These evaluations indicate the following Sensitivity and Specificity:

Sample Panel	Indian* Panel	W.H.O.** Panel
Sensitivity	100%	100%
Specificity	100%	100%

* Evaluation Reports of National HIV Reference Laboratories Govt. of India (AIIMS New Delhi, CMC Vellore, NICD New Delhi)

** As per evaluation of WHO; CH-1211, GENEVA, 27 SWITZERLAND, August 1999.

This information is provided for the Scientific Community Enquiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional purpose.

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of nine samples - two HIV negative, six HIV-1 positive (one strong, two medium and three weak sample) and one HIV-2 Positive. The C.V. (%) of all the ten samples were within 20%.

19. DISPOSAL

Discard the test device immediately after reading result. Before discarding it, add few drops of disinfectant on device membrane and on all other items used for handling serum. Put all items to be disposed in Disposable Bags and dispose off accordingly.

20. 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 for use, 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 manufacture'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 for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to 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.

21. REFERENCES

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in vitro diagnostic Reagent, not for medicinal use

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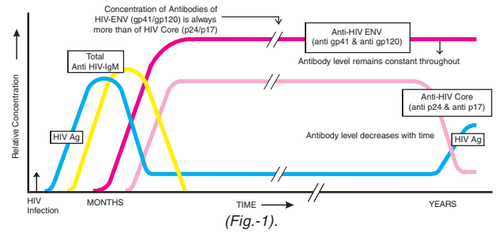
Rev. Date: May - 25 VER-02

HIV TRI-DOT

Rapid Visual Test for the Qualitative and differential Detection of Antibodies to HIV-1 & HIV-2 in Human Serum/Plasma (Separate Dots for HIV-1, HIV-2 & Control)

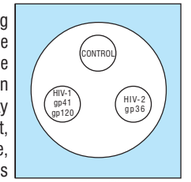
1. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

First confirmed case of AIDS was identified in 1983 and by 1984 the etiologic agent, the Human Immunodeficiency Virus (HIV), subsequently named HIV-1 was isolated. Shortly afterwards in 1985 another retrovirus subsequently named HIV-2 was isolated in Africa. These two viruses belong to the retrovirus group and are slow viruses. The structure, gene organisation and serological behaviour of HIV-1 & HIV-2 and their complete nucleotide sequence has been determined. This knowledge has laid a foundation for the development of a new assay based on Recombinant DNA technology leading to the differential detection of antibodies to HIV-1 & HIV-2 (if present) in Human Serum or Plasma. Research has shown that antibodies produced against envelope gene are found in infected people as shown in graph, (Fig.-1).



(Fig.-1).

HIV TRI-DOT has been developed and designed using gp41, C terminal of gp120 & gp36 representing the immunodominant regions of HIV-1 & HIV-2 envelope gene structure respectively. The device (an immunofiltration membrane) includes a "Built-in Quality Control DOT" which will develop colour during the test, thereby, confirming proper functioning of the device, reagents and correct procedural application. This CONTROL DOT is the "Built-in Quality Control." (Fig.2)



(Fig.-2).

HIV TRI-DOT has been specially researched, developed and engineered using several thousands of serum/plasma specimens. It has also been evaluated by UNAIDS (WHO) Geneva, using samples of European, Asian, Latin American & African origin. The Sensitivity and Specificity has been extremely high in these samples of diverse origin.

The panel used for evaluation of HIV TRI-DOT by Institute of Tropical Medicine, WHO Collaborating Centre in AIDS, Belgium also included HIV-O Virus, which was found reactive with HIV TRI-DOT.

2. INTENDED USE

The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2 and is for in vitro diagnostic use only.

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 European Standard EN ISO 15223-1:2021.

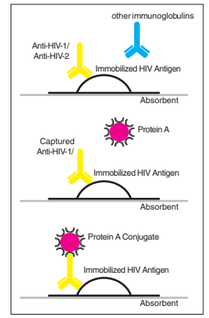
	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

4. PRINCIPLE OF THE TEST

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent.

As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens.

Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple DOT(s) against a white background. (Fig.-3)



(Fig.-3).

5. KIT DESCRIPTION

COMPONENTS	CONTENTS	PREPARATION
HIV TRI-DOT Test Device	Packed individually. Device has membrane with 1 Control & 2 Test Dots, one each for HIV-1 & HIV-2.	Cut open the pouch before use.
Buffer Solution	Buffer containing BSA and sodium azide.	Puncture the nozzle before use.
Protein-A Conjugate	Protein-A Conjugate in liquid form containing sodium azide.	Puncture the nozzle before use.
Control — (Available on request)	Control tested negative for HBsAg, HCV, HIV-1 & HIV-2 and contains presevative.	Ready to use.
Control + (Available on request)	Control tested Positive for antibodies to HIV-1 and contains presevative.	Ready to use.
Sample Dropper	Long Plastic dropper provided for adding the sample.	

