BHAT BIO-TECH INDIA (P) LTD.

CE 1023

PAREEKSHAK®

HIV - 1/2 ELISA

An ELISA for the detection of antibodies to HIV - 1 and HIV - 2 in Human Serum / Plasma
HIV-1 Antigens for gp 120, gp41
HIV-2 Antigen for gp36

For Professional Use IVD

READ THIS PACK INSERT
CAREFULLY BEFORE PERFORMING THE TEST

CATALOGUE No.: PEL

INTENDED USE : The Pareekshak HIV ½ ELISA kit is a sloid phase immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum or plasma.

INTRODUCTION : The epidemiological evidence indicates that an infectious agent transmitted through intimate contact, intravenous drug use, or use of infected blood or blood products leads to Acquired Immunodeficiency Syndrome (AIDS). This disease affects T-Cell mediated Immunity, resulting in severe lymphopenia and a reduced subpopulation of helper T- lymphocytes. Destruction of this T-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient response to subsequent infections. Consequently, infections become more severe and may cause death.

The etiological agent has been identified as a reterovirus, Human Immunodeficiency virus type-1 (HIV-1). A closely related, but distinct second type of immunodeficiency virus, designated HIV-2, has been isolated and causes a disease that is indistinguishable from AIDS. Serological cross- reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample. This variability necessitates the inclusion of antigens to both HIV-1 and HIV-2 for the detection of antibodies to HIV-1 and HIV-2.

The HIV genome has outer structural (env-gp120,gp41), inner structural (gag p17, p24, p7,p6), pol-viral enzymes (Protease, reverse transcriptase, integrase) and regulatory proteins (Tat, Rev, Vif, Vpu, Vpr, Nef) and long terminal repeats on either end.

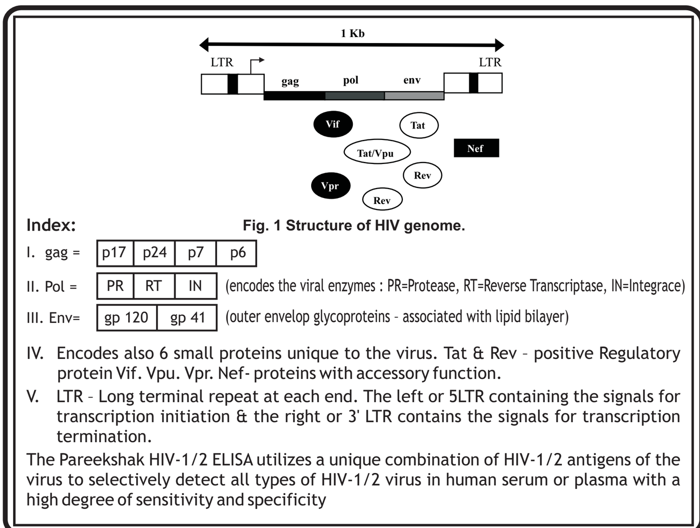


Fig.1 Structure of HIV genome.

Index:

I. gag = p17 p24 p7 p6

II. Pol = PR RT IN (encodes the viral enzymes : PR-Protease, RT-Reverse Transcriptase, IN-Integrace)

III. Env= gp 120 gp 41 (outer envelop glycoproteins - associated with lipid bilayer)

IV. Encodes also 6 small proteins unique to the virus, Tat & Rev - positive Regulatory protein Vif, Vpu, Vpr, Nef- proteins with accessory function.

V. LTR - Long terminal repeat at each end. The left or 5LTR containing the signals for transcription initiation & the right or 3' LTR contains the signals for transcription termination.

The Pareekshak HIV-1/2 ELISA utilizes a unique combination of HIV-1/2 antigens of the virus to selectively detect all types of HIV-1/2 virus in human serum or plasma with a high degree of sensitivity and specificity.

