. The problem is probably caused by the tagents and the addition. The problem is probably caused by the tagents and the reagents. Conjugate control washing. The problem is probably caused by the tagents and between assay steps. Strips not properly mashed be for eagents during agent. Conjugate contaminated with woring contracted of denatured hytes reagents during agent. Strips tagengent(s) or water have beard freeze-thaminated with exprese in the addition. Strips tagenge					negative							
1. Strips was lipped over during assay. 1. They are cracked. 1. Strips after after by by cracked or scatched. 3. Poor dissolution of Botting Powder. 2. Trays not properly washed before use. 2. Sample cross reacts with He proteins present in viral preparation (eg. HLA, ABC, DP) interference during manufacturing. 1. Strips again are sample. 1. They are cracked. 1. Strips after a scatched. 1. Bacterial or fungal contamination of test sample. Provides present in viral preparation (eg. HLA, ABC, DP) manufacturing. 1. Overdeveloped strips (deglycosylated envelope antigen has been indentified at around 80-90kD in some test sample. 1. Overdeveloped strips (contaminated 1. Strips assay. 3. Here of transport Positive control weak Positive control Weak Positive control OK 1. Overdeveloped strips (cased or scratched. 1. Strips manufacturing assay. 1. Serum not added. 1. Serum not added. 4. Strips toriped over during assay between assay steps. 1. Norog test sample or conjugate dilution. 1. Strips tample dilution. 1. Serum not added. 1. Wong test sample or conjugate dilution. 5. Thest sample or conclude in proper temperature exposure. 1. Norog test sample or conjugate dilution. 1. Strips tample or curing assay. 2. Control temperature greater than 30°C. 1. Thest sample or conjugate di				sample or erro	neous higher				3			
1. Strips was lipped over during assay. 1. Bacterial or fungal contamination of test sample. 2. Precipitation of immune complexes in aged test sample. 2. Strips lipped over during assay. 1. Reagents such as Blotting Buffer and Working Positive control weak by the reagents. Positive control weak by the reagents. Positive control weak by the reagents. 1. Wrong test sample dilution. 1. Serum not added. 2. Strips flipped over during assay. 1. They are cracked. 1. Strip develop sometime caused by test sample dilution. 2. Strips flipped over during cracked or storage. 1. Meagents such as Blotting Buffer and Working 1. Wrong test sample dilution. 1. Serum not added. 2. Strips flipped over during assay. 3. Incomplet washing. 1. Their is and preferagent incubation too long. 1. This is not gp41 as app41 is a diffuse band. 4. Strips flipped over during assay. 2. Strips flipped over during ass				, .				develop				
during assay. .2. Trays not properly washed before use. Note:: The is not caused by book effect because hook effect will result in false negative result. Band 1. They are cracked. 1. Strips after bubbles which cause the appearance of addi- streps contamination of test sample. 1. They are cracked. 1. Strips after bubbles which cause the appearance of contamination of test sample. 1. Bacterial or fungal contamination of test sample. 2. Strips for control Weak Positive control OK 1. Deredveloped strips (deglycosylated envolope antigen) has been indentified at around 80-90kD in some test sample. Tray wells or Contamination of test sample. 2. Precipitation of immune complexes in aged test sample. Positive control OK 1. Overdveveloped strips (deglycosylated envolope antigen) has been indentified at around 80-90kD in some test sample. The problem is probably caused to improper strip cracked or scratched. The problem is probably caused to improper straps (cracked or scratched. The problem is probably caused to improper straps (cracked or scratched. 1. Wrong test sample eliution. 1. Sarum not added. 1. This is not gp41 as gp41 is a diffuse the user's site. 3. Instrast maples 1. Unstrap result in dials reagents. 3. Test sample for gasay. 3. Conjugate int added. 1. This is not gp41 as gp41 is a diffuse the user's site. 4. Substrate not added. 1. Straps flipped over during assay. 3. Incomplete washing during assay. 1. This is possibly a cell inteprotein. p42.		1. Strip:	s was flipped over		king buffer or over							
