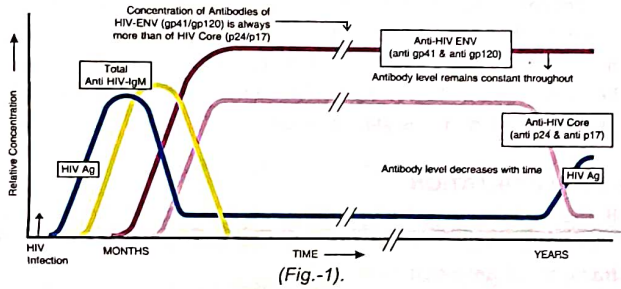
HIV TRI-DOT

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

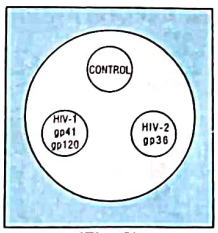
I. HISTORICAL REVIEW AND AETIOLOGY OF AIDS (Acquired Immuno Deficiency Syndrome)

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 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

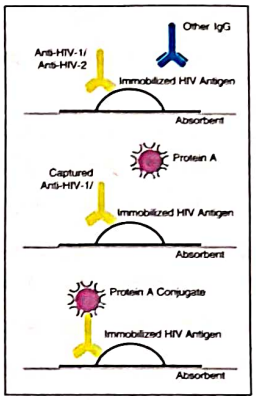
The HIV TRI-DOT Test is a visual, rapid, sensitive and accurate immunoassay for the differential detection of HIV-1 & HIV-2 antibodies (IgM, IgG & IgA) in Human Serum or Plasma using HIV-1 & HIV-2 Antigens immobilized on an immunofiltration membrane. The test is a screening test for anti-HIV-1 & anti-HIV-2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

8. 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 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.
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.

9. 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. Place white nozzle cap on Buffer Solution vial and red cap on Protein-A Conjugate Vial after use.
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.

10. SPECIMEN/SAMPLE COLLECTION & STORAGE

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

11. 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.

12. BEFORE YOU START

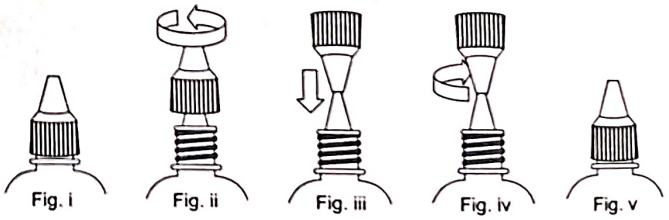


Fig. i Fig. ii Fig. iii Fig. iv Fig. v

The Buffer Solution and Protein-A Conjugate vials are provided with closed nozzle and screw cap with pin(outside), then punture the nozzle before use as given below:

1. Before using reagents, keep the vial vertically straight and tap down gently on the working platform, so that reagents come down at the bottom of the vial.
2. To orifice the closed nozzle, press the inverted cap on the respective closed nozzle and give a half turn twist to ensure nozzle is properly orificed/ punctured as illustrated below in Fig. iii & iv:

13. ASSAY PROCEDURE

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. DO NOT HEAT OR REPEATEDLY FREEZE/THAW SPECIMEN.

R.T. 20-30°

2. Place the required number of HIV TRI-DOT test devices at the working area.

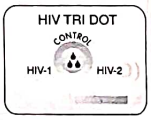
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.
4. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.
   However drops of each solution should be added in continuous stream to wet the entire area of membrane.
5. If the solution does not soak-in within 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".

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. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.

14. 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 (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.

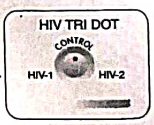
Read results immediately and discard the device considering it to be potentially infectious.

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

15. INTERPRETATION OF RESULTS

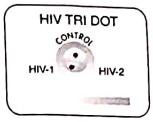
NON-REACTIVE

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.

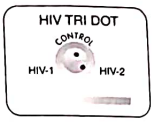


REACTIVE

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



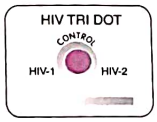
2. If two DOTS, one for the control and the other for HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-2.



3. If all the three DOTS, one each for control, HIV-1 & HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1 & 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/reagens or particulate matter in the specimen. The specimen should be centrifuged at 10,000 rpm for 15 minutes and re-run the test using new device (Refer Specimen/ sample processing).



(If the problem persists, please call our Technical/ Customer service cell, Parwanoo, Himachal Pradesh, Phone: 01792-232253).

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.***

16. 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.

17. PERFORMANCE CHARACTERISTICS

Performance of the HIV TRI-DOT with reference to sensitivity and specificity has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1325 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HIV status were fully comparable with those of HIV TRI -DOT. The results of the in-house study done are as follows:

No. of Samples	Status	HIV TRI-DOT	HIV TRI-DOT
		+ ve	- ve
50	ELISA +ve	50	-
1275	ELISA -ve	1	1274

Sensitivity: 100%

Specificity: 99.84%

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of 10 samples: 7 HIV-1 positive (1 strong, 1 moderate & 5 weak), 1 HIV-2 positive and 2 HIV negative. The C.V.(%) of all the samples were within 10%.

18. 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.

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

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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.

