HIV 1&2 Western Blot

Western-Blot assay for the Detection of HIV Antibodies in Human Serum/Plasma

(Confirmatory Test for detection of antibodies to HIV-1 and indicative for HIV-2 antibodies)

1. INDICATIONS FOR USE

HIV 1&2 Western Blot is an in vitro qualitative immunoassay for the detection of antibodies to HIV-1 & HIV-2 in human serum / plasma. It is manufactured by J. Mitra & Co. Pvt. Ltd. based on lab research at CRI, Mumbai, under sponsorship from DBT (Department of Biotechnology) Govt. of India, New Delhi. It is intended to be used as a more specific & supplemental assay on samples found initially reactive using ELISA and other screening tests and is a confirmatory test for detection of antibodies to HIV-1 and indicative for HIV-2 antibodies.

2. INTRODUCTION

Human Immunodeficiency Virus (HIV) is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS). HIV infection is now recognised worldwide as a major public health problem. Hence screening methods play an important role in disease detection. The most common immunoassay used for the detection of antibodies to HIV-1 & HIV-2 are the Enzyme-Linked Immunosorbent Assay (ELISA), rapid tests and the Immunoblot or Western Blot assay which are easy to perform.

The Western Blot test can be used as a more specific and supplemental assay on human serum or plasma specimen found repeatedly reactive using ELISA. The HIV-1 viral antigens are separated by gel electrophoresis, electrically transferred to nitrocellulose membrane strip which is impregnated with a specific HIV-2 antigen band. Each strip also has an internal serum inbuilt quality control band. The serological events following HIV infection is represented graphically in fig. 1. In individuals infected with HIV, antigen appears first before anti-HIV but due to sero conversion, the antigen is lost and antibody develops within 1-2 months after infection and thereby the level of the antibody increases. However, p24 antibodies level decrease with time in advance stage of infection as shown in the graph (pink colour line). Hence, in advance stages of the infection, the p24 band on the HIV Western Blot strip may either be light or absent.

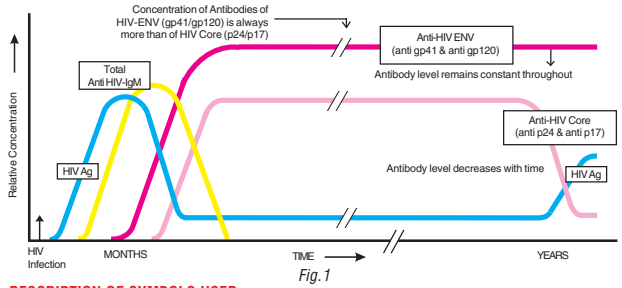


Fig.1

3. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard BS EN 15223-1:2012.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	8°C 2°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight

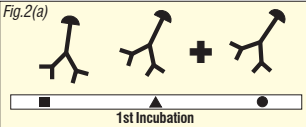
4. PRINCIPLE OF THE TEST

The HIV 1&2 Western Blot is manufactured from HIV-1 cell line. The HIV-1 viral antigen is purified and then separated by SDS gel electrophoresis. SDS denatures viral components and yields proteins which migrate in the gel according to their molecular weight to produce various bands. Low molecular weight components migrate faster and are found at the bottom of the gel, while high molecular weight proteins remain near the top. They are then transferred from SDS-PAGE gel on to nitrocellulose membrane which is also impregnated with HIV-2 antigen (gp 36) and a control band. The membrane is cut and packaged as strips. To perform the assay, the strip is incubated with the patient serum/plasma diluted in a buffer. Antibodies to HIV-1 & 2 if present, bind to viral antigens located on the strip. Unbound material is washed off and then the strip is incubated with anti-human IgG conjugated to alkaline phosphatase.

After washing the unbound conjugate, substrate (BCIP/NBT) is added which results in the staining of bands. If antibodies to HIV-1 antigens are present in the sera, any two ENVELOPE and more of the following bands will be seen : p17, p24, p31, gp41, p51/p55, p66, gp120 & gpl60.

If antibodies to HIV-2 antigen is present, HIV-2 band is also observed along with some of the other bands. If HIV specific antibodies are not present, the band pattern does not meet the required criteria.