2. Trays not properly washed before use. affect because hook effect will result in false negative result. affect because hook effect will result in false negative result. 2. They contain air affect bubbles which cause step additions properly washed before use. 1. Bacterial or fungal contamination of test sample. 2. Eactorian since bubbles which cause the appearance of contaminated. Tray wells or Control may have been crossed contaminated. Tray wells or Control may have been crossed contaminated. 2. They show dark spots due to fungal contaminated. 3. Legitimate bands (deglycosylated envelope antigen) has been infinite at around 80-90kD in some test sample. Tray wells or Control may have been crossed contaminated. The yrbole may have been crossed contaminated. 3. They show dark spots due to fungal contaminated. 3. They show dark spots due to fungaper opening of the tube the trap per strage. 1. Bacterial or fungal contamination on strip due to improper storage. Positive control OK 1. Overdeveloped strips caused by test sample. 1. Serum not added. 3. Every site. 1. Reagents such as Blotting to timpoper typerated. 1. Wrong test sample dilution. 3. Test sample severely more or properly prepared. 3. Conjugate not added. 1. Wrong test sample or conjugate dilution. 2. Wrong conjugate contaminated to improper temperature exposure. 2. Norage conditions at the user's site. 3. They show are due to improper storage. 3. Incorect substrate pH due to morpore tamperature exposure to storag UV		durin	g assay.				Control		1 Thou are a	 	1 String le	off to dry
washed before use. result in false negative result. a. Proof dissolution of Blotting Powder. bubbles which cause step addition 4. Electrotransbiot interference during manufacturing. 2. Sample cross reacts with H-9 proteins present in viral preparation (eg. HLA, ABC, DR) Tray wells or Control may have Tray wells or Control may have bubbles which cause step addition 1. Bacterial or fungal contamination of test sample. 1. Bacterial or fungal contamination on strip due to improper storage. Positive control OK 1. Overdeveloped strips sample. The yshow dark spots develop sometime tater after initial opening of the strip tubes. 3. They show dark spots develop sometime tater after initial opening of the strip tubes. 3. Bacterial or fungal contamination on strip due to improper storage. Positive control Weak Positive control OK 1. Overdeveloped strips caused by test sample. 1. Serum not added. 3. They show dark spots develop sometime tater after initial opening of the tube then the problem is probably caused by test sample dilution. 1. Serum not added. 1. Serum not added. 1. This is not gp41 as gp41 is a diffuse band. 2. Wrong conjugate dilution. 3. Unstable reagents due to improper storage. 3. They show for develop sometrue conjugate contaminated with human lgG. 3. Tost sample igG 4. Substrate not added. 1. Wrong test sample or conjugate dilution. 1. Serum not added. 1. This is possibly a cell incubation too long. <td< th=""><td></td><td>,</td><td></td><td></td><td></td><td>Ballu</td><td></td><td></td><td></td><td></td><td>· ·</td><td>e-soaking</td></td<>		,				Ballu					· ·	e-soaking
3. Poor dissolution of Bioting Power. 2. Sample cross reacts with interference during manufacturing. 2. Sample cross reacts with H-9 proteins present in viral preparation (eg. HLA, ABC, DR) Tray wells or Control may have been crossed Tray wells or Control may have been crossed 3. They show dark spots 1. Bacterial or fungal contamination of test sample. Positive control OK 1. Overdeveloped strips (stop reaction sooner). 3. They show dark spots 2. Precipitation of immune complexes in aged test sample. Positive control OK 1. Overdeveloped strips (stop reaction sooner). The problem is probably caused the tropper ty mergared. Positive control OK 1. Overdeveloped strips (stop reaction sooner). However, if dark spots develop sometime tater after initial opening of the strip tubes. 3. Bacterial or fungal contamination on strip due to improper storage. 1. Reagents such as Blotting Buffer and Working Conjugate Solution are not properfy prepared. 1. Wrong test sample contaminated 2. Strips flipped over during assay. 1. Serum not added. 1. This is not gp41 as gp41 is a diffuse babween assay steps. 4. Conjugate contaminated to improper typerated. 2. Noragles contaminated to improper typerated. 3. The stample for conjugate folution. 2. The stample for conjugate folution. 3. The stample reactive storage conditions at the user's site. 5. Rotary platform used to improper typerature exposure to storage UV light or reducing apent. 5. Test sample fore corocute substrate pH due to impore typeratu											step prid	
A. Electroitansiold interference during manufacturing. 4. Electroitansiold interference during manufacturing. H-9 proteins present in viral preparation (eg. HLA, ABC, DR) 3. Leglimate bands (deglycosylated envelope antigen) has been indentified at around 80-90kD in some test sample. Tray wells or Control may have been crossed contaminated. white spots in reactive control may have been crossed contaminated. White spots in reactive contaminated. White spots in reactive c					0						adding I	Blotting
 interference during manufacturing. interence during manufacturing. interend terference							Tray well	sor	white spots	s in reactive	Buffer.	
