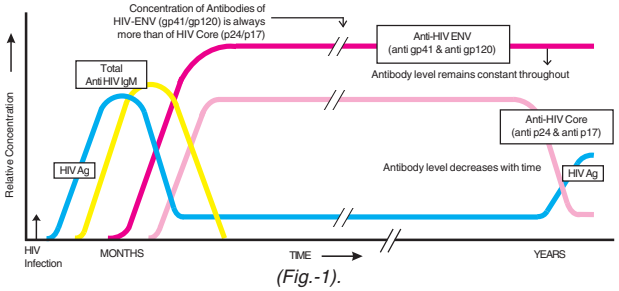
DIAGNOS HIV BI-DOT

Rapid Visual Test for the Qualitative Detection of Antibodies to HIV-1 & HIV-2 in Human Serum/Plasma

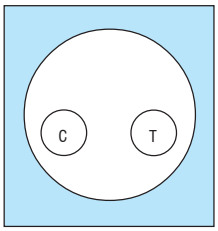
I. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

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. This knowledge has laid a foundation for the development of a new assay based on Recombinant DNA technology leading to the 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).

DIAGNOS HIV BI-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 "C" is the "Built-in Quality Control." (Fig.2)



(Fig.-2).

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

2. INTENDED USE

The DIAGNOS HIV BI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the detection of HIV-1 & HIV-2 antibodies (IgG) 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 the British and European Standard EN ISO 15223-1:2016.

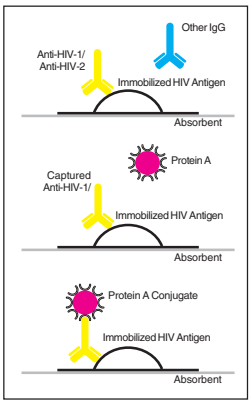
	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	8°C 2°C	Temperature Limitation
	Manufacturing Date		Caution see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight

4. PRINCIPLE OF THE TEST

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 against a white background. (Fig.-3)



(Fig.-3).

5. KIT DESCRIPTION

COMPONENTS	CONTENTS	PREPARATION
1. DIAGNOS HIV BI-DOT Test Device	Packed individually. Device has membrane with 1 Control & Test Dot.	Cut open the pouch before use.
2. Buffer Solution	Buffer containing BSA and sodium azide.	Ready to use.
3. Protein-A Conjugate	Protein-A Conjugate in liquid form containing sodium azide.	Ready to use.
4. Sample Dropper	Long Plastic dropper provided for adding the sample.	

Store the kit at 2-8°C in the driest area available.
Bring all reagents and test components to room temperature (20-30°C) before use. Return entire kit at 2-8°C when not in use. DO NOT FREEZE TEST COMPONENTS.

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 kit at 2-8°C in the driest area available. **The shelf life of the kit is 15 months from the date of manufacturing.**

Do not use the kit beyond the expiry date mentioned on it. Before running the test bring all the kit components to room temperature (20-30ºC) for best results. Return the entire kit to 2-8ºC when not in use. DO NOT FREEZE KIT COMPONENTS.

8. KIT PRESENTATION

10 Test Pack 50 Test Pack

9. WARNING FOR USERS

1. The use of disposable gloves is STRONGLY RECOMMENDED during the test.
2. In case there is a wound or cut in the hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. This Kit is for *in vitro* diagnostic use only.
5. All the samples to be tested should be handled as though capable of transmitting infection.
6. Spills should be decontaminated promptly with disinfectant.
7. Dispose of all specimens and materials used to perform the test appropriately using disinfectant in accordance with established safety procedures and guidelines.
8. The Protein-A Conjugate and Buffer Solution contain Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amount 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).
9. Thoroughly wash hands with soap after the use of this kit. In case of a needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.

10. PRECAUTIONS

1. Do not combine reagents from different batches during the same series, as they are optimized for individual batch to give best result.
2. 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. **Place white nozzle cap on Buffer Solution vial and red nozzle cap on Protein-A Conjugate Vial.**
3. Use a separate sample dropper for each sample and then discard it as biohazardous waste.
4. Avoid several times freezing and thawing of the sample to be tested.
5. 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.
6. Avoid microbial and cross contamination of reagents.

11. SPECIMEN / SAMPLE COLLECTION

Collect blood in a clean dry sterilized 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 2-8ºC or frozen at -20ºC. Serum may be stored at 2-8ºC for upto 3 days and stored frozen at -20ºC for 3 months. Only serum or plasma should be used for the test.

Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

12. SPECIMEN / SAMPLE PROCESSING

Though DIAGNOS HIV BI-DOT 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 SAMPLE:

(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 10,000 r.p.m. for 15 minutes.

(ii) Insert the dropper just below the top surface of the sample and withdraw one drop 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 10,000 r.p.m. for 15 minutes and retested on a fresh device to avoid inconsistent results.

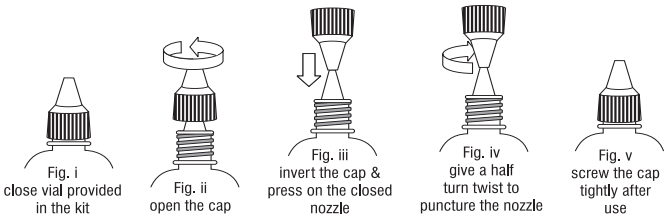
(C) TRANSPORTATION

(i) The WHO guidelines for the safe transport of specimen (WHO/EMC/97.3) should be read carefully by the laboratory staff as these guidelines hold equally good for Hepatitis samples.