Fig.2(a)

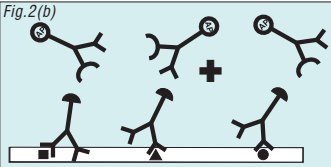


1st Incubation

A test strip loaded with HIV antigens is incubated for 1 hour / overnight with diluted serum or plasma.

Wash 3 times for 5 minutes each.

Fig.2(b)

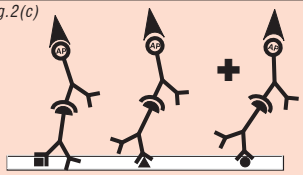


2nd Incubation

Alkaline phosphatase conjugated anti-human IgG is added and is Incubated for 1 hour.

Wash 4 times for 5 minutes each.

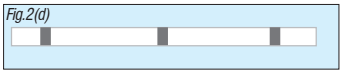
Fig.2(c)



Colour Reaction

Substrate solution (BCIP+NBT) is added and incubated for 0.5-15 minutes. Insoluble coloured bands develop at the sites on the test strip occupied by antibodies. Strips are washed with distilled water.

Fig.2(d)



Read Results

5. ADVANTAGES OF HIV 1&2 WESTERN BLOT ASSAY

- Separate disposable trays with covers are provided so that each test can be run individually without any cross contamination.
- Since one disposable tray is provided for one test therefore there is no need to store the infectious tray after performing the test.
- Easy to run and available in convenient packsize: 5 Test Pack and 25 Test Pack.
- Easy & clear interpretation through easy-to-read bands.
- No special equipment is required.
- Internal inbuilt control line is incorporated for the validity of the test.
- No weighing of blotting powder is required.
- Substrate is ready to use.

6. KIT PRESENTATION

- 5 Test Pack
- 25 Test Pack

7. MATERIALS PROVIDED

	5 Tests	25 Tests
HIV Test Strips Strips blotted with HIV-1 Viral Lysate and specific HIV-2 antigen & Anti-human IgG as control line.	5 Nos.	25 Nos.
Wash Buffer Concentrate (20x)	10 ml. (1 Vial)	50 ml. (1 Vial)
Diluent Buffer Concentrate (10x)	3 ml. (1 Vial)	15 ml. (1 Vial)
Blotting Powder	0.5 gm. 5 Nos.	0.5 gm. 25 Nos.
Enzyme Conjugate Concentrate (100x) Rabbit Anti-Human IgG conjugated with alkaline phosphatase.	0.15 ml. (1 Vial)	0.15 ml (1 Vial)
Substrate (Ready to use) BCIP + NBT (5-Bromo, 4-chloro, 3-indolyl phosphate+Nitroblue tetrazolium)	12 ml. (1 Vial)	60 ml. (1 Vial)
Control – Normal human serum containing sodium azide as preservative. Non reactive for HIV-1 & HIV-2, HCV and HBsAg.	0.1 ml. (1 Vial)	0.1 ml. (1 Vial)
Control + Inactivated human serum with antibodies to HIV-1. Non reactive for HBsAg and HCV.	0.1 ml. (1 Vial)	0.1 ml. (1 Vial)
Incubation Trays One tray with lid cover for one strip	5 Trays	25 Trays
Forcep	1 No.	1 No.
Measuring Spoon for bloting powder	1 No.	1 No.
Band Monitor Scale	1 No.	1 No.
Instruction Manual	1 No.	1 No.

8. STORAGE OF THE KIT

(i) Store the kit at 2-8°C in the driest and darkest area available. Do not freeze kit components.

(ii) Do not use the kit beyond the expiry date mentioned on it.

(iii) Remove required number of strips from the container quickly and replace the rest with desiccant intact.

(iv) Do not expose substrate to light.

(v) Do not store the opened Blotting Powder Pouch. Discard the pouch after use as sufficient Blotting Powder is provided with the kit i.e., one pouch/strip can be used.

9. ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

(i) Rotary Shaker (60-70 r.p.m.)
(ii) Pipette and tips
(iii) Timer
(iv) Vortex mixer/Magnetic stirrer
(v) Aspirator with Sodium Hypochlorite/suitable disinfectant

10. SPECIMEN COLLECTION, PREPARATION & STORAGE

Serum/ Plasma samples shall be used with this test. Collect blood in a clean dry sterlized vial and allow it to clot. Separate the serum by centrifugation at room temperature. It is recommended that FRESH samples should be used. If serum is not to be assayed immediately, it should be stored at 2-8°C or frozen at -20°C. Serum may be stored at 2-8°C for upto 3 days and stored frozen at -20°C for 3 months. Bring specimen (serum/plasma) to room temperature (20-30°C) and ***mix each specimen thoroughly prior to use***. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. Controls and other reagents contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Center for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. All materials used in the assay and samples should be disposed off in the manner that will inactivate virus.