R-07

in vitro diagnostic reagent, not for medicinal use

Manufactured & Marketed By:

DIAGNOSTIC ENTERPRISES

Plot No.: 26, Indl. Estate, Sector-1, Parwanoo - 173 220, (H.P.)

Phone: 01792-232253 E-mail: de@diagnosticenterprises.com

MN/HTD/001

Quality approved by SD BIOSENSOR / For *In vitro* use only

STANDARD Q

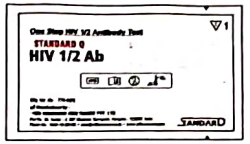
HIV 1/2 Ab Test

STANDARD Q HIV 1/2 Ab Test

PLEASE READ BACK PAGE CAREFULLY BEFORE YOU PERFORM THE TEST

STANDARD

[Materials Provided]



Cassette

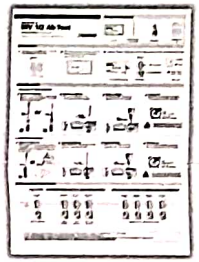
Specimen transfer device (10 µl)

Buffer Bottle

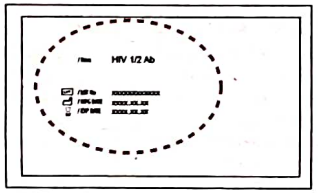
Instructions for use

[Preparation]

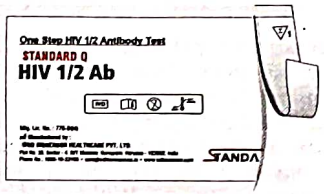
1 Carefully read instructions for using the STANDARD Q HIV 1/2 Ab Test.



2 Check the expiry date at the back of the cassette packaging. Use another lot, if expiry date has passed.



3 Open the Cassette Packaging, and check the Cassette and the color indicator silica gel.



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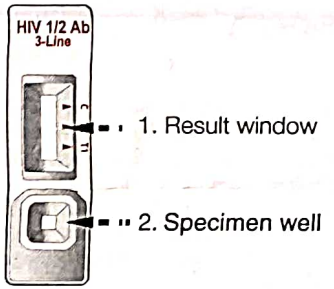

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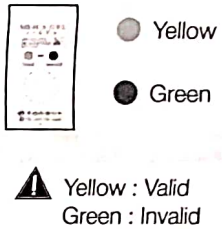
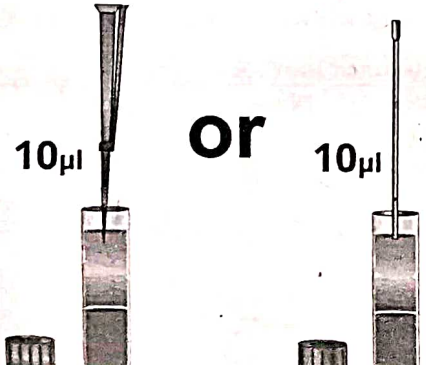


<Silica gel>

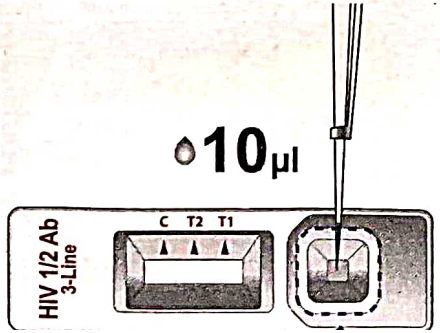
[Test Procedure]

1. For Serum or Plasma specimen

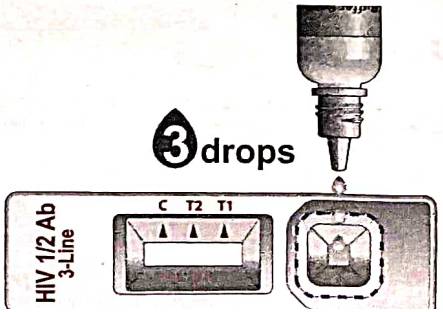
1 Specimen Collection
Using a micropipette or specimen transfer device collect 10µl (till the black line) of serum or plasma.



2 Specimen Addition
Add serum or plasma to the specimen well of the cassette.



3 Buffer Addition
Add 3 drops of buffer into the specimen well of the cassette.



4 Reading Time
Read the test results after 10 minutes.
The test can be read up to 20 minutes.

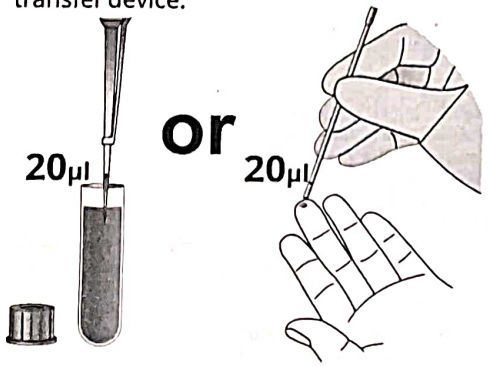
Read
After 10 mins
Do not read
After 20 minutes

CAUTION

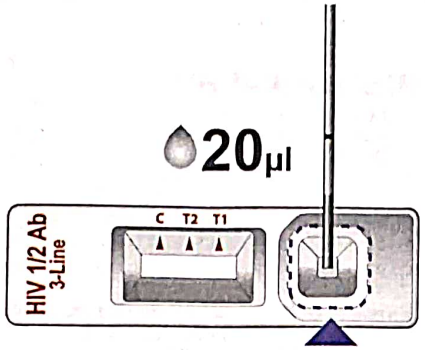
Do not read test results after 20 minutes. It may give false results.

