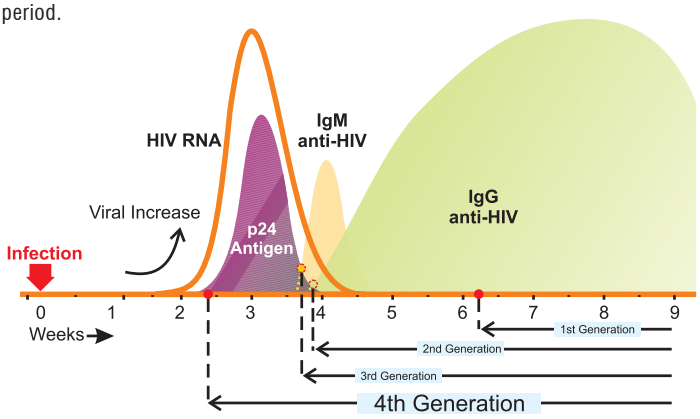
4th Generation
HIV TRI-DOT + Ag

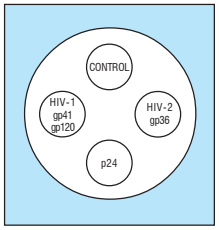
Rapid Visual Test for the Qualitative Differential Detection of HIV-1 p24 antigen and Antibodies (IgM, IgG & IgA) to HIV-1 & HIV-2 in Human Serum/Plasma

I. 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. The serological events following HIV infection are represented graphically in fig.1. In individuals infected with HIV, antigen appears first (after 2 weeks) before anti-HIV but due to seroconversation, the antigen is lost and antibody develops (after 4 weeks) after infection and thereby the level of the antibody increases. The sensitivity of the tests can be improved by the incorporation of HIV antigen (p24) detection alongwith HIV antibody to reduce the window period.



HIV TRI-DOT + Ag has been developed and designed using anti-p24, 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 sequential addition of reagent (Fig.2).



(Fig.-2).

HIV TRI-DOT + Ag has been specially researched, developed and engineered using several thousands of serum/plasma specimens.

2. INTENDED USE

The 4th Generation HIV TRI-DOT + Ag Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 p24 antigen and HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma. The test is a screening test for p24 antigen (HIV-1), anti-HIV-1 & anti-HIV-2 and is for *in vitro* diagnostic use only. It is intended for screening of blood donors or others individual at risk for HIV-1 & HIV-2 infection and for clinical diagnostic testing.

3. DESCRIPTION OF SYMBOLS USED

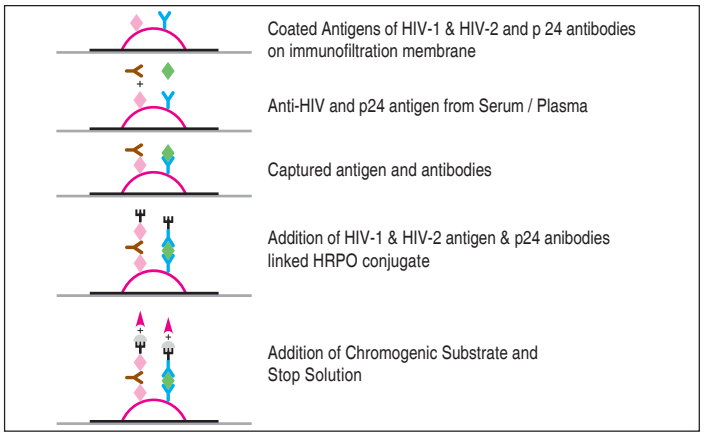
The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

Manufactured By		*In vitro* diagnostic medical device	
No. of tests		See Instruction for use	
Lot Number Batch Number		Temperature Limitation	
Manufacturing Date		Caution see instruction for use	
Expiry Date		Catalogue Number	
Do not use if package is damaged		Keep away from sunlight	
Single use only		Keep Dry	

4. PRINCIPLE OF THE TEST

HIV TRI-DOT + Ag test is an enzyme immuno assay based on "Sandwich Immunoassay". HIV antigens and p24 antibody is immobilized on a porous immunofiltration membrane.