Test for the antibodies to HIV was proved to be highly valuable in diagnosis and study of the infection. The antibodies produced against HIV proteins are found in infected people and are useful tools for the diagnosis of HIV in blood as shown in Fig.2

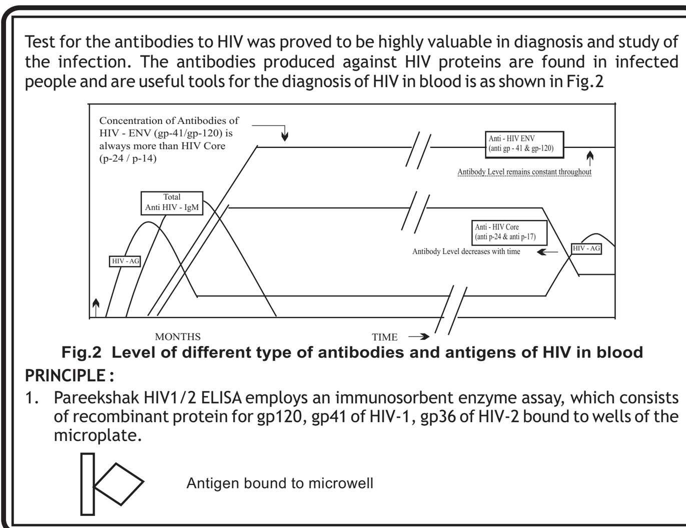


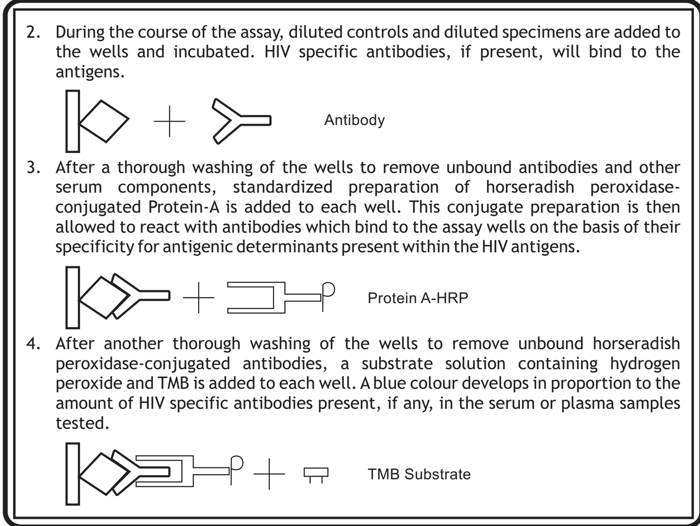
Fig.2 Level of different type of antibodies and antigens of HIV in blood

PRINCIPLE :

1. Pareekshak HIV1/2 ELISA employs an immunosorbent assay, which consists of recombinant protein for gp120, gp41 of HIV-1, gp36 of HIV-2 bound to wells of the microplate.

Antigen bound to microwell

2. During the course of the assay, diluted controls and diluted specimens are added to the wells and incubated. HIV specific antibodies, if present, will bind to the antigens.



Antibody

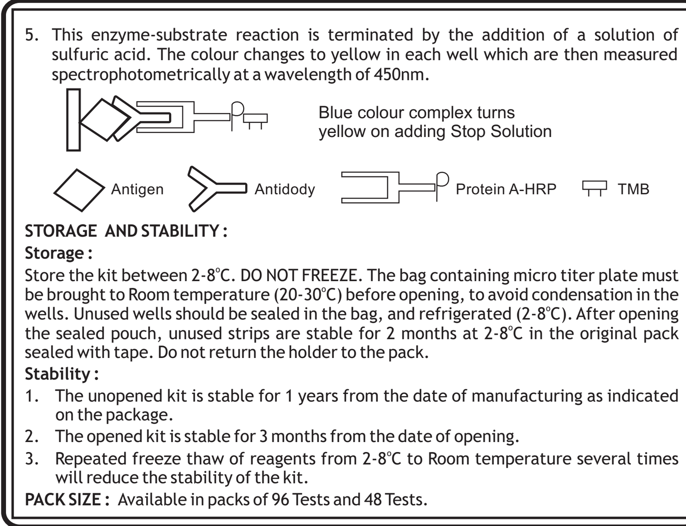
3. After a thorough washing of the wells to remove unbound antibodies and other serum components, standardized preparation of horseradish peroxidase-conjugated Protein-A is added to each well. This conjugate preparation is then allowed to react with antibodies which bind to the assay wells on the basis of their specificity for antigenic determinants present within the HIV antigens.