2. For whole blood specimen

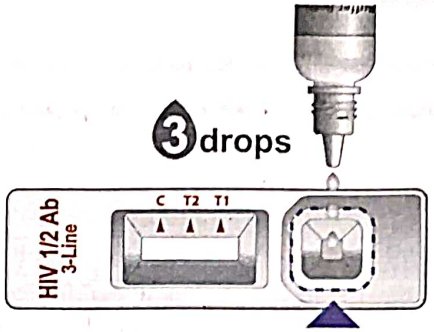
1 Specimen Collection
Collect 20µl of whole blood by using a micropipette or collect two times 10µl of whole blood till the black line of specimen transfer device.



2 Specimen Addition
Add collected 20 µl whole blood to the specimen well of the Cassette.



3 Buffer Addition
Add 3 drops of buffer into the specimen well of the cassette.

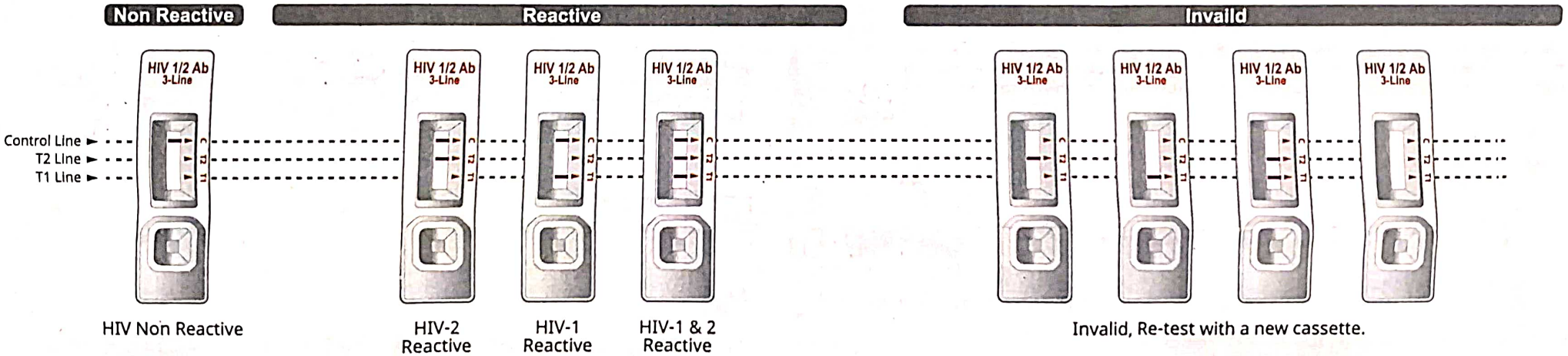


4 Reading Time
Read the test results after 10 minutes.
The test can be read up to 20 minutes.

Read
After 10 mins
Do not read
After 20 minutes

CAUTION
Do not read test results after 20 minutes. It may give false results.

[Interpretation of Test Result]



HIV Non Reactive | HIV-2 Reactive | HIV-1 Reactive | HIV-1 & 2 Reactive | Invalid, Re-test with a new cassette.

1. A colored band will appear in the top section of the result window to show that the test is working properly. This band is the control line (C).
2. A colored bands will appear in the lower section of the result window. These bands are test lines (T1 and T2).
3. Even if the test line is faint, or the test line is not uniform, the test should be considered to be performed properly and the test result should be interpreted as a reactive result.

* **Reactive results should be considered in conjunction with the clinical history and other data available to the clinician.**

EXPLANATION AND SUMMARY

[Introduction]

AIDS is caused by two known types of HIV (human immunodeficiency virus), HIV type 1 and HIV type 2. HIV type 1 (HIV-1) is found in patients with AIDS, AIDS-related complex (ARC), and asymptomatic infected individuals at high risk for AIDS. The virus is transmitted by sexual contact, by exposure to infected blood or blood products, or from an infected mother to her fetus or infant. The infection of HIV type 2 (HIV-2) is endemic only in West Africa, and it has been identified in individuals who had sexual relations with individuals from that geographic region. HIV-2 is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism, and the modes of transmission appear to be identical. Within the two major HIV types, there is significant variation. HIV-1 has been divided into four groups: group M (for major)-including at least ten subtypes, group O (for outlier), group P, and group N (for non-M, non-O). Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E). STANDARD Q HIV 1/2 Ab Test can detect both HIV-1 antibody and HIV-2 antibody, which first appear significantly later, 20-45 days after infection. Detecting HIV earlier with STANDARD Q HIV 1/2 Ab Test is helpful to prevent future transmission during extremely infectious stage.

[Intended use]

STANDARD Q HIV 1/2 Ab Test is a rapid, qualitative immunoassay to detect circulating antibodies against HIV in human serum, plasma or whole blood. The test is for in vitro diagnostic use and is intended as an aid to early diagnosis of HIV infection. This is intended for professional use, only for an initial screening test.

