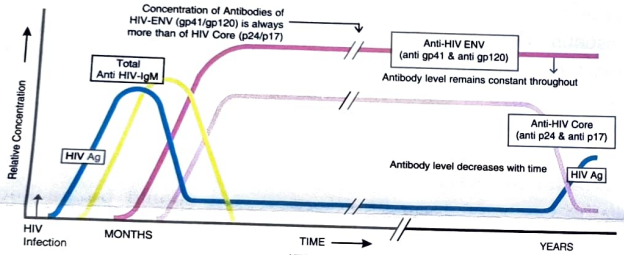
HIV TRI-DOT

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

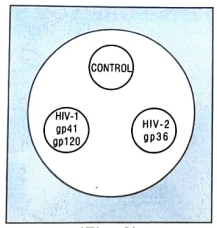
1. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

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

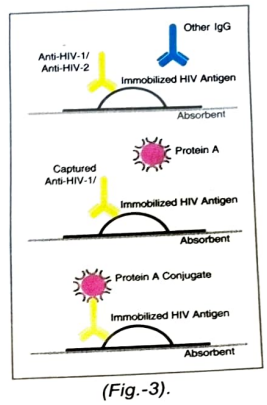
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2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

3. 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).

4. KIT DESCRIPTION

COMPONENTS	CONTENTS	PREPARATION
1. 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.
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.

5. 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.

6. STORAGE

Store the entire kit at 2-8°C in the coolest and driest area available. The components are stable for 24 months from the date of manufacturing, when stored at 2-8°C. Do not use the kit beyond the expiry date. DO NOT FREEZE THE KIT COMPONENTS.

7. KIT PRESENTATION

50 Test Pack
100 Test Pack
200 Test Pack

Signal® HIV 3D Ver. 1.0

Flow Through Anti-HIV Spot / Immunodot Test Kit

REF : 51FT102-10, 51FT102-50
Σ : 10, 50

IVD +2 °C +8 °C

INTENDED USE

Signal® HIV 3D Ver. 1.0 - Flow Through Anti-HIV Spot / Immunodot Test Kit is an *in vitro* qualitative screening test for diagnosis of HIV-1 (including Group O) and HIV-2 infection using human serum or plasma.
The assay is intended for use by skilled Health Care Workers / Laboratory Professionals, for an *initial* screening only. Reactive samples should be confirmed by a confirmatory assay.

INTRODUCTION

HIV infection, without treatment, progresses through three stages : acute infection, clinical latency and AIDS (Acquired immunodeficiency syndrome). Globally, 36.7 million people are living with HIV infection.[1] Although HIV-1 infection is associated with most of the global AIDS pandemic, HIV-2 is an important cause of disease in West Africa where it is endemic, but has now spread to other parts of Africa, Europe, India and United States.[2] By crude estimates, approximately one to two million of these people are infected with HIV-2 including some who are dually infected with both viruses.[3]
HIV-1 and HIV-2 belong to retrovirus group and are slow viruses. The modes of transmission for HIV-1 and HIV-2 are the same, namely sexual contact, blood-borne exposure (blood transfusion, shared needles), organ or tissue transplant and perinatal transmission.[2] The clinical manifestations of HIV-2 AIDS are similar to those for HIV-1 and only minor differences in pathology resulting from HIV-2, compared to HIV-1, infection have been observed Most of the assays utilized to detect nucleic acids (NAT) are complex, technically demanding or inappropriate for non-specialist diagnostic laboratories. Serological testing identifies HIV antigen and/or antibody generated as part of the immune response to infection with HIV. Serological studies indicate that HIV-1 and HIV-2 share multiple common epitopes in their core region but the envelope glycoproteins are fairly conserved and much less cross-reactive.[4]
Signal® HIV 3D Ver. 1.0 - Flow Through Anti-HIV Spot / Immunodot Test Kit explores combination of recombinant antigen gp41 of HIV-1 and gp36 of HIV-2 *which detects anti-HIV antibodies including* antibodies to HIV-1 O group *offering high specificity and sensitivity.*

PRINCIPLE

1. Mixture of recombinant antigens of HIV-1 and HIV-2 [◇, ○] are immobilised at 'T' region and recombinant HIV-1 antigen [○] is immobilised at '1' region on the porous membrane of the Test Device.
2. As the sample flows through the membrane, if anti-HIV-1 and / or 2 [and / or] antibodies are present in the specimen, they get trapped by immobilised antigens. Non-specific antibodies are filtered through in washing step.
3. Colloidal gold protein A reagent [■] is added in the next step which gets bound with immobilised HIV antigen anti-HIV antibody complex and give pinkish red colour dot / dots at test 'T' and / or '1' regions.