Protein A-HRP

4. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibodies, a substrate solution containing hydrogen peroxide and TMB is added to each well. A blue colour develops in proportion to the amount of HIV specific antibodies present, if any, in the serum or plasma samples tested.

TMB Substrate

5. This enzyme-substrate reaction is terminated by the addition of a solution of sulfuric acid. The colour changes to yellow in each well which are then measured spectrophotometrically at a wavelength of 450nm.



Blue colour complex turns yellow on adding Stop Solution

Antigen Antibody Protein A-HRP TMB

STORAGE AND STABILITY :

Storage :

Store the kit between 2-8°C. DO NOT FREEZE. The bag containing micro titer plate must be brought to Room temperature (20-30°C) before opening, to avoid condensation in the wells. Unused wells should be sealed in the bag, and refrigerated (2-8°C). After opening the sealed pouch, unused strips are stable for 2 months at 2-8°C in the original pack sealed with tape. Do not return the holder to the pack.

Stability :

1. The unopened kit is stable for 1 years from the date of manufacturing as indicated on the package.
2. The opened kit is stable for 3 months from the date of opening.
3. Repeated freeze thaw of reagents from 2-8°C to Room temperature several times will reduce the stability of the kit.

PACK SIZE : Available in packs of 96 Tests and 48 Tests.

CONTENTS OF THE KITS :

Materials	96 Tests	48 Tests	480 Tests
Microtiter Plate	12x8 wells strip	6x8 wells strip	5x12x8 wells Strip
Dilution buffer (Ready to use)	25ml	12ml	5x25 ml
Wash solution (Concentrated 10X)	100 ml	50 ml	5x100 ml
HRP Conjugate (Concentrated 100X)	0.3 ml	0.2 ml	5x0.3 ml
HRP Conjugate diluent	15 ml	8 ml	5x15 ml
TMB Substrate (Ready to use)	11 ml	6 ml	5x11 ml
Stop solution (Ready to use)	12 ml	6 ml	5x12 ml
Positive control	1 ml	0.5 ml	5x1 ml
Negative control	1 ml	0.5 ml	5x1 ml
Adhesive slips	3 Nos.	2 No.	5x3 Nos.

MATERIALS REQUIRED BUT NOT PROVIDED :

1. Distilled or Deionised water, preferably sterile
2. Graduated cylinders for reagent dilutions.
3. Vials to store the diluted reagents.
4. Precision pipettes.
5. Paper towels or absorbent paper.
6. Timer.
7. ELISA Reader.
8. ELISA Washer.
9. Sodium hypochlorite solution (free available chlorine 50-500mg/dl)
10. Disposable latex gloves.

SPECIMEN COLLECTION AND HANDLING :

Specimens must be centrifuged before use (e.g. 3000 RPM, 10 minutes), especially citrate plasma specimens will cause false HIV reactive results, if not centrifuged properly.

Serum and plasma (preferably EDTA) samples may be stored for up to 7 days at 2-8°C or atleast 6 months as frozen (-20°C or -70°C). Samples should not be repeatedly frozen and thawed.

Do not use heat-inactivated samples. Especially heat inactivated plasma specimens will cause false HIV reactive results.

Do not use sodium azide as preservative because it inactivates horseradish peroxidase.