[Test principle]

STANDARD Q HIV 1/2 Ab Test has "T1", "T2" and "C" line pre-coated with recombinant HIV-1 gp41 protein / recombinant HIV-1 subtype O gp41, recombinant HIV-2 gp36 protein and monoclonal anti-chicken IgG respectively. The anti-HIV-1/anti-HIV-1 subtype O in patient sample interacts with the recombinant HIV-1 gp41-gold / recombinant HIV-1 subtype O gp41-gold and the anti-HIV-2 in patient sample interacts with the recombinant HIV-2 gp36-gold in the conjugation pad. The complex of gold conjugated antigens and antibodies moves along the membrane chromatographically to the membrane with assay diluent and is captured by the HIV antigens on the test regions (T1 and T2). If the antibodies against HIV are in the patient sample, visible lines are formed in the test region. The control line should always appear if the test procedure is performed properly.

[Materials Provided]

Components	
Cassette	Specimen transfer device (10 µl)
Buffer Bottle	Instruction for use

KIT STORAGE AND STABILITY

Store the RDT Box at room temperature, 2-40°C / 36-104°F out of direct sunlight. Materials provided are stable until the expiration date printed on the RDT box. Do not freeze.

SPECIMEN COLLECTION AND PREPARATION

[Serum]

1. Collect the whole blood into the commercially available plain tube, NOT containing anti-coagulants such as heparin, EDTA or sodium citrate, by venipuncture and leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
2. If serum in the plain tube is stored in a refrigerator at 2-8°C / 36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen in the long-term keeping more than 1 week can cause non-specific reaction. For prolonged storage, it should be at below -20°C / -4°F.
3. It should be brought to room temperature prior to use.

[Plasma]

1. Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin, EDTA or sodium citrate by venipuncture and centrifuge blood to get plasma specimen.
2. If plasma in an anti-coagulant tube is stored in a refrigerator at 2-8°C / 36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen in the long-term keeping more than 1 week can cause non-specific reaction. For prolonged storage, it should be at below -20°C / -4°F.
3. It should be brought to room temperature prior to use.

[whole blood]

- **Capillary whole blood**

1. Capillary whole blood should be collected aseptically by fingertip.
2. Clean the area to be lanced with an alcohol swab.
3. Squeeze the end of the fingertip and pierce with a sterile lancet.
4. Collect the capillary whole blood till the black line of the specimen transfer device for testing.
5. The capillary whole blood must be tested immediately after collection.

- **Venous whole blood**

1. Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin, EDTA or sodium citrate by venipuncture.
2. If venous whole blood in an anti-coagulant tube is stored in a refrigerator at 2-8°C / 36-46°F, the specimen can be used for testing within 1-2 days after collection.
3. Do not use hemolyzed blood specimens

CAUTION

- Anticoagulants such as heparin or EDTA do not affect the test result.
- As known relevant interference, haemolytic specimen, rheumatoid factors-contained specimen and lipaemic, icteric specimen can lead to impair the test results.
- Use separate disposable materials for each specimen in order to avoid cross-contamination which can cause erroneous results.

TEST PROCEDURE

[Preparation]

1. Carefully read instructions for using the STANDARD Q HIV 1/2 Ab Test.
2. Check the expiry date at the back of the cassette package. Use another lot, if expiry date has passed.
3. Open the cassette packaging, and check the cassette and the silica gel pack in the cassette packaging.
4. Methods for following steps can be changed depending on the specimen or specimen transfer device.

[Test Procedure]

- **For serum or plasma specimen**

1. Using a micropipette or specimen transfer device collect 10µl (till the black line) of serum or plasma.
2. Add the collected serum or plasma to the specimen well of the cassette.
3. Add 3 drops (90 µl) of buffer into the specimen well of the cassette.
4. Read the test results after 10 minutes. Test can be read up to 20 minutes.

- **For Whole blood specimen**

1. Collect 20µl of whole blood by using a micropipette or collect two times 10µl of whole blood till the black line of specimen transfer device.
2. Add the collected whole blood to the specimen well of the cassette.
3. Add 3 drops (90 µl) of buffer into the specimen well of the cassette.
4. Read the test results after 10 minutes. Test can be read up to 20 minutes.

CAUTION

- Do not read test results after 20 minutes. It may give false results.

INTERPRETATION OF TEST RESULTS

Non-Reactive

The presence of only control line (C) within the result window indicates that the specimen is non-reactive for antibodies to HIV-1 and/or HIV-2.

Reactive

1) The presence of two lines as control line (C) and test line (1) within the result window indicates that the specimen is reactive for antibodies to HIV-1.
2) The presence of two lines as control line (C) and test line (2) within the result window indicates that the specimen is reactive for antibodies to HIV-2.
3) The presence of three lines as control line (C), test line (1) and test line (2) within the result window indicates that the specimen is reactive for antibodies to HIV-1 and HIV-2.

Invalid

If the control band ("C" Control line) is not visible within the result window, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. Re-test with a new cassette.