(ii) If the specimen is to be transported, it should be packed in compliance with the current Government regulations on 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:



14. RECOMMENDATION FOR USER

(i) **The procedural sequence of additions should be strictly adhered to avoid any discrepant results.**

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

R.T.
20-30º

(iii) ***Mix each specimen thoroughly prior to use***. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.

(iv) Place the required number of DIAGNOS HIV BI-DOT test devices at the working area.

(v) Cut open the pouch and take out the device for performing the test. Write the sample identification number to be tested on the device for correct correlation with results.

(vi) 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. If the sample does not soak-in within 40-60 seconds,observe the sample for any suspended particulate matter.

If present, centrifuge the sample at 10,000 r.p.m. for 15 mins. and use a fresh device to re-run the test. Refer to "SAMPLE / SPECIMEN PROCESSING".

(vii) All solutions and sample should be added to the CENTRE OF MEMBRANE.

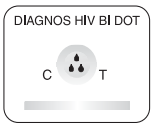
(viii) For consistent results ensure FREE FALLING OF DROPS on the membrane holding the vial / dropper vertically for proper volume.

(ix) Disinfect and DISCARD THE USED DEVICES IMMEDIATELY AFTER READING RESULT considering it as potentially infectious.

15. TEST PROCEDURE

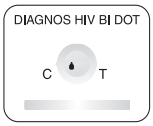
Step-1

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



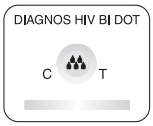
Step-2

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



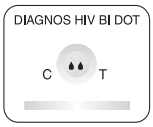
Step-3

3. Add **5** drops of Buffer Solution.



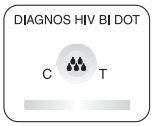
Step-4

4. Add **2** drops of Liquid Conjugate directly from the conjugate vial.



Step-5

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



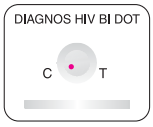
Read results immediately and discard the device considering it to be potentially infectious.

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

16. INTERPRETATION OF RESULTS

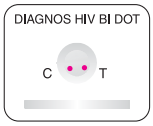
NON-REACTIVE

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



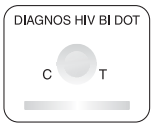
REACTIVE

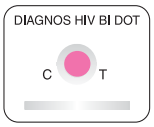
1. If two DOTS, one for the control and the other for test appear as shown in Fig., the specimen is reactive for antibodies to HIV-1 &/or HIV-2.



INVALID TEST

If no DOT appears after the test is complete, either with clear background or with complete pinkish/purple 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 tested on a new device.





(If the problem persists, please call our Technical/ Customer service cell, New Delhi, Phone: 0091-11-26818971, 26818972, 26818973).

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 dot 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 test for HIV-1& /or 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, 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.

18. PERFORMANCE CHARACTERISTICS

The performance of DIAGNOS HIV BI-DOT is evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 2112 nos. of known serum samples (including 576 tough sera). The results of all the sera with a defined HIV status were fully comparable with DIAGNOS HIV BI -DOT. The results of the in-house study done are as follows:

No. of Samples	Status	DIAGNOS HIV BI-DOT	DIAGNOS HIV BI-DOT
		HIV Positive	HIV Negative
539	Western Blot Positive	539	-
1573	EIA Negative	-	1573

Sensitivity : 100% (539/539 Positive sera)
Specificity : 100% (1573/1573 Negative sera)

* Performance of the test has been also determined by National Aids Research Institute (NARI), Pune. The results are as follows:

Sensitivity : 100%
Specificity : 100%

Performance of the test has been determined by DCG(I), Drug Controller General of India and is approved for use in Blood Bank.

** This information is provided for the scientific community Equiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional propose.*

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

19. 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.

20. REFERENCES

1. Ajaka Z.L. et.al. (1994). HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients. The Lancet., 343, 1393-1394.
2. Aman E., Brosius J., (1985): "ATG Vectors" for Regulated High level Expression of Cloned Genes in *E. coli* Gene, 40, 183-190.
3. Dolt J., Sotz T., Schafer, T., Bergmann, C., (1968): Expression, Secretion and Processing of Hirudin in *E. coli*, using the Alkaline Phosphatase Signal Sequence. FEBS Letter, 202, 373-377.
4. Guyader, M, Emerman, M, Sonigo, P., Clavel. F, Montaginer L., Alizon M., (1987): Genome Organization and Transactivation of the Human Immunodeficiency Virus Type 2. Nature, 326, 662-669.
5. Kan N.C. Franchini G., Wong-Staal F., Dukbois, G.C., Robey. W.G., Lautenber, J.A., Papas T.S., (1986): Identification of HTLV-III/LAV SOR Gene Product and Detection of Antibodies in Human Sera. Science, 231, 1553-1555.
6. Schauder B., Blocker H., Frank. R; Mc Carthly. J.E.G., (1987): Inducible Expression of Cloned Genes in *E. coli* Gene., 40, 183-190.
7. Schumacher T.T., Garrett P.E., Tegtmeyer G.E., Thomas D., (1988): Comparative Detection of Anti-HIV in Early HIV seroconversion. J. Clinical Immunoasay, 11, 130-131.
8. Smith B.B., Johnson K.S., (1988): Single-Step Purification of Polypeptides W Expressed in *Escherichia coli* fusion with Gluthathione S-Tranferase. Gene., 52, 279-283.
9. Gurtler L.G. et.al. (1994). A new subtype of Human Immunodeficiency virus type 1 (MVP-1580) from conversion. J. of Virology., 68, 1581-1585.

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