Microbially- contaminated, grossly haemolysed, icteric or hyperlipemic serum and plasma specimens may give erroneous results.

PRECAUTIONS :

1. For in vitro diagnostic use only.
2. The positive control contains inactivated Hepatitis B virus, However, it should be treated as infectious. The Negative serum also should be treated as infectious.
3. All human serum and plasma samples should be considered potentially infectious. It is recommended that all specimens of human origin should be handled as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease control/National Institute of Health Manual "Biosafety in Microbiological and Biomedical Laboratories". 1984.
4. Never pipette by mouth.

5. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Wear disposable latex gloves while handling specimens and kit reagents. Afterwards wash hands carefully with disinfectants. Avoid splashing or forming aerosols. Discard all materials and specimens capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500mg/dl available chlorine. Allow 30 minutes for decontamination to be completed.

NOTE :

1. Liquid wastes containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
2. Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.
3. Deterioration is indicated by a significant decrease in the absorbance level of positive control.
4. Avoid exposure of TMB solution to intense source of light. Oxidising agents, metallic ions or soap remaining in glassware containers can interfere with the TMB reaction. In order to avoid this problem rinse the glassware thoroughly with 1N acid (HCL or H2SO4) followed by several washes with distilled water before use.
5. Reagents are stored between 2-8°C. Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB. Storage of reagents and samples in self-defrosting freezers is not recommended.
6. Do not use reagents after expiration date mentioned on the label.
7. Do not mix or interchange reagents from different kit or kit lots. Cross contamination of reagents or samples can cause erroneous results.

8. Stop solution contains sulphuric acid. Avoid contact with skin and eyes.
9. Do not interchange vial caps. 10. When removing aliquots from the reagent vials, use aseptic technique to avoid contamination, otherwise incorrect results may occur. Use a new pipette tip for each sample. Optimal results will be obtained by strict adherence to the protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements are essential.
11. Once the assay has been started, all steps should be performed without interruption.
12. Do not touch the wells or scratch the wells while pipetting.
13. Do not let wells dry, once the assay has started.
14. Reusable glassware's must be disinfected, washed out and rinsed free of detergents.

INDICATIONS OF INSTABILITY AND DETERIORATION OF REAGENTS :

1. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use reagents, which are visibly turbid.
2. The TMB SUBSTRATE solution should be colorless for proper performance of the assay. Any color may indicate deterioration of the TMB substrate.

TEST PROCEDURE

Preliminary preparations

1. Wear disposable latex gloves throughout the procedure.
2. Bring all reagents and Microwells to Room temperature (25-30°C) before starting the assay. Gently mix all liquid reagents before use.
3. Dilute the wash solution 1/10" with distilled or deionised water. Diluted wash

If the concentrated solution shows any crystals, dissolve them by warming in a water bath at 37°C before dilution.

4. Preparation of working HRP conjugate: Dilute 100X HRP conjugate diluent. For eg. For 8 wells mix 10ul of 100X HRP conjugate and 1 ml of HRP conjugate diluent.

NOTE : Prepare working HRP conjugate solution freshly every time.

TEST PROCEDURE :

1. Set up the microtitration wells in the frame provided and label each well. Label one well as reagent blank and two wells each as Positive and Negative controls.
2. Add 200 ul of Ready to use dilution buffer into required number of wells.
3. In duplicate, add 20 ul of Positive and Negative controls to appropriately labeled wells of the microtiter plate.
4. Add 20 ul of samples into each well and mix thoroughly by gentle swirling.
5. Cover the wells with adhesive slips.
6. Incubate at RT for 30 minutes (25-30°C).
7. Aspirate and dispose the samples along with microtips into a container containing 0.5 percent sodium hypochlorite (ordinary bleach).
8. Wash the microplate 5 times with approximately 300 ul per well of working wash solution. Care should be taken to avoid overfilling and cross contamination.
9. Add 100 ul of the working HRP conjugate solution in the same order.
10. Incubate at RT for 30 minutes (25-30°C).
11. Wash the microplate 5 times with approximately 300 ul per well of working wash solution.
12. Add 100ul of Ready to use TMB substrate to each well in the same order.
13. Incubate at RT for 30 minutes (25-30°C). (Avoid exposure to light).
14. Stop the reaction by adding 100 ul of the stop solution to each well in the same order.