Samples/ specimen and reagents pass through membrane followed by addition of enzyme conjugate (HIV antigens & p24 antibody linked with HRPO). A sandwich complex is formed on membrane where HIV-1 & HIV-2 antibodies or p24 antigen (from serum sample) is sandwiched between the antigen & antigen HRPO and antibody & antibody HRPO conjugate. The membrane is then washed with wash solution and wash solution absorbed into the underlying absobent. Finally substrate solution containing chromogen & hydrogen peroxide is added to the membrane. If p24 antigens and/or HIV-1 & HIV-2 antibodies are present in patient samples, a blue test dots at respective position appear with control dot. The color intensity of test dot is directly propotional to the amount of HIV-1 and/or HIV-2 and/or p24 antigen present in the specimen. The reaction is stopped by stop solution and read visually.



5. KIT DESCRIPTION

COMPONENTS	CONTENTS	PREPARATION
HIV Tri-Dot + Ag Test Device	Packed individually. Device has membrane caoted with 1 Control & 3 Test Dots, one each for p24 antigen, HIV-1 & HIV-2.	Cut open the pouch before use.
Wash Buffer	Buffer containing BSA and preservative.	Ready to use.
Enzyme Conjugate	HIV-1 & HIV-2 antigens & p24 antibodies linked with HRPO with protein stablizer.	Ready to use.
Sample Diluent	Buffer containing protein & preservative.	Ready to use.
TMB Substrate	TMB conainting H_2O_2 & chromogen.	Ready to use.

Stop Solution	Buffer containing HCl and presevative,Ready to use.
Antigen Control +	Inactivated p24 Antigen positive control with preservative.
Antibody Control +	Inactivated diluted human serum positive for HIV antibody and non reactive for HBsAg & HCV with preservative. **(Available on request)**.
Sample Dropper	Long Plastic dropper
Transfer Dropper	Small Plastic dropper
Sample Cup	For sample Dilution.

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 10 minutes a timer or stop watch is not essential.

7. STORAGE

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

8. KIT PRESENTATION

10 Test Pack 50 Test Pack

9. WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES AND POSITIVE CONTROL 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. 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 off 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. Avoid strong light exposure during the assay.
10. **Do not mix antibody and antigen positive control. Both positive control should be tested seperately.**

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.

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 2-8°C or frozen at minus 20°C (-20°C). Only human serum or plasma should be used for the test.

Do not use Sodium Azide as preservative because it inactivates Horseradish peroxidase.

12. SPECIMEN/SAMPLE PROCESSING

(A) FROZEN SAMPLE:

The HIV TRI-DOT + Ag 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. PROCEDURAL PRECAUTIONS

Take care of the following points before starting the test.

1. Bring all the reagents and specimens to room temperature (20°C-30°C) before beginning the test. The immunological sequence of reactions which take place during different procedural steps shows best performance at room temperature.

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

4. 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.

5. If the solution does not soak-in within 90-120 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".

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

7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. The liquid conjugate should not be subjected to frequent temperature fluctuations.
9. Both the positive control provided in the kit should be run independently as p24 antibody present in **Antibody Positive Control** will react with p24 **Antigen Positive Control** and will neutralise p24 antigen in the control and hence will give negative result.
10. **The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.**
11. **Important:** To avoide cross contamination, all components have different color nozzle caps and place the nozzle caps on their respective vials after use as given below:
    1. Wash Buffer - Transparent Cap
    2. Enzyme Conjugate - Black Cap
    3. TMB Substrate - Brown Cap
    4. Sample Diluent - Yellow Cap
    5. Stop Solution - Blue Cap

14. BEFORE YOU START

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

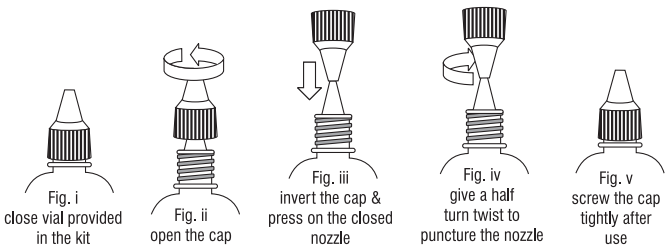


Fig. i close vial provided in the kit

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

15. TEST PROCEDURE

STEP-1

1. Add **3** drops of sample to Sample cup using sample dropper provided. (Use seperate dropper for each sample)

STEP-2

2. Add **3** drops of sample diluent to each of the above Sample cup containing sample.

STEP-3

3. Add **3** drops of Enzyme Conjugate to above Sample cup containing sample & sample diluent and mix properly & make sure bubbles are not formed while mixing.