Caution: There is an homology in the amino acid sequence between HIV-1 and HIV-2 due to which they have a cross reactivity of 30-70%. Hence, appearance of test lines for both HIV-1 and HIV-2 antibodies on the cassette does not necessarily imply co-infection from HIV-1 & HIV-2. To determine the virus type or diagnose a co-infection accurately, a confirmatory test such as Western Blot or PCR must be performed.

LIMITATION OF TEST

1. The test should be used for the detection of antibodies to HIV in human serum, plasma or whole blood specimens.
2. Neither the quantitative value nor the rate of antibodies to HIV concentration can be determined by this qualitative test.
3. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
4. A negative test result may occur if the level of extracted antibody in a sample is below the sensitivity of the test or if a poor-quality specimen is obtained.
5. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
6. The test result must always be evaluated with other data available to the physician.

PERFORMANCE CHARACTERISTICS

1. The sensitivity of STANDARD Q HIV 1/2 Ab Test : Total 35 specimen were evaluated for sensitivity. The STANDARD Q HIV 1/2 Ab Test Kit got a high correlation with CLIA test.

Reference		STANDARD Q HIV 1/2 Ab		Total Result
		Reactive	Non reactive	
CLIA Analyzer	Positive	35	0	35
	Negative	0	0	0
Total Result		35	0	35
Sensitivity		35/35 (100.0%)		

2. The specificity of STANDARD Q HIV 1/2 Ab Test : Total 165 specimen were evaluated for specificity. The STANDARD Q HIV 1/2 Test Kit got a high correlation with CLIA test.

Reference		STANDARD Q HIV 1/2 Ab		Total Result
		Reactive	Non reactive	
CLIA Analyzer	Positive	0	0	0
	Negative	2	163	165
Total Result		2	163	165
Specificity		163/165 (98.8%)		

WARNINGS AND PRECAUTIONS

1. Do not re-use the kit.
2. Do not use the kit if the cassette package is damaged or the seal is broken.
3. Do not use the buffer bottle of another lot.
4. Do not smoke, drink or eat while handling specimen.
5. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after the tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout testing procedures.
9. Dispose off all specimens and materials used to perform the test as bio-hazard waste. Laboratory chemical and bio-hazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
10. Silica gel in cassette packaging is to absorb moisture and keep humidity from affecting products. If the moisture indicating silica gel beads change from yellow to green, the cassette in the pouch should be discarded.
11. Discard the cassette immediately after reading result.

BIBLIOGRAPHY

1. Report of the WHO evaluation (Phase 1) of the SD Bioline HIV-1/2 3.0 at WHO collaborating center for Transfusion Transmitted Infectious Department of Institute of Tropical Medicine in Antwerp, Belgium. (2002)
2. Owen SM et al. Alternative Algorithms for Human Immunodeficiency Virus Infection Diagnosis Using Tests That Are Licensed in the United States. J Clin Microbiol 46:1588-1595, 2008.
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Product Disclaimer

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the SD BIOSENSOR and distributor and the result may accordingly be affected by environmental factors and/or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

Warning

The SD BIOSENSOR and distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect of consequential arising out of or related to an incorrect diagnosis, whether reactive or non reactive, in the use of this product.

CAT **RK019-30**
Issue date : 2018. 01

SD/019/IN/01-01

Manufactured by

SD BIOSENSOR HEALTHCARE PVT. LTD.

Corporate Office
Unit No-202 A-D, 2nd Floor, Tower-A, Unitech Signature Towers South City 1, Gurugram, Haryana-122001, India

Manufacturing site
Plot No. 38, Sector - 4, IMT Manesar, Gurugram, Haryana-122052, India

Any inquiries regarding the instruction provided should be addressed to:
care@sdbiosensor.co.in

www.sdbiosensor.com

Toll Free No.
1800-10-23105

CAT Catalogue number | Note | IVD In vitro Diagnostics | Consult Instructions for Use | Contains Sufficient for <n> Tests | Caution | Do not re-use | To indicate the temperature limitation in which the package has to be stored | Date of manufacture | Use by | LOT Batch code | Manufacturer

WARRANTY

This product is designed to perform as described on the label and pack[illegible]nsert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-111) from patients with AIDS and Pre-AIDS, Mikulas Popovic, et al., Science, Vol. 224, 497-500, 4 May 1984. (2) Frequent detection and isolation of Cytopathic Retroviruses (HTLV-111) from patents with AIDS and at risk of AIDS. Robert C. Gallo, et al., Science, Vol. 224, 500-502, 4 May 1984. (3) Serological analysis of a subgroup of Human T-Lymphotropic Retroviruses (HTLV-111) associated with AIDS, Jorg Schupbach, et al., Science, Vol. 224, 503-505, 4 May 1984. (4) Retroviruses (HTLV-111) in the serum of patients with AIDS, M.G. Sarangadharan, et al., Science, Vol. 224, 506-508, 4 May 1984. (5) A field test for the detection of antibodies to Human Immunodeficiency Virus Types 1 and 2 in Serum or Plasma, Anthony Burgess-Cassler, et al., Clinical and Diagnostic Laboratory Immunology, Vol. 3 No.4, 480-482, 1996. (6) Current concepts in Human Immunodeficiency Virus Infection and AIDS, Stanley A.Schwartz, Madhavan P.N.Nair, Clinical and Diagnostic Laboratory Immunology, Vol.6 No.3, 295-305, May 1999. (7) Diagnosis of Human Immunodeficiency Virus Type 1 infection with different subtypes using rapid tests, Susan Phillips, et al., Clinical and Diagnostic Laboratory Immunology, Vol.7 No.4, 698-699, July 2000. (8) Principle and Practice of Infectious Diseases, Mandell, Bennett and Dolin, 5th Ed., Vol 1-Part 11, 1332-1528, 2000, Churchill Livingstone Publications. (9) Rebecca D. Saville, Niel T Constatine, Farley R. Cleghorn et.al., Fourth generation Enzyme-linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody, Journal of Clinical Microbiology, July 2001, p 2518-2524. (10) Data on file: Viola Diagnostic Systems.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use		Date of Manufacture
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device		This side up
	Use by	REF	Catalogue Number	DEVICE	Device
Σ	Contains sufficient for <n> tests	LOT	Batch Number / Lot Number	CON	Protein A Gold Conjugate
2	Do not reuse	BUF	Wash Buffer		

