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

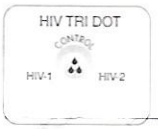
3. Tear off the pouch and take out the device for performing the test. Write the sample number to be tested on the device.
4. While adding sample/reagents to the device, be sure to ALLOW EACH SOLUTION TO SOAK IN BEFORE ADDING THE NEXT SOLUTION.
   However drops of each solution should be added in continuous 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.

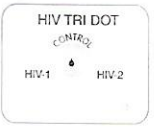
7. For consistent results, ensure FREE FALLING OF DROPS on the membrane.
8. Do not use kit components beyond the expiration date.
9. The liquid conjugate should not be subjected to frequent temperature fluctuations.
10. The procedural sequence of reagent addition should be strictly adhered to avoid any discrepant results.

14. TEST PROCEDURE

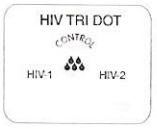
1. Add 3 drops of Buffer Solution to the centre of the device



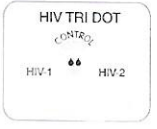
2. Hold the dropper vertically and add 1 drop of patient's sample (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).



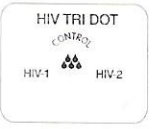
3. Add 5 drops of Buffer Solution.



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.*

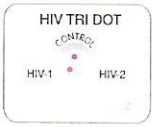
15. INTERPRETATION OF RESULTS

NON-REACTIVE

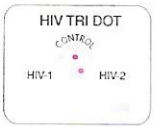
1. If only One DOT (only the Control Dot) appears as shown in fig., the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.

REACTIVE

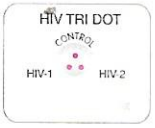
1. If two DOTS, one for the control and the other for HIV-1 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1.



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.

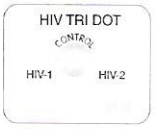


3. If all the three DOTS, one each for control, HIV-1 & HIV-2 appear as shown in Fig., the specimen is reactive for antibodies to HIV-1 & HIV-2.



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

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.

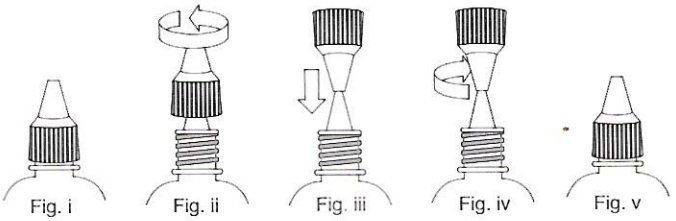
(B) THICK OR VISCOUS SAMPLES:

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



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°

3. The control band in fresh unused membrane test devices is blue coloured and changes to deep purple colour after test performance.
4. The control band would not develop if the sample addition has not been done.
5. 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 package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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(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
	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.

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 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.
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 material.
8. After use, the kit components must be returned to the recommended storage temperature immediately.
9. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.

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.

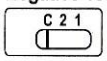
4. Do not use haemolysed, clotted, contaminated, viscous / turbid specimen.
5. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only used for testing.
6. Do not heat -inactivate the specimen.
7. Frozen samples for retrospective studies must be centrifuged 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.

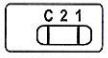
INTERPRETATION OF RESULTS

Negative Test Result

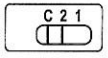


Appearance of only one control band corresponding to control region 'C'.

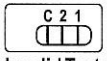
Positive Test Result



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

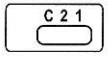


In addition to the control band 'C', appearance of reactive band at test region '2': Specimen positive for Antibodies to HIV 2.



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 tested.

Sensitivity in HIV-1 non-B specimens:

Retroquic- HIV was evaluated to check the sensitivity in 40 HIV-1 samples belonging to different serotypes. The results of 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 m be recentrifuged appropriately so as to pellet invisible particulate matter. Test should be rerun with the clear supernatar
3. The presence of antibodies to HIV 1 / 2 indicates previous exposure to HIV 1 and / or HIV 2 virus but does not constitu 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 collect 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 diagnosi 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 reacti 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 t sample.
12. Testing of pooled specimen is not recommended.