12. PRECAUTIONS

Handle all patient samples, positive and negative controls as potentially infectious agents.

(i) **Optimal assay performance requires strict adherence to the assay procedure described in the insert.**
(ii) Bring all kit components and samples to room temperature before use.
(iii) Do not use kit components beyond the expiration date, which is printed on the kit.
(iv) Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
(v) Due to interchange of caps of the vials, the reagents may get contaminated. Care should be taken while handling the reagent caps to avoid cross contamination of the reagents.
(vi) Avoid microbial and cross contamination of reagents.
(vii) Discard the incubation tray after each use.
(viii) Use only the controls provided with the kit.
(ix) Use only deionized/distilled water to dilute wash buffer and diluent buffer concentrate in order to get correct results.
(x) After addition of substrate, the strips must be monitored closely and continuously.
(xi) All aspirated fluid (serum/plasma/wash buffer etc.) should be discarded in a container with sodium/calcium hypochlorite solution.
(xii) One blotting powder pouch should not be used for more than 2 tests.
(xiii) The working Wash Buffer and working Diluent Buffer prepared during assay should be used within 24 hours when stored at 2-8°C.
(xiv) The cap of the plastic container should be tightly closed after removing the required no. of stips.
(xv) Unused strips should be stored at 2-8°C, with dessicant in plastic container and seal the plastic container in an aluminium pouch with clamp & rod. Stips are stable for 90 days at 2-8°C from the date of opening of sealed pouch, when stored with desicant along with clamp & rod.
(xvi) ***Always keep the substrate bottle tightly closed.***

13. PREPARATION OF THE REAGENTS

Bring all reagents to room temperature (20-30°C) before use. Prepare the following reagents just before starting assay procedure and use within 24 hrs.

a. Preparation of Working Wash Buffer: For each strip 20ml working wash buffer is required. Dilute 1ml, wash buffer concentrate (20X) to 20ml with distilled water and mix well. (Mix 1ml. of Wash Buffer Concentrate (20x) with 19ml. of deionized/ distilled water)

b. Preparation of Sample and Conjugate Diluent Buffer: The working diluent buffer is used at sample and conjugate dilution step only. Please refer Table 1 given below for amount of reagent to be prepared for different number of strips. For the preparation of the working diluent buffer, take the required volume of diluent buffer concentrate (10X) and add required volume of distilled water to this concentrate. Then add required amount of blotting powder using the measuring spoon only given in the kit. Mix the working diluent buffer properly before use.

c. Preparation of working conjugate: ***It should be prepared fresh just before use***. Dilute enzyme conjugate 1:100 with working diluent buffer. e.g. add 20µl of conjugate (100x) to 2ml of working diluent buffer.

d. Substrate Solution: Substrate solution is ready to use. Pipette required volume directly from bottle using a clean pipette and cap tightly after use.

14. AMOUNT OF REAGENTS REQUIRED FOR DIFFERENT NUMBER OF TESTS TO BE RUN AT ONE TIME

TABLE-1

No.of Strips	Working Wash Buffer							Working Diluent Buffer								Working Conjugate		Substrate ml.
	Wash Buffer Conc. (20x)	+	Distilled Water ml.	=	Total vol. ml.			Diluent Buffer (10x)	Conc.+	Distilled Water ml.	=	Total vol. ml.		No.of Spoons of Blotting Powder		Working Diluent Buffer (ml)	100x Conjugate (µl)	
1	1	+	19	=	20			0.5	+	4.5	=	5		2		2	20	2
2	2	+	38	=	40			0.9	+	8.1	=	9		3		4	40	4
3	3	+	57	=	60			1.5	+	13.5	=	15		5		6	60	6
4	4	+	76	=	80			1.8	+	16.2	=	18		6		8	80	8
5	5	+	95	=	100			2.1	+	18.9	=	21		7		10	100	10
10	10	+	190	=	200			4.2	+	37.8	=	42		14		20	200	20
15	15	+	285	=	300			6.3	+	56.7	=	63		21		30	300	30
20	20	+	380	=	400			8.4	+	75.6	=	84		28		40	400	40
25	25	+	475	=	500			10.5	+	94.5	=	105		35		50	500	50