manufacturing. 3. Legitimate bands (deglycosylated envelope antigen) has been indentified at around 80-90kD in some test sample. been crossed contaminated. prevent any detection. 1. Bacterial or fungal contamination of test sample. Precipitation of immune complexes in aged test sample. Positive control OK 1. Overdeveloped strips (stop reaction sooner). 3. They show dark spots due to fungal contaminated. However, if dark spots develop sometime later after initial opening of the strip tubes. 9. Bacterial or fungal contamination on strip due to improper storage. Positive control OK 1. Overdeveloped strips (stop reaction sooner). 2. Incomplete washing. However, if dark spots develop sometime later after initial opening of the tube tube to pering of the tube storage conditions at the user's site. 4. Strips physically damaged cracked or scratched. 1. Wrong test sample dilution. 1. Serum not added. 1. This is not gp41 as gp41 is a diffuse voring assay. 2. Wrong conjugate dilution. 3. Intest sample diffuse to improper temperature exposure. 3. They show fark spots (stop reaction sooner). 1. This is not gp41 as gp41 is a diffuse voring deteriorated or denatured due to repeated freeze- to improper temperature exposure to storog UV light or reducing agent. 1. This is possibly a cell instead of Rocking platform. 2. They show seropsative results are 2. They show seropsative results are 6. Trays, reagent(s) or water having high phosphate concentration. 5. Rotamplatform. 5. Rotamplatform. <td></td> <td></td> <td></td> <td>preparation (eg</td> <td>g. HLA, ABC, DR)</td> <td></td> <td>Control n</td> <td>nay have</td> <td>, v</td> <td>•</td> <td></td> <td></td>				preparation (eg	g. HLA, ABC, DR)		Control n	nay have	, v	•		
(deglycosylidated envelope antigen) has been indentified at around 80-90kD in some test sample. contaminated. 3. Iney show dark spots due to fungal growth some test sample. 2. Precipitation of immune complexes in aged test sample. Positive control Weak Positive control OK 1. Overdeveloped strips (stop reaction sooner). However, if dark spots develop sometime later after initial opening of the strip tubes. 3. Bacterial or fungal contamination on strip due to improper storage. The problem is probably caused by the reagents. The problem is probably caused by test sample. I. Nerong test sample dilution. I. Serum not added. I. Serum not added. 2. Wrong conjugate follution. Unstable reagents due to improper temperature exposure to storag UV light or reducing agent. I. The is not gp41 as munne-complexed. S. Strips flipped over during assay. I. Wrong test sample igG deteriorated of featured deteriorated of featured thew or improper storage. I. This is possibly a cell incubation too long. 6. Incorrect substrate pH due to exposure to storag UV light or reducing agent having high phosphate concentration. S. Rotary platform. S. Test sample is HIV serongeative by Western Biot, the screening assay. S. Test sample reactive with non-viral proteins. S. Test sample reactive with non-viral proteins. S. Test sample reactive with non-viral proteins. S. This is possibly a cell incubation temperature greater than 30°C.			9	0								
1. Bacterial or fungal contamination of test sample. at around 80-90kD in some test samples. upon initial opening of the stip tubes. 2. Precipitation of immune complexes in aged test sample. Positive control Weak Positive control OK 1. Overdeveloped strips (stop reaction sooner). Upon initial opening of the stop sometime later after initial opening of the tube. 3. Bacterial or fungal contamination on strip due to improper storage. The problem is probably caused by test sample. The problem is probably caused by test sample. 2. Incomplete washing. 2. Incomplete washing. 2. Incomplete washing. 4. Strips physically damaged, cracked or scratched. 5. Strips on toproperly washed between assay steps. 1. Wrong test sample contaminated with human IgG. 1. Wrong test sample severely innune-complexed. 1. Serum not added. 1. Wrong test sample or tincubation too long. 5. Incorrect substrate pH due to exposure to storage UV light or reducing agent. 5. Incorrect substrate pH due to exposure to storage UV light or instead of Rocking platform. 3. The sis possibly a cell incubation too long. 3. This is possibly a cell incubation too long. 6. If sample is probably caused by test sample is not improper togenet to substrate pH due to exposure to storage UV light or reducing agent. 6. If sample is HIV servere storage. 3. This is possibly a cell incubation too long. 3. This is possibly a cell incubation too long. 6. Is ample is HIV seromegative by Westerm having high phosphate conc			3				contamir	ated.				
