HIV-1/2 TRISPOT TEST KIT

For Professional Use

AIDSCAN®

IVD

A Rapid Trispot Test to detect of antibodies to HIV 1 & 2 in Human Serum or Plasma

Read pack Insert before use provided along with the kit

REF ATS

INTENDED USE: AIDSCAN® HIV-1/2 TRISPOT is an immuno concentration based assay for the detection of antibodies to HIV-1 & HIV-2 in Human Serum or Plasma.

INTRODUCTION: AIDSCAN® HIV-1 / 2 TRISPOT Test is an immunoassay which employs r-proteins for the detection of antibodies to HIV in human serum or plasma. These proteins, which are corresponding to highly antigenic segments of both the structural and non-structural proteins of the HIV constitute the solid phase antigenic absorbent. The use of r-proteins offers the advantage of high degree of specificity and sensitivity due to multiple epitopes. 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 infection affects T-Cell mediated immunity, resulting in severe lymphopenia and a reduced sub-population of helper Tlymphocytes. Destruction of this T-lymphocyte population by the virus cause an irreversible damage to immune system, resulting in a reduced or deficient response to subsequent infections and hence the patient becomes vulnerable (prone) to all kind of infections and shows bizzare symptoms hence this condition called as Syndrome. Consequently, infections become more severe and may cause death. At present there is no successful treatment for AIDS. The etiological agent has been identified as a retrovirus, human immunodeficiency virus type 1 (HIV-1). A closely related, but distinct second type of immunodeficiency virus, designated as HIV-2, has been isolated and causes a disease that is indistinguishable from AIDS. The only difference is in the potentiality of infection. Serological cross reactitivity 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 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).

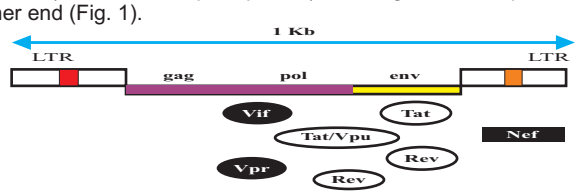

Fig. 1 **Structure of HIV genome**

Index:

I. gag = p17 p24 p7 p6 (Inner structural proteins of the retro virion)

II. Pol = PR RT IN

(encodes the viral Enzymes: PR - protease, RT = Reverse Transcriptase, IN = integrase)

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

AIDSCAN®HIV-1/2 TRISPOT Test utilizes a unique combination of HIV-1 & 2 antigens of the virus to selectively detect all subtypes of HIV-1 & 2 Virus in human serum/plasma with a high degree of sensitivity and specificity. The level of different type of antibodies and antigens of HIV in blood is as shown in Fig. 2

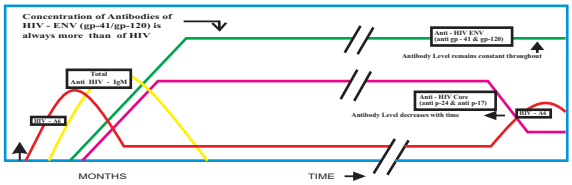

Fig. 2 **Level of different type of antibodies and antigens of HIV in blood**

TEST PRINCIPLE:

HIV-1 & HIV-2 antigens (HIV-1 & HIV-2) and a Control antigen (C) are immobilized on a porous immuno filtration membrane. Sample and the reagent pass through the membrane and are absorbed into the underlying absorbent pad. As the patient's sample drains through the membrane, HIV antibodies if present in serum / plasma, bind to the corresponding immobilized antigens. Unbound serum / plasma proteins are washed off in the subsequent washing step. Addition of the protein-A conjugate results in binding of HIV to give distinct Red Spot near the test region (HIV-1 & HIV-2). At the control region ("C") a "Built in-Quality Control Spot" has been coated to confirm the proper functioning of the device, reagent and correct procedural application.

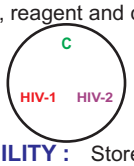

Fig. 3

STORAGE AND STABILITY : Store the test devices at 2-30°C temperature. Store the Buffer Solution and Gold Conjugate bottles at 2-8°C temperature. Do not use the kit beyond the expiry date mentioned on it. Before running the test bring all the kit components to room temperature (25+5°C) for best results. Return the Buffer Solution & Gold Conjugate bottles to 2-8°C. when not in use. DO NOT FREEZE KIT COMPONENTS.