15. PROCEDURAL NOTES

(i) ***Bring the test kit to room temperature (20-30°C) before use.***
(ii) Strictly follow the protocol given in the Instruction manual.
(iii) Using forceps carefully remove required number of strips, place into individual trays and note down the numbers printed on the strip.
(iv) Ensure that the numbered side of the strips are facing up.
(v) Proper and gentle shaking (60-70 rpm) of the trays is extremely important. If shaking is not proper, the sensitivity of the test may get affected.
(vi) Add reagents and samples to ends of the trays and not directly onto strips.
(vii) While running the assay :
  (a) For rapid method : Take out kit and kit components from 2-8°C on the same day.
  (b) For Over night method : Take out all components from 2-8°C except conjugate and substrate on the same day.
(viii) Use separate tips for all reagents including serum/plasma.
(ix) The wash buffer conc. (20x) may crystalize or turn cloudy at 2-8°C. Allow it to reach room temperature and mix thoroughly and then dilute with deionized water to prepare working wash buffer.
(x) Aspirate the solution from wells completely into the discarding container containing hypochlorite/ suitable disinfectant prior to next step of reagent addition.
(xi) The time required for development of bands in substrate depends on the sample. For e.g.. the strong positive sample shows up all the bands within 0.5 minutes whereas the weak positives may require almost 15 minutes. Therefore, stopping the reaction of the strips is entirely on the technician's judgement. If the strip reacted with a strong serum is left in substrate for 15 minutes, a very dark background may develop making it difficult to read the result. Such over exposed strip with complete dark background will make the bands invisible. On the other hand, if a strip reacted with a weak serum is taken out of the substrate within two minutes, the results may be erroneously interpreted as negative/indeterminate. Hence a careful decision has to be made while performing the last step. However none of the strips should be left in the substrate for more than 15 minutes.

16.A ASSAY PROCEDURE-RAPID ASSAY

NOTE : Bring the test kit and sample to Room Temperature (20-30°C) before use and all incubations are to be carried out on a Rotary shaker (60-70 r.p.m.) at Room Temperature.

(i) Remove required number of strips and trays from the kit. Place one strip in each tray with numbered side up. Note down the strip number with respect to samples & control on the worksheet for correct identification. Always include strips for positive and negative controls with each run.
(ii) Prepare working wash buffer according to the number of tests to be run.
(iii) Add 2ml of working wash buffer to each tray and incubate the strips for at least 5 minutes at room temperature. Remove buffer by aspiration.
(iv) Prepare working diluent buffer according to the no. of tests to be run.
(v) Add 2ml of working diluent buffer to each tray, add 20µl of patient sera and controls to appropriate wells.
(vi) Cover trays and incubate for 1 hr. at room temperature (25-30°C) on a Rotary Shaker. Take care to mark the cover also, to prevent interchange of covers which may lead to cross contamination.
(vii) Carefully remove covers, aspirate solution completely from tray and discard into sodium/calcium hypochlorite solution or any suitable disinfectant.
(viii) Wash each strip with 2ml working wash buffer **3 times** for 5 minutes each with shaking.
(ix) Prepare working conjugate solution according to the number of tests to be run.
(x) Add 2 ml. of working conjugate solution to each tray. Cover tray with corresponding cover and incubate on Rotary Shaker for 1 hour. Never interchange the cover of trays to avoid contamination.
(xi) Aspirate conjugate, wash each strip with 2 ml working wash buffer **4 times** for 5 minutes each with shaking. Aspirate wash solution completely from the tray at the end of the last washing.
(xii) Add 2 ml. substrate solution to each tray, cover tray and incubate for 0.5-15 minutes away from the light preferably in dark till bands develop. Make a careful decision to decide the time of incubation from 0.5 min. to 15 min.
(xiii) Continue to observe the reaction till gp160/gp120/gp41 appear and stop the reaction after their appearances so as to avoid excessive background making the observation difficult.
  However, in case the above bands do not appear, then continue the reaction upto the point
  (a) before strong background is formed on the strip
  (b) upto 15 minutes, whichever is earlier.
(xiv) Aspirate substrate, add distilled water and wash strips to stop the reaction. Remove the strips on paper towels and mount on worksheet keeping numbered side up. Observe band pattern and grade the results. For storage keep strips in dark.