6. MATERIAL REQUIRED BUT NOT PROVIDED

The kit contains all the items required to perform this test. But if the sample is viscous/turbid/contains particulate matter, a centrifuge will be required, to separate off the suspended matter. Since the test is completed in less than 5 minutes a timer or stop watch is not essential.

7. STORAGE

Store the entire kit at 2-8ºC in the coolest and driest area available. Expiry date on the kit indicates the date beyond which components should not be used. DO NOT FREEZE THE KIT COMPONENTS.

8. KIT PRESENTATION

10 Test Pack 50 Test Pack

100 Test Pack

9. WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT 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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. 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 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures and guidelines.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Protein-A Conjugate and Buffer Solution 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" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)

10. PRECAUTIONS

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
3. 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.
4. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
5. Avoid several times freezing and thawing of the sample to be tested.
6. Always allow each reagent to fall freely from the dropper tip. Do not touch the dropper tip to any surface; this may contaminate the reagent.
7. Avoid microbial and cross contamination of reagents.
8. Follow the standardized test procedure strictly.
9. Return entire kit at 2-8ºC, when not in use.

11. SPECIMEN/SAMPLE COLLECTION

Collect blood in a clean dry sterile vial and allow to clot or separate the serum by centrifugation at room temperature. It is recommended that fresh sample should be used if possible. If serum is not to be assayed immediately it should be stored at 2-8ºC or frozen at minus 20ºC (-20ºC) . Only human serum or plasma should be used for the test. Haemolyzed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

12. SPECIMEN/SAMPLE PROCESSING

(A) FROZEN SAMPLE

The HIV TRI-DOT Test is best when used with fresh samples that have not been frozen and thawed. However, most frozen samples will perform well if the following suggested procedure is followed.

1. Allow the sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample can be centrifuged at 10,000 r.p.m. for 15 min.
2. Insert the dropper just below the top surface of the sample and withdraw one drop of sample. If the above procedure still yields a high background, dilute 1 drop of sample with 2 drops of normal saline. Use 1 drop of this diluted sample in the test.

(B) THICK OR VISCOUS SAMPLES:

Whenever possible, clear specimens 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 10,000 r.p.m. for 15 min. and retested on a fresh device to avoid inconsistent results.

(C) TRANSPORTATION

If the specimen is to be transported it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

13. BEFORE YOU START

The Buffer Solution & Protein A Conjugate provided in the kit has closed nozzle and screw cap with pin (outside). Before using Buffer Solution & Protein A Conjugate, keep the vial vertically straight and tap down gently on the working platform, so that the reagents comes down at the bottom of the vial. To orifice/puncture the closed nozzle, follow the instruction as illustrated below:

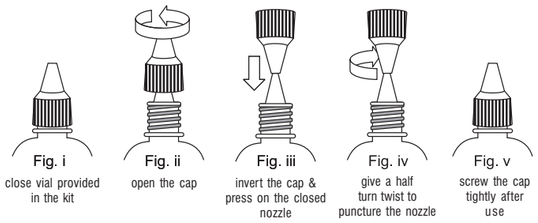


Fig. i close vial provided in the kit | Fig. ii open the cap | Fig. iii invert the cap & press on the closed nozzle | Fig. iv give a half turn twist to puncture the nozzle | Fig. v screw the cap tightly after use

14. PROCEDURAL PRECAUTIONS

Take care of the following points before starting the test.

1. The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.
2. Bring all the reagents and specimens to room temperature (20ºC-30ºC) before beginning the test. The immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature.

3. Place the required number of HIV TRI-DOT test devices at the working area.
4. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.

5. Do not run more than 5 devices at a time.
6. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.

   However drops of each solution should be added in continuous stream to wet the entire area of membrane.
7. If the solution does not soak-in within 40-60 seconds; observe the sample for any suspended particulate matter. If it is present, centrifuge the sample at 10,000 r.p.m. for 15 min. and use a fresh device to re-run the test. Refer to "SPECIMEN / SAMPLE PROCESSING".

8. All solutions and sample should be added to the CENTRE OF MEMBRANE.

9. For consistent results ensure FREE FALLING OF DROPS on the membrane, holding the vial/dropper vertically for proper volume.
10. Do not run more than 10 device at a time.
11. Do not use kit components beyond the expiration date.
12. The Protein-A conjugate should not be subjected to frequent temperature fluctuations.