STABILITY

1. The un-opened kits are stable for 1½ year from the date of manufacturing as indicated on the package.
2. Opened kits must be used with in 18 months of opening. Test device once opened from the pouch must be used immediately.
3. Repeated 'freeze-thaw cycles' i.e., bringing the kits to room temperature and back to the refrigerator several times will reduce the stability of the kit.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use gold conjugate which are turning black or precipitating.

PACK SIZE : Available in packs of 10's, 20's, 50's and 100's.

KIT CONTENTS

Pack Size	10 Test	25 Test	50 Test	100 Test
1. Test Device	10 Nos.	25 Nos.	50 Nos.	100 Nos.
2. Buffer Solution (Ready to Use)	1 x 6.0 ml	1 x 15.0 ml	2 x15.0 ml	4 x15.0 ml
3. Gold Conjugate (Ready to use)	1 x 1.5 ml	1 x 3.0 ml	2 x 3.0 ml	4 x 3.0 ml
4. Dropper	10 Nos.	25 Nos.	50 Nos.	100 Nos.
5. Pack Insert	1 No.	1 No.	1 No.	1 No.

MATERIAL REQUIRED BUT NOT PROVIDED:

- Sodium hypochlorite solution (free available chlorine 50-500 mg/l).
- Disposable latex gloves.

WARNINGS

1. For *in vitro* diagnostic use only.
2. Wear disposable latex gloves while handling specimens and kit reagents.
3. After the test, wash hands carefully.
4. Reagents have to be stored between +2° C and +8° C.
5. **Prewarm all reagents to 25+5° C before use at RT.**
6. The expiration date is printed on each component and on the package.
7. Do not expose the conjugate to excessive light and high temperature.
8. Once opened, the components must be closed tightly.

PRECAUTIONS :

For in vitro diagnostic use only.
All human serum and plasma samples should be considered as 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. Never pipette by mouth.
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. Avoid splashing or forming aerosols. Discard all materials and specimens as if 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 50-500 mg/l available chlorine. Allow 30 minutes for decontamination to be completed.
NOTE.: Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. 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 bio hazardous waste matter for proper disposal.
Store reagents between +2°C and +8°C. Avoid unnecessary exposure to light. The light sensitive reagents is the conjugate. Storage of samples in self defrosting freezers is not recommended.
Do not use reagents after the expiration date printed on the label.
Do not mix or interchange reagents from different kits or kit lots.
Cross contamination of reagents or samples can cause erroneous results.
Do not interchange vial caps. Use a new dropper for each sample.
Optimal results will be obtained by strict adherence to the test protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements, are essential. Once the assay has been started. all steps should be performed without interruption. Reusable glassware must be disinfected. washed out and rinsed free of detergents.

2. SPECIMEN PROCESSING

AIDSCAN ®HIV-1/2 TRISPOT Test works best when used with fresh samples, however the frozen or viscous samples can also perform well if the following instructions are strictly adhered to
A. Frozen samples : (I) Allow the sample to thaw in a vertical position in the rack. Mix the sample thoroughly. If particles are seen, allow them to settle at the bottom or if a centrifuge is available, the sample can be centrifuged at 5,000 r.p.m. for 15 minutes.
(ii) Insert the dropper just below the top surface of the sample and withdraw two drops of the sample.
B. Thick or viscous samples : Whenever possible, clear specimen should be used.However, viscous, thick or turbid samples which may sometimes take more than 40- 60 seconds to flow through the membrane should be centrifuged at 5,000 r.p.m. for 15 minutes and retested on a fresh device to avoid inconsistent results.

TEST PROCEDURE

1, Bring all the reagents. devices and specimens to room temperature (25+5°C).
2. Add 2 drops of buffer solution to the test device.
3. Add 2 drops of either serum or plasma.
4. Add 4 drops of buffer solution.
5. Add 2 drops of gold conjugate.
6. Add 4 drops of buffer solution.
Reading of the Results Read the result immediately. Do not read after 5 minutes.