16.B. ASSAY PROCEDURE-OVERNIGHT ASSAY

NOTE : Bring the test kit and sample to Room Temperature (20-30°C) before use and all incubations are to be carried out on a Rotary Shaker (60-70 r.p.m.) at Room Temperature.

Overnight assay procedure reduces number of indeterminate interpretations for detection of weak and early sero-conversion sample, as more time is available for antigen-antibody reaction.

(i) Remove required number of strips and trays from the kit. Place One strip in each tray with number side up. Note down the strip number with respect to samples and control on the work sheet for correct Identification. Always include strips for positive and negative controls with each run.

(ii) Prepare working wash buffer according to the number of tests to be run.

(iii) Add 2ml of working wash buffer to each tray and incubate the strips for at least 5 minutes at room temperature. Remove buffer by aspiration.

(iv) Prepare working diluent buffer according to the no. of tests to be run.

(v) Add 2ml of working diluent buffer to each tray, add 20μl of patient sera and controls to respective trays.

(vi) Cover trays and incubate for overnight (16-20 hrs), at room temperature (25-30^{o}C) on a Rotary Shaker. Take care to mark the cover also to prevent interchange of covers which may lead to cross contamination.

(vii) Follow step 16.A. (vii) to step (xiv) as mentioned above in **RAPID ASSAY PROCEDURE**.

16.C. QUICK REFERENCE PROTOCOL

The Western blot assay protocol is summarised in the flow chart below:

Strip + 2ml working wash buffer per strip

⇩

Incubate for 5 min. on rotary shaker & Aspirate wash buffer

⇩

Strip+2ml of working diluent buffer per strip + 20μl serum/plasma per strip

⇩

Incubate for 1 hr./ Overnight (16-20 hrs.) on rotary shaker.

⇩

Wash 3 times with 2 ml working wash buffer per strip for 5 minutes each on rotary shaker.

⇩

Add 2 ml of working conjugate per strip.

⇩

Incubate for 1 hour on rotary shaker.

⇩

Wash 4 times with 2 ml working wash buffer per strip for 5 minutes each on rotary shaker.

⇩

Add 2ml ready to use substrate per strip.
Incubate for 0.5-15 min.

⇩

Wash the strip with distilled water & ***Read Results***

17. QUALITY CONTROL WITH EVERY RUN

Use of negative and positive controls is recommended with every run irrespective of the number of samples being tested. For the results to be considered valid, the following conditions must be met.

1. Negative control

No HIV-1 & HIV-2 specific bands should be observed on the negative control strips. Only the band for serum control should be visible (Fig. 3-b).

2. Positive control

Almost all the virus specific bands at positions gp160, gp120, p66, p55/51, gp41, p31, p24 & p17 specific band should be visible along with the serum control band as seen in (Fig. 3-a) (can be used as a guide for relative positioning of the bands).

18A. READING & INTERPRETATION OF RESULTS

The presence or absence of antibodies to HIV-1, in a serum sample is determined by comparing each strip with the negative and positive control strips. The description of the various bands is given below:

Description of bands observed on strip reacted with positive control

TABLE-2

Molecular Wt. (kDa.)	Gene	Antigen	Description
gp160	ENV	Polymeric form of gp 41	Broad diffused band
gp120	ENV	Outer membrane	
p 66	POL	Reverse Transcriptase	Discreet band
p 55	GAG	Precursor protein	Fused
p 51	POL	Reverse Transcriptase	Spread band/Single band
gp 41	ENV	Transmembrane	Appears as 2-3 different bands/diffused band
p 31	POL	Endonuclease	Single band
p 24	GAG	Core Protein	Broad Band
p 17	GAG	Core Protein	Broad Band

18B. HOW TO INTERPRET

(a) Align your strip with serum control band keeping the numbered end of the strip at the bottom shown in the figure. Validate that the serum control band is visible.
If the control band is not seen, the results should be considered **invalid** as this indicates technical error like not adding serum, conjugate or substrate. It could also be due to strip/kit deterioration not maintaining cold chain i.e. 2-8^{o}C temperature.

