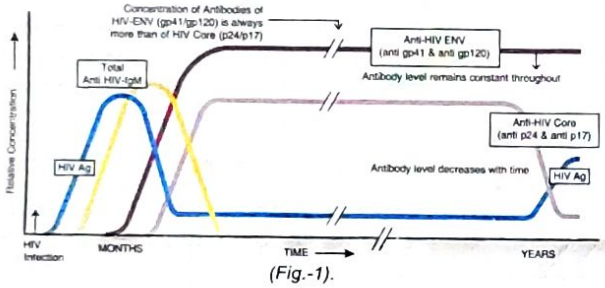
HIV TRI-DOT

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

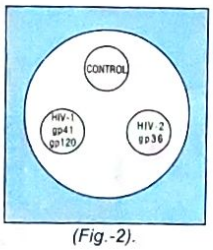
1. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

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

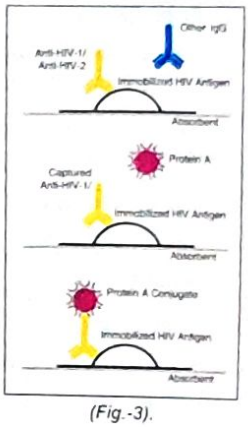
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2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

TRUSTline HIV 1/2 Ab Rapid Test – Cassette

REF AR0011C IVD

for the Detection of Antibodies to HIV 1 and 2 in Human Serum / Plasma / Whole Blood

INTENDED USE

The TRUSTline HIV 1/2 Ab Rapid Test is intended for use by healthcare professionals and is a rapid, qualitative, screening, lateral flow immunoassay for the simultaneous detection and differentiation of HIV-1 and HIV-2 antibodies (IgG, IgM, IgA) in human serum, plasma or whole blood. The test kit is not automated and does not require any additional instrument. Any reactive specimen with the TRUSTline HIV 1/2 Ab Rapid Test must be confirmed with alternative testing method(s) such as ELISA, Western Blot assay or PCR.

SUMMARY AND EXPLANATION OF THE TEST

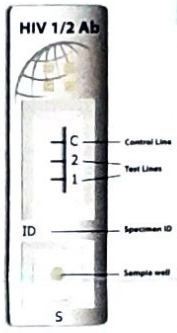
Human immunodeficiency virus type I and type II (HIV-1 and HIV-2) are enveloped, single-stranded, positive-sense RNA viruses. The causative relationship between HIV-1 and HIV-2 virus and acquired immunodeficiency syndrome (AIDS) has been established over several decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex and from healthy individuals with a high risk for developing AIDS[1]. HIV-2 has been isolated from West African AIDS patients and from sero-positive asymptomatic individuals[2].

The two types of HIV have significant variation in sequences. HIV-1 has been divided into three groups: group M (for major) including at least ten subtypes (A through J); group O (for outlier); and group N (for non-M, non-O). Similarly, HIV-2 has been classified into at least five subtypes (A through E). Some HIV-1 variants share up to 50% homology in their envelope genes with the sequences of more common prototype strains.

Both HIV-1 and HIV-2 can elicit strong immune responses including the production of anti-virus antibodies[3]. Presence of specific anti-HIV-1 and/or anti-HIV-2 in blood, serum or plasma indicates exposure of an individual to HIV-1 and/or HIV-2 and thus is of great value for clinical diagnosis[4].

The TRUSTline HIV 1/2 Ab Rapid Test was developed to detect and differentiate anti-HIV-1 and anti-HIV-2 (IgG, IgM, IgA) in serum, plasma or whole blood. The test can be performed by minimally trained personnel and without cumbersome laboratory equipment.