15. TEST PROCEDURE

1. Add 3 drops of Buffer Solution to the centre of the device.

2. Hold the dropper vertically and add 1 drop of patient's sample 50μl (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).

3. Add 5 drops of Buffer Solution.

4. Add 2 drops of Protein-A Conjugate directly from the conjugate vial.

5. Add 5 drops of Buffer Solution and read results.

6. **Read results immediately and discard the device considering it to be potentially infectious.**

IMPORTANT: ***IT IS IMPORTANT TO ALLOW EACH SOLUTION TO SOAK IN THE TEST DEVICE BEFORE ADDING THE NEXT SOLUTION.***

16. INTERPRETATION OF RESULTS

NON-REACTIVE

1. If only one Dot (only the Control Dot) appears as shown in fig.a, the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.

Fig. a

REACTIVE

1. If two Dots, one for the control and the other for HIV-1 appear as shown in Fig.b, the specimen is reactive for antibodies to HIV-1.

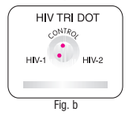


Fig. b

2. If two Dots, one for the control and the other for HIV-2 appear as shown in Fig.c, the specimen is reactive for antibodies to HIV-2.

Fig. c

3. If all the three Dots, one each for control, HIV-1 & HIV-2 appear as shown in Fig.d, the specimen is reactive for antibodies to HIV-1 & HIV-2.

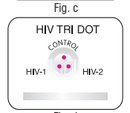


Fig. d

INVALID TEST

If no Dot appears after the test is complete, either with clear background or with complete pinkish/purple background as shown in Fig. e & f, the test indicates ERROR. This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. The specimen should be centrifuged at 10,000 rpm for 15 minutes and tested again on a new device.

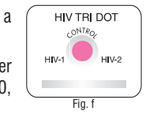


Fig. f

(If the problem persists, please call our Technical / Customer Service Cell at New Delhi, Phone: +91-11-47130300, 47130500)

Fig. e

IMPORTANT

1. All initially reactive samples should be subjected to centrifugation at 10,000 r.p.m. for 15 min. It is recommended that this centrifugation step should be carried out prior to sending the sample for the Western Blot. The test should be repeated with supernatant collected after centrifugation. If no dot appears on repetition, it indicates a falsely reactive sample. A truly reactive dot will not show much change in its colour intensity after centrifugation. The false reactivity of the sample is generally due to the presence of suspended particulate matter in the serum which may or may not be visible to the naked eye.

   This critical step of centrifuging a reactive sample should be faithfully followed. Its correct application makes the test EXTREMELY SENSITIVE and completely eliminates the possibility of false reactivity.
2. 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 10,000 r.p.m. for 15 min. Use a fresh device to re-run the test.
3. Test dots HIV-1 and HIV-2 either dark or light in pink colour should be considered reactive.
4. **Sample found to be reactive by the above screening test must be confirmed by standard supplemental assay, like Western Blot.**

17. LIMITATIONS OF THE TEST

1. The kit works best when used with fresh samples. Samples 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 difficult.
2. Optimum test performance depends on strict adherence to the test procedure as described in this manual. Any deviation from test procedure may lead to erratic results.
3. HIV-1 and 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%. Appearance of dots for HIV-1 and HIV-2 antibodies on the test device does not necessarily imply co-infection from HIV-1 & HIV-2.
4. Some samples show cross reactivity for HIV antibodies. **Following factors are found to cause false positive HIV antibody test results:** Naturally occurring antibodies, Passive immunization, Leprosy, Renal Disorders, Tuberculosis, Myco-bacterium avium, Herpes simplex, Hypergamma-globulinemia, Malignant neoplasms, Rheumatoid arthritis, Tetanus vaccination, Autoimmune diseases, Blood Transfusion, Multiple myeloma, Haemophelia, Heat treated specimens, Lipemic serum, Anti-nuclear antibodies, T-cell leukocyte antigen antibodies, Epstein Barr virus, HLA antibodies and other retroviruses.
5. **This is only a screening test. All samples detected reactive must be confirmed by using HIV 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.
6. The test is only validated for serum and plasma from individual bleeds and not for pools of serum or plasma or other body fluids.
7. A non-reactive result does not exclude the possibility of exposure to or infection with HIV.

18. PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity studies were carried out on samples fresh as well as frozen from low risk as well as high risk groups. Performance of the test with reference to sensitivity and specificity has been determined by NATIONAL HIV REFERENCE CENTRES of Govt. of India and WHO, Geneva using various testing panels.