RESULTS

Interpretation of Results
1. NEGATIVE: If only one red spot (control spot) appears at the control region "C" indicates that the specimen does not contain antibodies either to HIV-1 or HIV-2. (Fig. a)
2. POSITIVE: (a) If two red spots (Control spot and HIV-1 or HIV-2 Spot) appear at the control region "C" and test region HIV-1 and/or HIV-2 indicates that the specimen is reactive for antibodies to HIV-1 and/or HIV-2. (Fig. b)
(b) If three red spots (Control. HIV-1 & HIV-2 Spot) appear at the control region "C" and test region HIV-1 & HIV-2 indicates that the specimen is reactive for antibodies to HIV-1 & HIV-2. (Fig. c)
3. INVALID RESULT
If no spot appears after the completion of test. either with Clear background or with complete reddish background the test indicates ERROR. (Fig. d) This may indicate a procedural error or deterioration of specimen / reagents or particulate matter in the specimen. The specimen should be retested on a fresh device.
IMPORTANT
(i) Test spot on "HIV-1 & HIV-2" area either dark or light in color (red) should be considered reactive for antibodies to HIV.
(ii) Sometimes. if the sample solution does not soak-in within 40-60 seconds. the sample should be observed for any suspended particulate matter; if it is present. centrifuge the sample at 5000 r.p.m. for 15 minutes. Use a fresh device to rerun the test.
.(iii) Sample found to be reactive by the above screening test must be confirmed by standard supplemental assay. like ELISA Radio immuno assay or Western blot.

Fig. a Fig. b Fig. C Fig. D

TROUBLESHOOTING

FALSE POSITIVE

Cause / Error	Remedy
Addition of more than 2 drop of sample or gold conjugate	Use only 2 drops of sample or gold conjugate

WEAK INTENSITY OF CONTROL SPOT

Cause / Error	Remedy
Very cold reagent	Bring the sample, test device, buffer and gold conjugate to room temperature before testing (25 ± 5°C)

POOR SENSITIVITY

Cause / Error	Remedy
Frozen sample not mixed properly after thawing.	Mix well sample before pipetting.

PERFORMANCE CHARACTERISTICS

Accuracy : AIDSCAN® HIV-1/2 TRISPOT Test meets the requirements when tested against DCI approved kit.

Specificity

No. of Negatives Tested	No. of Negatives by AIDSCAN® HIV-1/2 TRISPOT Test	Specificity (%)
481	480	99.7%

Sensitivity

a)

No. of HIV-1 Positive Samples Tested	No. of Positives by AIDSCAN® HIV-1/2 TRISPOT Test	Sensitivity (%)
86	86	100

b)

No. of HIV-2 Positive Samples Tested	No. of Positives by AIDSCAN® HIV-1/2 TRISPOT Test	Sensitivity (%)
14	14	100

LIMITATIONS OF THE TEST

1. AIDSCAN® HIV-1/2 TRISPOT Test detects anti-HIV antibo dies in human serum or plasma and is only a screening test. All reactive samples should be confirmed by supplemental assays like ELISA, RIA or Western Blot. Therefore, for a definitive diagnosis, the patient's clinical history, symptomatology. as well as serological data should be considered. The results should be reported only after complying with above procedure.
2. The assay is only validated for serum and plasma from individual bleeds and not for pools of serum or plasma or other body fluids.
3. A non-reactive result does not exclude the possibility of exposure to or infection with HIV.
4.It should be noted that repeated false reactive result may occur due to non-specific binding of the sample to the membrane or due to cross-reaction of non-specific antibodies to the HIV antigen.
5.The presence of anti-HIV does not imply a HIV infe ction but may be indicative of recent and / or past infection by HIV.
6. Patients with auto-immune liver disease may show falsely reactive results.
7.The kit work best when used with fresh sample and when all the kit component are at room temperature (25+5 C). Sample which have been frozen and thawed several times contain particulates which can block the membrane, hence resulting in improper flow of reagents and high background colour which may make the interpretation of results dificult..
8 Optimum test performance depends on strict adherence to the test procedure as described in this manual. Any deviation

REFERENCES

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BS ISO-15223-1:2012(E) MEDICAL DEVICES SYMBOL

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

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