Reading of the results: Read the absorbance at 450nm on an ELISA reader within 30 minutes.

RESULTS

QUALITY CONTROL VALUES

NEGATIVE CONTROL MEAN (Ncx)

Individual negative control values should be less than or equal to 0.250 when the photometer is blanked against air. If one of the values is outside the acceptable range, discard this value and recalculate the mean. If two of the values are out of range, the test should be repeated.

POSITIVE CONTROL MEAN (Pcx)

PCx value has been defined based on lot to lot follow up. To achieve the expected detection limit the value of PCx minus NCx should be greater than or equal to 0.9. If not, the technique may be suspected and the assay should be repeated.

Calculation of the results

CALCULATION OF THE NEGATIVE CONTROL MEAN (Ncx)

Determine the mean of the negative control values.

Example :

Negative control Sample No.	Absorbance
1	0.110
2	0.130
Total	0.240

$$Ncx = \frac{\text{Total absorbance}}{2} = \frac{0.240}{2} = 0.120$$

In the above example, all negative control values are within quality control range and the NCx need not be revised.

CALCULATION OF THE POSITIVE CONTROL MEAN (Pcx)

Determine the mean of the positive control values.

Example :

Positive control Sample No.	Absorbance
1	1.780
2	1.820
Total	3.600

$$PCx = \frac{\text{Total absorbance}}{2} = \frac{3.600}{2} = 1.800$$

In the above example PCx minus NCx is greater than 0.900, thus the technique is acceptable and data should be considered valid.

CALCULATION OF THE CUT-OFF VALUE (CO)

The cutoff value is calculated by multiplying the average absorbance value of the HIV Positive control by 0.1 and adding the average absorbance value of the negative control.

Example : Positive Control Average Absorbance = 1.800
Negative Control Average Absorbance = 0.120
Cutoff value = (0.1x1.800) + 0.120 = 0.300

RESULTS :

1. Non-Reactive : A test sample is considered to be non-reactive for HIV antibodies if the resulting absorbance value is less than the cut-off value.

2. **Reactive :** A test sample is considered to be reactive for HIV antibodies if the resulting absorbance value is greater than or equal to the cut-off value.

INTERPRETATION OF RESULTS :

1. Specimens with absorbance value less than the cut-off value are considered non-reactive by the criteria of the HIV-1/2 ELISA and may be considered negative for antibodies to HIV. Further testing is not required.
2. Specimens with absorbance value greater than or equal to the cut-off value are considered initially reactive. These specimens should be retested in duplicate (using the original sample) for the final confirmation of the result.
3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV. Further testing is not required.
4. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatedly reactive for antibodies to HIV.
5. **Specimens which have been found repeatedly reactive are interpreted to be positive for the presence of antibodies to HIV. In most settings it is appropriate to investigate repeatedly reactive specimens by additional more specific tests such as Radio-immunoassay, peptide based neutralization EIA and other peptide based EIAs that are capable of identifying antibodies to specific gene products of HIV. As in any diagnostic enzyme immunoassay, there is a possibility that repeatable reactions may occur for the following reasons.**
   - Inadequate washing
   - Contamination of reaction well with Protein-A-HRP conjugate
   - Contamination of substrate solution with conjugate or with oxidizing agents.