Manufactured by:

Viola Diagnostic Systems

A Division of Tulip Diagnostics (P) Ltd.

Plot No. 33, Sector-3, I.I.E. SIDCUL, Pantnagar, U. S. Nagar, Uttarakhand - 263 153. INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

0117/VER-01

Retroquic® HIV

RAPID IMMUNOCONCENTRATION TEST FOR HIV 1 AND HIV 2 ANTIBODIES

DEVICE

INTRODUCTION

Retroquic-HIV is a membrane based flow through immunoassay for the detection of antibodies to HIV 1 and HIV 2 in human serum and plasma. Highly purified synthetic peptides of gp 120 and gp 41 (HIV 1) and gp 36 (HIV 2) corresponding to the immunodominant regions of the HIV 1 and HIV 2 utilized in the test system assist in visual, qualitative, simultaneous detection and differentiation of antibodies to HIV 1 and 2.

SUMMARY

Acquired Immuno Deficiency Syndrome (AIDS) is caused by at least two retroviruses, the HIV 1 and the HIV 2, collectively referred to as HIV 1/2. Antibodies to HIV 1 envelope protein (gp 120), transmembrane protein (gp 41) and HIV transmembrane protein (gp 36) are prevalent in sera of individuals with AIDS or ARC or who are at high risk of contracting AIDS. Detection of these antibodies indicates exposure to the HIV 1/2 virus.

PRINCIPLE

Retroquic-HIV test comprises of a test device striped with distinct bands of purified gp 120 and gp 41 synthetic peptides specific to HIV 1 at test region '1' and gp 36 synthetic peptide specific to HIV 2 at test region '2'. The third band striped at region 'C' corresponds to the assay performance control. First the membrane assembly is hydrated with wash buffer and then specimen is added. Antibodies to HIV 1 and/or 2 if present, are captured by the respective antigens. After washing with wash buffer, Protein A conjugated gold sol reagent is added to reveal the presence/ absence of bound antibodies. Post final wash positive reaction is visualized by the appearance of purple coloured bands at the test region '1' and/or '2'. The absence bands at test region '1' & '2' is a negative test result. The appearance of control band serves to validate sample addition reagent and assay performance.

REAGENTS AND MATERIALS SUPPLIED

Kit Components:

Retroquic-HIV immunoconcentration test kit for HIV1 and HIV 2 antibodies comprises of the following components:

1. DEVICE Ready to use individually pouched, flow through test devices striped with HIV 1 specific purified synthetic peptides at test region '1' and HIV 2 specific purified synthetic peptides at test region '2' and a blue dyed protein A based control band at region 'C' along with a specimen dropper and desiccant pouch.
2. BUF Dropper bottle with ready to use wash buffer solution.
3. CON Dropper bottle with ready to use protein A conjugated gold sol solution.

REF	402020010	402020025	402020050	402020100
Σ	10 Tests	25 Tests	50 Tests	100 Tests

STORAGE AND STABILITY

The unopened **Retroquic-HIV** kit, as well as kit components upon opening, must be stored at 2-8°C, till the duration of shelf life as indicated on the kit / kit component labels.

NOTE

1. In vitro diagnostic test. NOT FOR MEDICINAL USE.
2. Read instructions carefully before performing the test.
3. Do not use beyond expiry date.
4. Flow through device, wash buffer and protein A conjugate of the same lot are optimized as a system. It is important that the kit components of the same lot are used for achieving accurate and reproducible results. Do not intermix reagents from different lots.
5. The sequence of addition of reagents should be followed meticulously for achieving accurate results.
6. Handle all specimen as potentially infectious.
7. Follow standard bio safety guidelines for personal protection, handling and disposal of potentially infectious materials.
8. After use, the kit components must be returned to the recommended storage temperature immediately.

SPECIMEN COLLECTION AND PREPARATION

1. No prior preparation of the patient is required before sample collection by approved techniques.
2. Fresh serum / plasma is preferable. Serum / plasma may be stored at 2-8°C upto 24 hours in case of delay in testing. For long term storage, freeze the specimen at -20°C.
3. Repeated freezing and thawing of the specimen should be avoided.
4. Do not use haemolysed, clotted, contaminated, viscous / turbid specimen.