STEP-4

4. Add **3** drops of Wash Buffer to the centre of the Device.

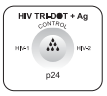
STEP-5

5. Transfer the complete volume of above reagent mixture (sample, sample diluent & enzyme conjugate) from Sample cup directly to Device or using Transfer dropper provided.

Note : Dispose off the Sample cup & droppers considering them biohazardous.

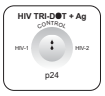
STEP-6

6. Add **6** drops of Wash Buffer to device.



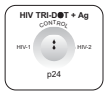
STEP-7

7. Add **2** drops of TMB Substrate to device.



STEP-8

8. Finally stop the reaction by adding **2** drops of Stop Solution to device and read results.



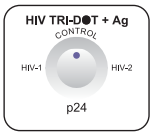
***Note:** Colour of Control & Test Dots will change from blue to bluish grey on addition of Stop Solution.*

Important Note : Do not read results after 10 minutes as due to backflow, membrane is likely to get colored making the result interpretation difficult. Read results immediately and discard the device considering it to be potentially infectious.

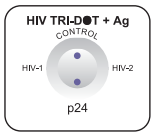
16. INTERPRETATION OF RESULTS

NON-REACTIVE

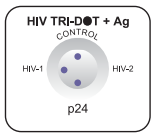
1. If only One DOT (only the Control Dot) appears as shown in fig., the specimen is non reactive for p24 antigen and antibodies to HIV-1 and HIV-2. Interpret sample as non-reactive.



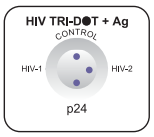
REACTIVE



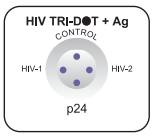
Reactive for p24 antigen



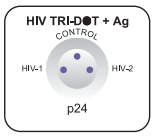
Reactive for p24 antigen and antibodies to HIV-1



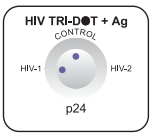
Reactive for p24 antigen and antibodies to HIV-2



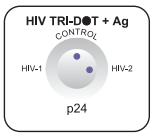
Reactive for p24 antigen and antibodies to HIV-1 & HIV-2



Reactive for antibodies to HIV-1 & HIV-2

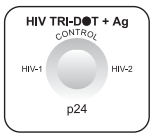


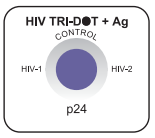
Reactive for antibodies to HIV-1



Reactive for antibodies to HIV-2

INVALID TEST





If no DOT appears after the test is complete, either with clear background or with complete Bluish/yellowish background 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 centrifused at 10,000 rpm for 15 minutes and re-run the test on a new device.
(If the problem persists, please call our Technical / Customer Service Cell at New Delhi, Phone: 011-47130300, 47130500)

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 p24, HIV-1 and HIV-2 either dark or light in bluish grey colour should be considered reactive.
4. ***Samples found to be reactive by the above screening test must be confirmed by standard supplemental assay, like Western Blot and PCR.***

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. p24 antigen shows cross reactivity to hetrophilic antibodies, RA factor etc.
5. 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.
6. **This is only a screening test. All samples detected reactive must be confirmed by using HIV Western Blot and/or PCR.** 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.

18. PERFORMANCE CHARACTERISTICS

1. **Analytical Sensitivity**: The sensitivity of the kit has been determined for p24 Antigen using WHO international standard: HIV-1 p24 antigen NIBSC Code No. 90/636 and it is equal to 1000 IU/ml.
2. 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 using a panel containing 5632 Nos. of known negative and positive antibodies samples and antigen control.
   The performance of the test was evaluated and compared with a licensed commercially available Elisa test. The results obtained are as follows:

Sample Type	No. of Samples tested	Licensed test	HIV TRI-DOT +Ag
Negative	5471	5471	5470
HIV Positive	161	161	161

Sensitivity : 100% Specificity : 99.98%

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - four negative, two HIV antigen (p24) postive, two HIV-1 positive and two HIV-2 Positive. The C.V. (%) of all the ten samples were within 10%.

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-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 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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VER-02 R-01

in vitro diagnostic Reagent, not for medicinal use

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MN/HTA/039
Rev. Date: Apr.-21