4. An inbuilt immobilised control at 'C' region ensures the test validity.

KIT CONTENTS AND DESCRIPTION

REAGENTS

Reagent No.	Reagent Name	Content
Reagent 1	Test Device	Plastic Device enclosing the nitrocellulose membrane onto which mixture of recombinant antigens for HIV-1 and HIV-2 are immobilised at 'T' region, recombinant HIV-1 antigen is immobilised at '1' region and Control Reagent is immobilised at 'C' region
Reagent 2	Wash Buffer	Physiological buffer containing detergent, protein stabiliser and preservative
Reagent 3	Signal Reagent	Protein-A Colloidal Gold reagent in a leakproof dropping bottle

CCESSORIES (*QS - Quantity Sufficient)
posable Plastic Droppers

REAGENT STORAGE AND STABILITY

All kit components are stable until the expiry date printed on the label, when stored between +2 °C to +8 °C. Store the kit in cool and dry place and protect it from direct sunlight.

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

1. Timer
2. Disposable Waste Bag

BIOSAFETY

1. Handle all the samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them off as biohazard waste.
3. Wear protective laboratory clothings in laboratory areas.
4. Do not smoke, eat or drink in area where samples are being handled.
5. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions.
6. Avoid spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of 5% freshly prepared sodium hypochlorite and dispose off the cleaning material by a suitable method.
7. Remnants of samples and used Test Devices should be collected in a waste container. Discard them as biohazard waste in a suitable container. The containers should be finally incinerated or autoclaved at 121 °C for 1 hour.
8. Any accessories which come in direct contact with specimen, and used Test Devices should be considered as contaminated product and should be treated appropriately.
9. Wash hands thoroughly with disinfectant after completion of the test.

SPECIMEN COLLECTION AND HANDLING

Specimen	Storage at	Stability	Remarks
Serum/ plasma	+2 °C to +8 °C	Short term	Samples containing clots or debris should be centrifuged prior to use. Use fresh non-hemolysed, unheated serum or plasma for performing the test. Grossly hemolysed / contaminated or lipemic samples should not be used. The test shows best results with freshly collected sample. The test performs well with frozen sample provided it is not repeatedly frozen and thawed.
	-20 °C or lower	Long term	

PRECAUTIONS

1. For every new kit, check the intactness of the reagent bottle, do not use the reagents if they are opened or leaked.
2. Do not use reagents after expiry date.
3. Do not use the reagents from different lots of kits.
4. Do not use the reagents, if they appear turbid or discoloured.
5. Open the sealed pouch at the time of assay performance only after it attains the *room temperature.
6. Gently mix the reagent and sample prior to use.
7. Do not over tighten the bottle cap, as it can lead to increased nozzle size of the bottle cap resulting in large drop volume.
8. Do not interchange vials or bottle caps.
9. Add all the reagents and sample to the center of the sample loading area of the Test Device.
10. Always allow each reagent to fall freely from the dropper tip by holding the bottle vertically over the Test Device.
11. Do not touch the dropper tip to the surface of nitrocellulose membrane in the device.

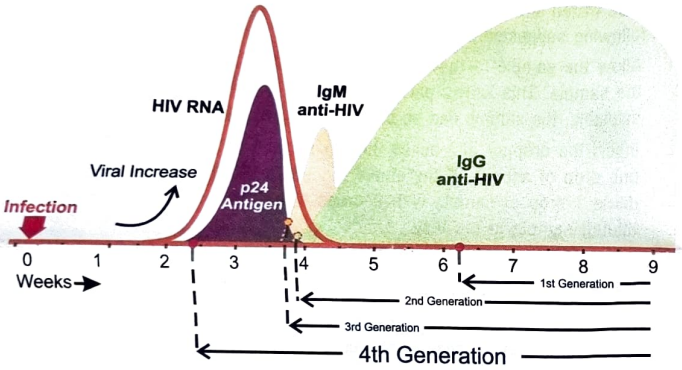
Third Line

4th Generation
HIV TRI-DOT + Ag

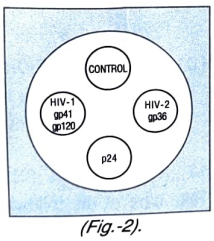
Rapid Visual Test for the Qualitative Differential Detection of HIV-1 p24 antigen and Antibodies (IgM, IgG & IgA) to HIV-1 & HIV-2 in Human Serum/Plasma

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. 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

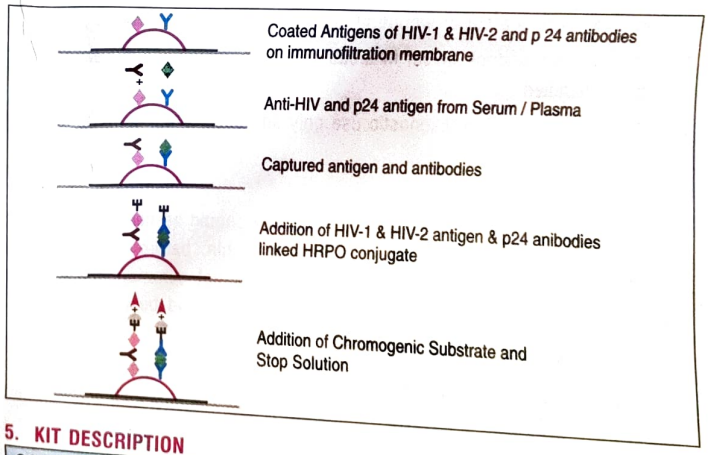
The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number / Batch Number		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
	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.