1. Decleration of urgal contamination of test sample. samples. samples. of the strip tubes. 2. Precipitation of immune complexes in aged test sample. Positive control OK 1. Overdeveloped strips (stop reaction sooner). develop sometime later after initial opening of the tube then the problem is due to improper strip storage conditions at the user's site. 3. Strips physically damaged, cracked or scratched. 1. Reagents such as Blotting Duffer and Working 1. Wrong test sample dilution. 1. Serum not added. 1. Serum not added. 2. Wrong conjugate dilution. 3. Test sample logG due to improper storage. 3. Test sample logG due to repeated freeze- to improper storage. 3. Test sample logG due to repeated freeze- thawnan ligG. 3. Incomplete washing. 3. The stops proper storage. 4. Conjugate contaminator with human logG. 5. Incorrect substrate pH due exposure 5. Reagent(s) or water having high phosphate contamination. 5. Test sample reactive with non-viral proteins. 3. The stample save HIV serropeative swith conjugate dilution.				0,								
Containination of test sample. Positive control weak Positive control OK 1. Overdeveloped strips (stop reaction sooner). However, if dark spots develop sometime later after initial opening of the tube then the problem is due to improper storage. 3. Bacterial or fungal contamination on strip due to improper storage. The problem is probably caused by the reagents. The problem is probably caused by test sample. 1. Overdeveloped strips (stop reaction sooner). 2. Incomplete washing. 5. Strips not properly washed between assay steps. 1. Reagents such as Blotting Suffer and Working 1. Wrong test sample dilution. 1. Serum not added. 2. Strips flipped over during assay. 3. Unstable reagents to improper temperature exposure to strong UV light or reducing agent. 3. Tact sample severely immune-complexed. 3. Toest sample or temperature exposure to storage. 3. Incomplete washing during assay. 1. Overdeveloped strips (stop reaction sooner). 1. Serum not added. 1. Wrong test sample or conjugate dilution. 2. Do not interpret assay. 5. Incorrect substrate pH due to reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 6. It sample is HIV seronegative by Westerm 6. It sample is are 1. Incubation temperature greater than 30°C. 1. Incubation temperatu		0			UKD IN SOME LESI				· ·			
 2. Precipitation of immune complexes in aged test sample. 3. Bacterial or fungal contamination on strip due to improper storage. 4. Strips spix-scally damaged, cracked or scratched. 5. Strips not properly washed between assay steps. 5. Strips not properly mashed between assay steps. 7. He agents such as Blotting at the contaminated with human IgG. 5. Incorrect substrate pH due to exposure to storage UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 7. Tray seagent(s) or water having high phosphate concentration. 7. Tray seagent(s) or water having high phosphate 7. Tray seagent(s) or water having h		n of test		Samples.								
complexes in aged test sample.In ortocorrol output (stop reaction soone).later after initial opening of the tube the thre problem is due to improper storage.3. Bacterial or fungal contamination on strip due to improper storage.The problem is probably caused by the reagents.The problem is probably caused by test sample.Inter after initial opening of the tube the thre problem is due to improper strip storage conditions at the user's site.1. Reagents such as Blotting batween assay steps.1. Wrong test sample contaminated with conjugate.1. Wrong test sample contaminated with conjugate.1. Serum not added.1. Serum not added.2. Unrong conjugate dilution. to improper temperature exposure.2. Test sample severely immune-complexed.1. Wrong test sample (JG due to improper temperature exposure.1. This is not gp41 as gp41 is a diffuse band.2. Conjugate contaminated with human IgG.1. Serum not added.1. Wrong test sample or assay.2. Do not interpret as conjugate dilution.5. Incorrect substrate pH due to or reducing agent.5. Rotary platform used instead of Rocking platform.5. Rotary platform. assays which gave HIV assays which gave HIV seropositive results are1. Test sample reactive with non-viral proteins.2. Test sample reactive with non-viral proteins.		ofimmune	Positive control weak	Positive control OK	1 Overde	veloped strips						
sample. 3. Bacterial or fungal contamination on strip due to improper storage. 4. Strips physically damaged, cracked or scratched. 5. Strips not properly washed between assay steps. 5. Strips not properly washed between assay steps. 5. Strips not properly mashed between assay steps. 5. Incorrect substrate pH due to exposure to storog UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 5. Test sample reactive with human lgG, 5. Incorrect substrate pH due to exposure to storog UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 5. Test sample reactive with human lgG, 5. Incorrect substrate pH due to exposure to storog UV light or reducing agent. 6. If sample is HIV serongative prover light phosphate concentration. 5. Test sample reactive with non-viral proteins. 5. Test sample reactive with non-viral proteins.									later after i	nitial		