TROUBLE SHOOTING :

BLANK HAS TOO HIGH ABSORBANCE VALUES

Cause/Error	Remedy
1. Substrate solution is contaminated 2. Contamination, spills from other wells 3. Washing solution has not been diluted correctly 4. Poor washing 5. HRP conjugate has not been diluted correctly.	Use fresh pipette tips every time Avoid contamination Should be diluted 1/10 (1+9) Check your washer Should be diluted 1/100 (1+99)

POSITIVE CONTROL HAS TOO HIGH ABSORBANCE VALUES

Cause/Error	Remedy
1. Substrate solution is contaminated 2. Interchange of controls from different lots 3. The pipetted volume is too high	Use fresh pipette tips every time Do not mix or interchange reagents from different lots. Volume should be as indicated

POSITIVE CONTROL HAS TOO LOW ABSORBANCE VALUES

Cause/Error	Remedy
1. Interchange of controls from different lots 2. The pipetted volume is too low 3. The volume of sample diluent is too high.	Do not mix or interchange reagents from different lots. Volume should be as indicated Volume should be 200 ul

4. Initially reactive specimens which are reactive in one or both of the repeat test are considered repeatedly reactive for antibodies to HIV.

5. SPECIMENS which have been found repeatedly reactive are interpreted to be positive for the presence of antibodies to HIV. In most settings it is appropriate to investigate repeatedly reactive specimens by additional more specific tests such as Radio-immuno-assay, peptide based neutralization EIA and other peptide based EIAs that are capable of identifying antibodies to specific gene products of HIV. As in any diagnostic enzyme immunoassay, there is a possibility that nonrepeatable reactions may occur for the

ALL ABSORBANCE VALUES VERY HIGH

Cause/Error	Remedy
1. Interchange of reagents from different lots. 2. Substrate solution is contaminated 3. Washing solution concentrate has not been diluted correctly. 4. Poor washing 5. Contaminated solution containers 6. HRP solution has not been diluted correctly.	Do not mix or interchage reagents from different lots. Use clean containers Should be diluted 1/10 (1+9) Check your washer Use clean containers Should be 1/100 (1+99)

ALL ABSORBANCE VALUES VERY LOW

Cause/Error	Remedy
1. Reagent solutions used after they have expired. 2. The reagents have not been warmed up to room temperature 3. Once opened microtiter foil package has not been resealed tightly and stored properly with dessicant. 4. Interchange of reagents from different lots 5. Substrate solution is exposed to direct sunlight. 6. Stop solution has not been mixed properly before measurement 7. Deterioration of reagents 8. Contamination of conjugate by human serum or plasma (usually from samples)	Do not use reagents after the expiration date Should be 25-30°C when starting the assay Once opened microtiter plate foil package has to be resealed tightly and stored properly with dessicant. Do not mix or interchange reagents from different lots Avoid unnecessary exposure to light Mix the plate before measuring Use aseptic technique. Do not pour used reagent back to vials. Even one microliter of human serum or plasma is enough to inhibit as much as 1 litre of conjugate. Never pour used reagent back to vial.

POOR SPECIFICITY

Cause/Error	Remedy
1. Washing solution has not been diluted correctly.	Should be 1:10 (1+9)

Cause/Error	Remedy
2. Salt crystals in the washing solution concentrate has not been redissolved before diluting. 3. Poor washing 4. Too low positive control value	Redissolve the crystals before diluting by warming and mixing the concentrate Check your washer See positive control has too low absorbance value

POOR SENSITIVITY

Cause/Error	Remedy
1. Too high positive value 2. Sample serum or plasma is not mixed properly with sample buffer 3. Frozen samples have not been mixed properly after thawing 4. Stop solution has not been mixed properly before measurement	See positive control has too high absorbance value While pipetting mix the sample with sample buffer Mix well before pipetting Mix the plate before measuring

PERFORMANCE CHARACTERISTICS :

Accuracy : The Pareekshak HIV 1/2ELISA meets the requirement for the third generation test when tested against approved kits.

A. Precision

The reproducibility of pareekshak HIV1/2 ELISA kit was checked by assaying three specimens.