WARRANTY

This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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SUMMARY

Acquired Immuno Deficiency Syndrome (AIDS) is caused by at least two retroviruses, the HIV 1 and the HIV 2, collectively referred to as HIV 1/2. Antibodies to HIV 1 envelope protein (gp 120), transmembrane protein (gp 41) and HIV 2 transmembrane protein (gp 36) are prevalent in sera of individuals with AIDS or ARC or who are at high risk of contracting AIDS. Detection of these antibodies indicates exposure to the HIV 1/2 virus.

PRINCIPLE

Retroquic-HIV test comprises of a test device striped with distinct bands of purified gp 120 and gp 41 synthetic peptide specific to HIV 1 at test region '1' and gp 36 synthetic peptide specific to HIV 2 at test region '2'. The third band striped at region 'C' corresponds to the assay performance control. First the membrane assembly is hydrated with wash buffer and then the specimen is added. Antibodies to HIV 1 and/or 2 if present, are captured by the respective antigens. After washing with wash buffer, Protein A conjugated gold sol reagent is added to reveal the presence/ absence of bound antibodies. Post final wash a positive reaction is visualized by the appearance of purple coloured bands at the test region '1' and/or '2'. The absence of bands at test region '1' & '2' is a negative test result. The appearance of control band serves to validate sample addition, reagent and assay performance.

REAGENTS AND MATERIALS SUPPLIED

Kit Components:

Retroquic-HIV immunoconcentration test kit for HIV1 and HIV 2 antibodies comprises of the following components:

1. DEVICE Ready to use individually pouched, flow through test devices striped with HIV 1 specific purified synthetic peptides at test region '1' and HIV 2 specific purified synthetic peptides at test region '2' and a blue dyed protein A based control band at region 'C' along with a specimen dropper and desiccant pouch.
2. BUF Dropper bottle with ready to use wash buffer solution.
3. CON Dropper bottle with ready to use protein A conjugated gold sol solution.

REF	402020010	402020025	402020050	402020100
Σ	10 Tests	25 Tests	50 Tests	100 Tests

STORAGE AND STABILITY

The unopened **Retroquic-HIV** kit, as well as kit components upon opening, must be stored at 2-8°C, till the duration of the shelf life as indicated on the kit / kit component labels.

NOTE

1. In vitro diagnostic test. NOT FOR MEDICINAL USE.
2. Read instructions carefully before performing the test.
3. Do not use beyond expiry date.
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5. The sequence of addition of reagents should be followed meticulously for achieving accurate results.
6. Handle all specimen as potentially infectious.
7. Follow standard bio safety guidelines for personal protection, handling and disposal of potentially infectious material.
8. After use, the kit components must be returned to the recommended storage temperature immediately.

SPECIMEN COLLECTION AND PREPARATION

1. No prior preparation of the patient is required before sample collection by approved techniques.
2. Fresh serum / plasma is preferable. Serum / plasma may be stored at 2-8°C upto 24 hours in case of delay in testing. For long term storage, freeze the specimen at -20°C.
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REF	402020010	402020025	402020050	402020100
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1. No prior preparation of the patient is required before sample collection by approved techniques.
2. Fresh serum / plasma is preferable. Serum / plasma may be stored at 2-8°C upto 24 hours in case of delay in testing. For long term storage, freeze the specimen at -20°C.
3. Repeated freezing and thawing of the specimen should be avoided.
4. Do not use haemolysed, clotted, contaminated, viscous / turbid specimen.

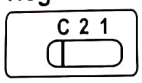
5. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
6. Do not heat -inactivate the specimen.
7. Frozen samples for retrospective studies must be centrifuged at 3000 rpm for 15 minutes and the clear supernatant must be used for tests.

TEST PROCEDURE

1. Bring all reagents and specimen to room temperature (25 - 30°C) before use. Tighten the Wash Buffer solution and Protein A Gold Conjugate dropper bottle caps in a clockwise direction to pierce the respective dropper bottle nozzles. The addition of specimen / reagents must be done at the centre of the reaction port, holding the sample dropper / dropper bottles in a vertical position. Ensure the drops are free falling. Use a new sample dropper for each specimen to avoid cross contamination.
2. Tear open the foil pouches and retrieve the required number of **Retroquic-HIV** membrane test devices and label appropriately.
3. Add two drops of wash buffer into the reaction port of the device and allow to soak through completely.
4. Using the sample dropper provided, add one drop of the serum / plasma specimen into the reaction port. Allow to soak through completely.
5. Add three drops of wash buffer to the reaction port and allow to soak through completely.
6. Add two drops of protein A gold conjugate to the reaction port and allow to soak through completely.
7. Add two drops of wash buffer and allow the wash buffer to soak through completely.
8. Read and record the results immediately.

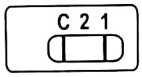
INTERPRETATION OF RESULTS

Negative Test Result

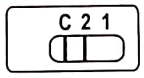


Appearance of only one control band corresponding to control region 'C'.

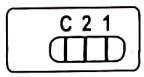
Positive Test Result



In addition to the control band 'C', appearance of reactive band at test region '1': Specimen positive for Antibodies to HIV 1.