3. Bacterial or fungal contamination on strip to improper storage. by the reagents. caused by test sample. 1. Wrong test sample. due to improper strip storage conditions at the user's site. 4. Strips physically damaged, cracked or scratched. 1. Reagents such as Blotting Buffer and Working 1. Wrong test sample dilution. 1. Serum not added. 1. Serum not added. 5. Strips not properly washed between assay steps. 2. Wrong conjugate dilution. 3. This is not gp41 as ummune-complexed. 3. Conjugate not added. 1. Wrong test sample or conjugate dilution. 1. Wrong test sample lgG 3. Unstable reagents due to improper temperature exposure. 4. Test sample lgG 3. This is possibly a cell incubation too long. 4. Conjugate contaminated with human lgG. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 5. Rotery platform used instab of properity weshed between assay, which gave HIV seronegative by Westerm having high phosphate concentration. 6. If sample is HIV seronegative by Western having high phosphate 6. If sample is HIV seronegative by Western having high phosphate 5. Test sample reactive with non-viral proteins. 5. Test sample reactive with non-viral proteins.		9	The problem is probably caus	ed The problem is probably	0 1	,						
contamination on strip due to improper storage. 1. Reagents such as Blotting Buffer and Working cracked or scratched. 1. Wrong test sample dilution. 1. Serum not added. 1. Serum not added. 1. This is not gp41 as gp41 is a diffuse band. 5. Strips not properly washed between assay steps. 1. Wrong conjugate dilution. 3. Test sample severely immune-complexed. 3. Test sample lgG deteriorated or denatured due to repeated freeze- thaw or improper storage. 1. Wrong test sample or conjugate dilution. 1. Wrong test sample or conjugate dilution. 2. Test sample contaminated with human lgG. 4. Test sample lgG deteriorated or denatured to improper storage. 1. Wrong test sample or conjugate dilution. 1. Wrong test sample or conjugate dilution. 2. Do not interpret as gp41. 5. Notary platform used or reducing agent. 5. Rotary platform used instead of Rocking platform. 3. Incubation temperature greater than 30°C. 3. Incubation temperature greater than 30°C. 3. Test sample reactive with non-viral proteins.		0				0						
1. Reagents such as Blotting Cracked or scratched. 1. Reagents such as Blotting Buffer and Working Conjugate Solution are not properly prepared. 1. Wrong test sample contaminated with conjugate. 1. Serum not added. 1. Strips flipped over during assay. 2. Strips not properly washed between assay steps. 1. Wrong conjugate dilution. 3. Test sample severely immune-complexed. 3. Test sample gG deteriorated or denatured deteriorated or denatured deteriorated or denatured deteriorated or denatured exposure. 1. Wrong test sample igG deteriorated or denatured deteriorated or denatured deteriorated or denatured due to repeated freeze- thaw or improper storage. 1. Wrong test sample value deteriorated or denatured deteriorated or denatured due to repeated freeze- instead of Rocking platform. 1. Wrong test sample value during assay. 2. Do not interpret as gp41. 3. Incomplete washing during agent. 5. Rotary platform used instead of Rocking platform. 3. Incomplete washing during assay. 3. Incomplete washing during assay. 3. Incomplete washing during assay. 3. Incomplete washing during assay. 6. Trays, reagent(s) or water having high phosphate concentration. Blot, the screening assays which gave HIV servopsuite results are 5. Test sample reactive with non-viral proteins. 5. Test sample reactive with non-viral proteins.			,									
 4. Strips physically darhaged, cracked or scratched. 5. Strips not properly washed between assay steps. 4. Conjugate dilution. 3. Unstable reagents due to improper temperature exposure. 4. Conjugate contaminated with human IgG. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 2. Test sample contaminated with human IgG. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 4. Substrate pH due to exposure to strong UV light or reducing agent. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 6. If sample is HIV servening 7. Test sample is HIV servening 8. If sample is HIV servening 9. Incubation temperature serve with non-viral proteins. 9. Incubation temperature serve with non-viral proteins. 		0	1. Reagents such as Blotting	1. Wrong test sample dil	lution.	1 Serum not	added		, U			