(b) Start reading the strip above control band form high molecular weight bands downward. Locate gp160/gp120/gp41/p24 and other bands.

(c) Align your developed strip with band monitor scale provided in the kit by matching the Control Band on the scale with Control Band on the developed strip. This scale is intended as an aid for identification of protein bands. **Protein bands are usually at a proportional distance similar but not necessarily exact to that on the scale**.

(d) Note down the bands visible on your strip in **HIV 1&2 Western Blot** report sheet. Interpret the results as per Table 3.

(e) Sometimes green colour tinge may be observed at p24 & p51/55 band with strong positive samples or when strip is overexposed for longer period after addition of substrate. However this does not interfere/affect the accuracy of results.

(f) HIV-2 indicated band alongwith serum control band should be observed on the strip with HIV-2 sample as shown in in (Fig. 3-c)

(g) The results should be interpreted as positive, indeterminate, negative or invalid.

TABLE-3

INTERPRETATION	PATTERN
POSITIVE : HIV-1 POSITIVE	a). 2 ENV (either of 2 ENV; gp160, gp41, gp120) + 1GAG (p17, p24, p55) or 1POL (p31, p51, p66)
HIV-1 POSITIVE with HIV-2 INDICATED	b). 2 ENV (either of 2 ENV; gp160, gp41, gp120) + 1GAG (p17, p24, p55) or 1 POL (p31, p51, p66) + HIV-2 Band
HIV-1 NEGATIVE with HIV-2 Indicated	Only Control band + HIV-2 BAND
INDETERMINATE	a). 1 ENV (either of 1 ENV; gp160, gp41, gp120) ± 1GAG (p17, p24) ± 1POL (p31)
Viral Specific bands present but pattern does not meet the criteria for POSITIVE	b). GAG (p17, p24) ± POL (p31) c). Only GAG (p17, p24) d). Only POL (p31)
INDETERMINATE with HIV-2 Indicated	Viral Specific bands present but pattern does not meet the criteria for POSITIVE + HIV-2 BAND
NEGATIVE	Only control band **or** control band with p51/ 55/ p66 band
INVALID	No Control band

Important Note:

Please carefully read the ESSENTIAL POINTS TO CONSIDER FOR INTERPRETATION before declaring the results.

18C WHEN TO REPEAT THE TEST

(i) If the sample shows indeterminate results with 1 hour procedure. Repeat the test with overnight procedure.

(ii) Overexposed test strips.
a. Dark colour background.
b. Greenish tinge on the bands as p24, p55/51 & p66 due to overexposure of strips to substrate buffer. Repeat the test avoiding the over exposure.

18D ESSENTIAL POINTS TO CONSIDER FOR INTERPRETATION

1. The presence of gp160/gp120+p24 is a strong indication for sero conversion (Interpretative Western Blot criteria for HIV-WHO, 1999).
2. Some serum samples (due to their specific characteristic) may result **INDETERMINATE** using 1 hour testing procedure. Such samples must be retested on overnight procedure to know the exact status of the samples.
3. There is also a high molecular weight nonspecific band above 160 KD that is presumed to be a GAG-POL precursor protein. This is seen with some high titered HIV-1/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.
4. Sometimes, reactivity of sera to bands that do not correspond to HIV-I antigen may occur. This is due to autoantibodies cross reactive with cellular proteins in the molecular weight range of 70K, 51-55K, 43K (i.e. HLA, actin, myosin) found in the mammalian host cells used to propagate the HIV-1 virus.
5. Most positive sera will react to most of its protein bands, but at early and late stage of the infection, an HIV infected person may lose reactivity to one or more of the bands.
6. Low risk individuals may have **indeterminate** reactions on the blot in the regions corresponding to p24 & p55.
7. Persons immediately after sero-conversion may display incomplete band patterns but when followed for a period of two to six months, evolve a complete band pattern.
8. Antibodies to p24 are in excess during the early asymptomatic stage. The onset and progression of the disease is preceded by marked decrease in antibody.
9. Antibodies to p31 could be low due to poor immunogenicity of this antigen.
10. Some specimens might continue to show absence of gp41 band because antibodies to gp41 may react with gp120 or gp160 instead of their own corresponding antigen. gp41 also undergoes polymerisation, therefore, antibodies to these multimeric forms may not recognise the monomeric form i.e. gp41. Thus the presence or absence of any band in a typical patient depends upon the stage of infection, the titre of individual antibodies and their intrinsic properties of polymerisation.