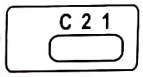


In addition to the control band 'C', appearance of reactive band at test region '2': Specimen positive for Antibodies to HIV 2.



In addition to the control band 'C', appearance of reactive bands at test region '1' and test region '2': Specimen positive for Antibodies to HIV 1 and HIV 2.

Invalid Test result



The test should be considered invalid if neither the test band nor the control band appears. In case of invalid results, the test should be repeated using a fresh device.

PERFORMANCE CHARACTERISTICS

In an in-house trial, one thousand and sixty four specimen negative for antibodies to HIV 1 and HIV 2 and hundred and forty five specimen positive for antibodies to HIV 1 and nineteen specimen positive for antibodies to HIV 1 and HIV 2 were run in parallel with a licensed, commercially available test and **Retroquic-HIV**. The results obtained are as follows:

Specimen	No. of samples	Licensed Test	Retroquic-HIV
Negative for Ab. to HIV 1/2	1064	1064	1064
Positive for Ab. to HIV 1	145	145	145
Positive for Ab. to HIV 1 & HIV 2	19	19	19

Based on the above study the sensitivity and specificity of **Retroquic- HIV** is 100% each.

External Evaluation:

Four hundred and forty four samples out of which, eighty anti HIV-1, eighty anti HIV-2 positive specimens and two hundred and eighty four anti-HIV negative specimens were tested with **Retroquic- HIV** at Institute of Tropical Medicine, AIDS Reference Laboratory, Belgium. The results of the evaluation are as follows:

Retroquic- HIV	Reference Results		
	HIV-1 positive	HIV-2 positive	HIV negative
HIV-1 positive	80	0	0
HIV-2 positive	0	70	0
HIV positive	0	10	0
HIV negative	0	0	284
Total	80	80	284

All 80 anti HIV-1 positive specimens and 80 anti HIV-2 positive specimens were detected as HIV-1 positive and HIV-2 positive by the assay, sensitivity 100%. The **Retroquic- HIV** was found to have a specificity (100%) on the HIV negative samples tested.

Sensitivity in HIV-1 non-B specimens:

Retroquic- HIV was evaluated to check the se[redacted]ity in 40 HIV-1 samples belonging to different serotypes. The results of the evaluation are as follows:

HIV Type	No. of Samples	Retroquic- HIV			
		HIV-1 positive	HIV-2 positive	HIV positive	HIV negative
HIV-1 positive	40	40	0	0	0

GAG	ENV	POL	No. of Samples
A	A		4
C	C		4
D	D		4
CRF01_AE	CRF01_AE		3
F	F		3
G	G		3
H	H		3
CRF01_AE			1
D	F		1
C	A		1
		J	3
		K	3
A	CRF01_AE		1
F	D		2
O	O		3
G	A		1
TOTAL			40

All 40 HIV-1 non-B were correctly positive identified as HIV-1 positive by **Retroquic- HIV**

Consistent performance by NARI Evaluation:

Year	Sensitivity	Specificity
2007	100%	100%
2008	100%	100%
2009	100%	100%

REMARKS

1. The addition of reagents must be accomplished without interruptions.
2. After addition of the wash buffer, in step 7 of the procedure, if the background in the reaction port is high, the samples must be recentrifuged appropriately so as to pellet invisible particulate matter. Test should be rerun with the clear supernatant.
3. The presence of antibodies to HIV 1 / 2 indicates previous exposure to HIV 1 and / or HIV 2 virus but does not constitute a diagnosis of AIDS.
4. Absence of antibodies to HIV 1 / 2 does not indicate that an individual is absolutely free of HIV 1 or HIV 2 as the collection of sample and its timing vis-à-vis sero-conversion will influence the test outcome.
5. Since HIV 1 and HIV 2 viruses are similar in genomic structure and morphology and antibodies to them have (30-70%) cross reactivity, reactive test bands for HIV 1 and HIV 2 do not necessarily imply mixed infection with HIV 1 & HIV 2.
6. Though **Retroquic-HIV** is a reliable and sensitive screening test, it should not be used as a sole criterion for diagnosis of HIV infection.
7. All positive specimen should be further tested using appropriate supplemental confirmatory tests.
8. As in all tests the results must be correlated with clinical findings before arriving at the final diagnosis.
9. Since various tests for HIV 1 / 2 differ in their performance characteristics and antigenic composition, the reactivity patterns may differ.
10. The results of **Retroquic-HIV** must be read within 30 minutes of test completion.
11. Do not compare the intensity of the test lines and the control lines to judge the concentration of the antibodies in the test sample.
12. Testing of pooled specimen is not recommended.
13. The control band in fresh unused membrane test devices is blue coloured and changes to deep purple colour after test performance.
14. The control band would not develop if the sample addition has not been done.
15. Sample volume (25µl) other than the prescribed volume if used may lead to discordent result.

WARRANTY

This product is designed to perform as described on the label and pack[illegible]nsert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up
Use by	REF — Catalogue Number	DEVICE — Device
Contains sufficient for <n> tests	LOT — Batch Number / Lot Number	CON — Protein A Gold Conjugate
Do not reuse	BUF — Wash Buffer	

Manufactured by:

Viola Diagnostic Systems

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