Intra-assay

The intra-assay variation of Pareekshak HIV1/2 ELISA was determined by assaying three samples (Two positive and one negative) in replicates of 16 in a single run.

Table 1 : Summary of the Intra-assay variation study of HIV1/2 ELISA

Serum sample	Mean (O.D)(A_{450nm})	Standard Deviation (SD)	Coefficient of Variation (%)
A	2.377	0.092	3.87
B	1.444	0.039	2.70
C	0.097	0.005	5.15

Inter-assay

The inter-assay variation of Pareekshak HIV1/2 ELISA was determined by assaying three samples (Two positive and One negative) in duplicate of 15 different runs.

Table 2 : Summary of the Inter-assay variation study of HIV1/2 ELISA

Serum sample	Mean (O.D)(A_{450nm})	Standard Deviation (SD)	Coefficient of Variation (%)
A	2.351	0.093	3.95
B	1.461	0.044	3.01
C	0.098	0.005	5.10

B. Sensitivity :

No. of Positive Samples tested	No. of Positives by Pareekshak HIV 1/2 ELISA Test	Sensitivity (%)
HIV-1 62	62	100
HIV-2 52	52	100

C. Specificity :

No. of Negative Samples tested	No. of Negatives by Pareekshak HIV 1/2 ELISA Test	Specificity (%)
150	150	100

VALIDATION :

Please refer to the schedule below for quality performance as tested with Boston Biomedica Inc. HIV Seroconversion panel PRB 932 and HIV Group O-JP8 & HIV-2,NIH Reference Panel No. 409 has been used.

BBI Panel Member	Result	Abbott ELISA Test	NIH No.	HIV - 2 Status	Result with Pareekshak HIV 1/2 ELISA
03	-	-	1493	+	+
04	-	-	1494	+	+
05	+	+	1495	+	+
06	+	+	1496	+	+
07	+	+	1497	+	+

BBI Panel Member	Result	Abbott ELISA Test	NIH No.	HIV - 2 Status	Result with Pareekshak HIV 1/2 ELISA
08	+	+	1498	+	+
09	+	+			
Group (O) 2701-0001	+	+			

REFERNCES :

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2. Barre-Sinoussi F.Chermann JC,Rey F.et al.Isolation of T-lymphotrophic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). Science 220:868-871,1983.
3. Gallo RC, Salahuddin SZ,Popovic M, et al: Frequent detection and iaolation of cytopathic retroviuses (HTLV-III_ from patients with AIDS. Science 224:500-503,1984.
4. Coffin J, Hasse A, Levy, et al: What to call the AIDS virus? Nature 321:10,1986.
5. Clavel F, Guetard D, Brun Vezinet F: Isoaltion of a new human reterovirus from west African patients with AIDS. Science 233:343-346,1986.

SUMMARY OF PROCEDURE :

Add 200ul of Ready to use dilution buffer
↓
Add 20ul of control or test sample
↓
Incubate at room temperature for 30 minutes
↓
Aspirate and wash 5 times with working wash solution
↓
Add 100ul of working HRP conjugate solution
↓
Incubate at room temperature for 30 minutes
↓
Aspirate and wash 5 times with working wash solution
↓
Add 100ul of ready to use TMB substrate
↓
Incubate at room temperature for 30 minutes
↓
Add 2 drops (100ul) of stop solution
↓
Read absorbance at 450nm

EN 980:2008 (E) MEDICAL DEVICES SYMBOL

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
2°C–30°C	Temperature Limitation	2001-06	Date of Manufacture	IVD	In vitro Diagnostic Device	LOT	Batch Code
	Company name & address		Refer Operating Instructions		Use by	Company	Company Name
EC REP	Authorised Representative in European Community		Do Not Reuse	Σ	Sufficient for		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	NON STERILE	NON-STERILE	CONTROL -	NEGATIVE CONTROL	CONTROL +	POSITIVE CONTROL

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