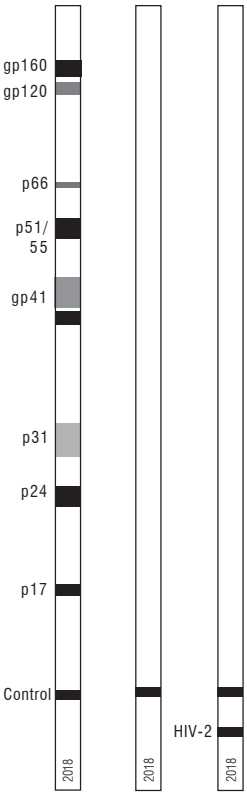


Fig. (3-a) **HIV POSITIVE** Fig. (3-b) **HIV NEGATIVE** Fig. (3-c) **HIV-2 POSITIVE**

11. Infected patients with malignancies and patients receiving immunosuppressive drugs may fail to develop a positive reaction.
12. p24 antibodies level decrease with time in advance stage of infection as shown in the graph (pink colour line) in fig. 1. Hence in advance stages of the infection, the p24 band on the HIV Western Blot strip may either be light or absent.
13. The cross reactivity of HIV-2 is variable but typically shows reactivity with GAG and/or POL antigens. However the cross reactivity with envelope bands is rare.
14. The HIV-2 band on the strip is just an indicative band and its intensity will be less than serum control band.
15. Since reactivity of any degree with any of the virus-specific proteins identified on the strip is presumptive evidence of antibodies to HIV-1, any such results **(Indeterminate)** must be taken as suspicious and should trigger **Repeat** testing and **Follow-up** testing. The corrective evaluation in such situations must be based on subsequent blot testing and clinical evaluation.

19. LIMITATIONS OF THE PROCEDURE

The test must be performed in strict adherence to the assay procedure and instructions to obtain reproducible results.

1. Although a blot positive for antibodies to HIV-1 indicates infection with the virus but diagnosis of AIDS can only be made clinically if a person meets the case definition of AIDS established by the World Health Organisation or other relevant authorities.
2. It is recommended that all indeterminate blots be repeated using original specimen and sequential samples. Blood donors with an indeterminate blot should be retested using a fresh specimen after two to six months.
3. It is also known that antibodies to p24 and p31 decrease during the course of AIDS, leading to a shift in blot interpretation from positive to indeterminate. Indetermination of results should then be based on subsequent blot testing and clinical evaluations in such situations.
4. **gp41:** Antibodies to gp41 may appear on the strip as (a) single band or may appear as (b) two separate bands or may appear as (c) three separate bands, depending upon antibody concentration in the sample. This is because gp41 antibodies are having tendency to aggregate.
5. Due to its highly specific nature, non-reactivity of samples with HIV-2 specific antigen 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 a HIV-2 Western Blot Kit.
6. HIV-1 & HIV-2 viruses share, many morphological and biological characteristics. It is likely that due to this, their antibodies have a cross reactivity of 30-70%

20. PERFORMANCE CHARACTERISTICS

Several known (ELISA positive sera /plasma samples as well as sera from known control subjects) and unknown sera/body fluids were tested on different batches of strips.

A total of 476 samples from different hospitals at Mumbai were tested initially at the Virology Laboratory, Cancer Research Institute, Mumbai. These samples included mainly sera but also included milk from HIV positive mothers, CSF from HIV positive children with neurological complications as well as sera from healthy volunteers. However, finally 250 coded sera originating from five different national institutes (National Institute of Communicable Diseases, New Delhi; PGIMER, Chandigarh; Christian Medical College, Vellore; National AIDS Research Institute, Pune and National Institute of Cholera and Enteric Diseases, Calcutta) were sent by Dept of Biotechnology (DBT), Govt. of India, New Delhi.

Results were compared with those obtained by the source institutes and the sensitivity & specificity were evaluated. The results showed **100% sensitivity & 100% specificity.** This compares excellently with the major commercially available kit performances.

The specificity of HIV 1&2 Western Blot was also checked with normal sera and sera with other viral infections. The results showed 100% Specificity.