TEST PRINCIPLE



The TRUSTline HIV 1/2 Ab Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant HIV-1 antigen conjugated with colloidal gold (HIV-1 conjugates), recombinant HIV-2 antigen conjugated with colloidal gold (HIV-2 conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (1 and 2) and a control line (C). Test line 1 is pre-coated with HIV-1 antigen for the detection of antibodies to HIV-1, test line 2 is pre-coated with HIV-2 antigen for the detection of antibodies to HIV-2, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the strip. HIV-1 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-1 conjugates. The immune-complex is then captured on the membrane by the pre-coated HIV-1 antigen forming a burgundy colored line at test line 1, indicating a HIV-1 antibody positive or reactive test result. Lack of color development on test line 1 suggests an HIV-1 antibody negative or non-reactive result.

HIV-2 antibodies, if present in the specimen, migrate through the conjugate pad where they bind to the HIV-2 conjugates. The immune-complex is then captured on the membrane by the pre-coated HIV-2 antigen forming a burgundy colored line at test line 2, indicating a HIV-2 antibody positive or reactive test result. Lack of color development on test line 2 suggests a HIV-2 antibody negative or non-reactive result.

The test contains an internal control (C line), which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of color development on the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Specimen transfer device
3. Sample diluent (5 mL/bottle)
   Package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test
3. Alcohol swab
4. Disposable gloves

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use the test device if pouch is not intact.
4. Do not use expired devices or components.
5. Bring all reagents to room temperature (15-30°C) before use.
6. Do not use the components of different lots and of any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolyzed blood for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. Handle the negative and positive controls in the same manner as the patient specimens.
13. The test result should be read 15 minutes after a specimen is applied to the sample well of the device. Reading the test result after 20 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.
15. Clean up spills thoroughly using appropriate disinfectant.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store test kit at 1-30°C. If stored at 2-8°C, ensure that all reagents are brought to room temperature before opening. The sample diluent (opened and unopened) and unopened test device is stable through the expiration date printed on the label, when stored at recommended temperature. Do not freeze the kit or expose the kit to temperatures above 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Blood

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®). Do not use hemolyzed blood for testing. Capillary blood (finger tip puncture) can be used directly without anticoagulant. Collect blood with Specimen transfer device and transfer it to sample well of device

Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.

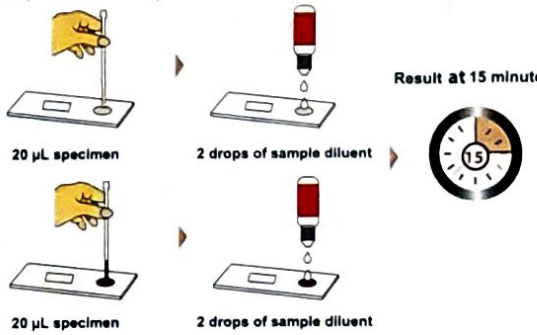
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the specimen transfer device with specimen (about 20 µL) not to exceed the specimen line as shown in the images below. **For better precision, transfer specimen using a pipette capable of delivering a 20 µL volume.**

Holding the specimen transfer device vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Immediately add 2 drops (60-80 µL) of sample diluent to the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Result should be read at 15 minutes.

Do not read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.

Signal® HIV

Flow Through HIV 1+2 Spot / Immunodot Test Kit

REF : 51FT100-10, 51FT100-50, 51FT100-60

Σ : 10, 50, 100

IVD +2 °C +8 °C

INTENDED USE

Signal® HIV - Flow through HIV 1+2 Spot / Immunodot Test Kit is a simple, rapid & an *in vitro* qualitative screening test for the detection of antibodies to HIV-1 and / or HIV-2 using human serum or plasma to diagnose HIV infection. The test does not require any additional instrument.

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

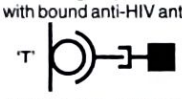
HIV infection, without treatment, progresses through three stages : acute infection, clinical latency and AIDS (Acquired immunodeficiency syndrome). Globally, 36.7 million people are living with HIV infection.[1] Although HIV-1 infection is associated with most of the global AIDS pandemic, HIV-2 is an 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 HIV2 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 HIV2 share multiple common epitopes in their core region but the envelope glycoproteins are fairly conserved and much less cross-reactive.[4]

Signal® HIV - Flow through HIV 1+2 Spot / Immunodot Test Kit uses a combination of recombinant gp 41 and gp 36 antigens derived from the conserved and immunodominant regions of HIV-1 and HIV-2 envelope proteins.