5. Strips not properly washed between assay steps. 5. Strips not properly washed between assay steps. 5. Strips not properly member between assay steps. 5. Unstable reagents due to improper temperature exposure. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. 5. Trays member between assay steps. 5. Strips not properly member thaving high phosphate concentration. 5. Strips not phote thaving high phosphat		, 0,	0		nated			าต				
between assay steps. 2. Wrong conjugate dilution. 3. Unstable reagents due to improper temperature exposure. 4. Conjugate contaminated with human IgG. 5. Incorrect substrate pH due to exposure to strong UV light or reducing agent. 6. Trays, reagent(s) or water having high phosphate concentration. between assay steps. 2. Wrong conjugate dilution. 3. Conjugate not added. 4. Substrate not added. 5. Conjugate dilution. 5. Test sample/reagent incubation temperature greater than 30°C. 5. Test sample reactive with non-viral proteins. 5. Test sample reactive with non-viral proteins.			, 0	, 0				5			01	
3. Unstable reagents due to improper temperature exposure.4. Test sample IgG deteriorated or denatured deteriorated or denatured2. Do not interpret as gp41.4. Conjugate contaminated with human IgG.due to repeated freeze- thaw or improper storage.2. Test sample/reagent incubation too long.3. This is possibly a cell line protein, p42.5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.5. Rotary platform used instead of Rocking platform.3. Incomplete washing during assay.1. Incubation temperature greater than 30°C.6. Trays, reagent(s) or water having high phosphate concentration.Blot, the screening assays which gave HIV seropositive results are5. Test sample reactive with non-viral proteins.					y	3. Conjugate	not added.			01	alliuse	
to improper temperature exposure.deteriorated or denaturedconjugate dilution.gp41.4. Conjugate contaminated with human IgG.due to repeated freeze- thaw or improper storage.2. Test sample/reagent incubation too long.3. This is possibly a cell line protein, p42.5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.5. Rotary platform used instead of Rocking platform.3. Incomplete washing during assay.line protein, p42.6. Trays, reagent(s) or water having high phosphate concentration.Blot, the screening assays which gave HIV seropositive results are5. Test sample reactive with non-viral proteins.on-viral proteins.						 Substrate r 	not added. 1	. Wrong test	sample or		erpret as	
exposure.due to repeated freeze- thaw or improper storage.2. Test sample/reagent incubation too long.3. This is possibly a cell line protein, p42.4. Conjugate contaminated with human IgG.5. Rotary platform used3. Incomplete washing during assay.1. Incubation too long.1. Ine protein, p42.5. Incorrect substrate pH due to exposure to strong UV light or reducing agent.instead of Rocking platform.4. Incubation temperature greater than 30°C.6. Trays, reagent(s) or water having high phosphate concentration.Blot, the screening assays which gave HIV5. Test sample reactive with non-viral proteins.			0		ured			conjugate di	ilution.			
 a. Solviggate containing the formation with the original starge. b. Name protein (p+2.) b. Incorrect substrate pH due to exposure to strong UV light or reducing agent. c. Trays, reagent(s) or water having high phosphate concentration. b. Trays results are c. Trays reagent (s) or water block (second strong starge). b. Trays reagent (second strong strong starge). c. Trays reagent (second strong strong strong starge). c. Trays reagent (second strong st				due to repeated freez	e-		2		•	3. This is po	ssibly a cell	
5. Inclusion used 5. Inclusion used during assay. 6. If sample is HIV 6. If sample is HIV 4. Incubation temperature greater than 30°C. 6. Trays, reagent(s) or water having high phosphate concentration. Blot, the screening serve sults are 5. Test sample reactive with non-viral proteins.					age.		0		0	line protei	in, p42.	
6. If sample is HIV 4. Incubation temperature greater than 30°C. 6. Trays, reagent(s) or water having high phosphate concentration. Blot, the screening assays which gave HIV serversults are			9	, i			3		0			
or reducing agent.seronegative by Westerngreater than 30°C.6. Trays, reagent(s) or waterBlot, the screening5. Test sample reactive with non-viral proteins.having high phosphate concentration.assays which gave HIV seropositive results arenon-viral proteins.				51	attorm.		4		·			
6. Trays, reagent(s) or water having high phosphate concentration. Blot, the screening assays which gave HIV seropositive results are 5. Test sample reactive with non-viral proteins.					tern							
having high phosphateassays which gave HIVnon-viral proteins.concentration.seropositive results are			0 0	0,			5	. Test sample	reactive with			
				, 0	IV			non-viral pro	oteins.			
7. Botary platform used instead actually false positive.			0 0 1 1	, 0								
			7. Rotary platform used instead	ad actually false positive								
of Rocking platform.			of Rocking platform.									