Specificity of HIV 1& 2 Western Blot with sera of normal donor & sera with other viral infections

TABLE-4

Type of serum samples*	Number	HIV 1&2 Wester Blot		
		Positive	Indeterminate	Negative
Normal Donors	200	0	0	200
Rheumatoid Arthritis (RA)	1	0	0	1
Antistreptolysin 'O' (ASO)	1	0	0	1
C-reactive protein (CRP)	1	0	0	1
Syphilis	3	0	0	3
Mycobacterium tuberculosis**	25	0	0	25
Herpes-simplex	1	0	0	1
Cytomegalo virus (CMV)	7	0	0	7
Rubella	2	0	0	2
Hepatitis B Virus	4	0	0	4
Non-Hodgkin's Lymphoma (NHL)	7	0	0	7
Hodgkin's Lymphoma (HL)	2	0	0	2
Chronic Myeloid Leukemia (CML)	4	0	0	4
Acute Myeloid Leukemia (AML)	3	0	0	3
Multiple Myeloma	1	0	0	1

* Tested in Cancer Research Institute, Mumbai

** p55 band was detected in 12 tuberculosis serum samples.

2. The performance of HIV 1&2 Western Blot was also evaluated in clinical studies and compared to one licenced Western Blot test. The results are as follows:

No. of Samples	ELISA		HIV 1&2 Western Blot			Licenced Western Blot		
	+ve	-ve	+ve	Id*	-ve	+ve	Id*	-ve
92	88	4	75	17	-	74	18	-
88	-	88	-	2	86	-	2	86

*Id : Indeterminate

The **HIV 1&2 Western Blot** was positive in 74/74 of cases identified as positive by Licenced Western Blot test, demonstrating 100% sensitivity. The **HIV 1&2 Western Blot** also showed 100% specificity when compared with Licenced Western Blot test.

21. TROUBLE SHOOTING CHART

TROUBLE	POSSIBLE REASONS	POSSIBLE REMED/ SOLUTION
1. Dark Spots develop on strips.	1. Contamination of test samples by bacteria. 2. Immune complexes in aged test sample are precipitating out.	Use fresh sample
2. Bands do not develop or show up weak.	1. Positive control may have degraded.	1. Test positive control by ELISA. O.D. should be above 1.0
	2. Conjugate may have degraded or improper conjugate dilution.	2. Check the conjugate & prepare the dilution carefully
	3. Regents may not have mixed correctly.	3. Mix reagents thoroughly before use.
	4. Substrate may have oxidised.	4. Substrate bottle cap should be kept tightly closed.
	5. Sample may be too diluted/inactivated/ spoiled/hemolysed.	5. Use fresh sample without dilution.
	6. Test sample may have been ELISA "False" positive.	6. Repeat ELISA
3. Non-specific bands develop and not HIV-2 indicative	1. Sample has antibodies which cross-react with non-specific proteins on the strip 2. Sample cross reacts with other cellular proteins present in viral preparation.	See ESSENTIAL POINTS TO CONSIDER FOR INTERPRETATION.
4. Strong background develops on strip in the presence or absence of positive bands.	1. Overdeveloped strips. 2. Incomplete washing.	1. Stop reaction sooner. 2. Wash thoroughly.
5. Absence of serum control band	1. Serum or conjugate or substrate not added.	Test should be considered invalid and repeated taking all precautions.
	2. Strips flipped over during assay.	
6. Bands other than the serum control band develop with negative control.	Tray wells or assay reagents may have been cross contaminated.	1. Change tips for every addition/ aspiration to avoid cross contamination. 2. Mark tray covers as well to avoid any mix up.

22. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly 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's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind whatsoever for an amount greater than the purchase price of the goods in respect of which damages may be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

23. REFERENCES

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24. HIV 1&2 WESTERN BLOT REPORT SHEET

Kit Lot No: _____ Technician: _____ Source: _____

TABLE-5

Run Date	Sample No.	Core Antigens (GAG)			Endonuclease Polymerase Antigens (POL)			Envelope Antigens (ENV)			Serum Control Band	HIV-2 Specific Band	Comments
		p17	p24	p55	p31	p51	p66	gp41	gp120	gp160			

This is a suggested specimen of the HIV 1&2 Western Blot REPORT SHEET which the user can prepare on their own record sheets.

VER-01 R-04

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