PRINCIPLE

1. Recombinant gp 41 and gp 36 antigens of HIV-1 and HIV- 2 [⊃] are immobilised at Test 'T' region on the porous membrane of the Test Device.
2. As the sample flows through the membrane, if anti-HIV-1 and / or anti-HIV-2 antibodies [⊃] 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 bound anti-HIV antibodies and give pinkish red colour dot at test 'T' region.



4. An inbuilt immobilised control at 'C' region ensures the test validity. A pinkish red coloured dot will always appear at the "Control area" during the test after application of test sample detecting presence of human immunoglobulins (IgG), irrespective of the presence or absence of HIV specific antibodies in the specimen.

KIT CONTENTS AND DESCRIPTION

REAGENTS

Reagent No.	Reagent Name	Content
Reagent 1	Test Device	Plastic Device enclosing nitrocellulose membrane onto which recombinant antigens cocktail specific to HIV-1 and HIV-2 is immobilised at 'T' region and control reagent 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 leak proof dropping bottle

ACCESSORIES (*QS-Quantity Sufficient)
Disposable Plastic Dropper and Rubber Teat

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT
Timer
Biohazard Disposable Container
Sharp Container
Pen / Pencil
Disposable Gloves

REAGENT STORAGE AND STABILITY

Storage at +2 °C to +8 °C, away from direct sunlight and excess humidity. The Test Device (Reagent 1), Wash Buffer (Reagent 2) & Signal Reagent (Reagent 3) are stable till expiry date in unopened condition. Once opened the Test Device should be used immediately. May be refrigerated. **Do not freeze**. The shelf life of the kit is as indicated on the outer package. **Do not use Test Device, Wash Buffer & Signal Reagent beyond the date of expiry.**

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 clothing in laboratory areas.
4. Do not pipette any reagent by mouth.
5. Do not smoke, eat or drink in area where samples are being handled.
6. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions.
7. Prevent splashing or 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.
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 (Up to 7 days)	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. Follow the assay procedure strictly, deviation will invalidate the results.
7. Once opened the Test Device should be used immdiately.
8. Test Device & disposable plastic droppers are single use items. DO NOT REUSE.
9. The reagent and sample should be brought to room temperature and shaken gently prior to use.
10. Do not over tighten the bottle cap, as it can lead to increased nozzle size of the bottle cap resulting in large drop volume.
11. Do not interchange vials or bottle caps.
12. Add all the reagents and sample to the center of the sample loading area of the Test Device.
13. Always allow each reagent to fall freely from the dropper tip by holding the bottle vertically over the Test Device.
14. Do not touch the dropper tip to the surface of nitrocellulose membrane in the device.
15. Caution should be taken while interpreting results with potentially interfering samples like hemolytic samples, Rheumatoid Factor containing samples, lipaemic samples, icteric samples & samples from neonates & newborn under the age of 14 months.

PROCEDURE

Note: Ensure that the Test Device and Reaction Buffer are at *room temperature before starting the assay procedure.

1. Remove the Test Device from pouch and label with the patient's identification number / name and keep it on a flat horizontal surface.
2. Add 2 drops (100 µL) of Wash Buffer to the Test Device.
   Note: After addition of sample or each of the reagent to Test Device, allow it to soak in completely before addition of next reagent.
3. Add 1 drop (50 µL) of patient's sample using the disposable plastic dropper (provided in the kit).
4. Add 2 drops (100 µL) of Wash Buffer.
5. Add 2 drops (100 µL) of Signal Reagent.
6. Add 3 drops (150 µL) of Wash Buffer.
7. Read the result within next 10 minutes.

Do not read beyond 10 minutes. READING TOO LATE CAN GIVE FALSE RESULTS.

***Note : As per USP room temperature is (+15 °C to +30 °C).**