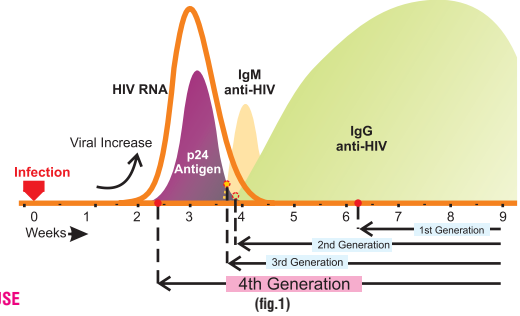
HIV Ag & Ab Card (4th Generation)

Rapid visual test for the qualitative detection of HIV-1 p24 Antigen and Antibodies (IgM, IgG & IgA) to HIV-1& HIV-2 in Human Serum/ Plasma/ Whole Blood

INTRODUCTION

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 organization and serological behavior of HIV-1 & HIV-2 and their complete nucleotide sequence has been determined. The serological events following HIV infection are represented graphically in fig.1. During initial infection, the HIV-1 p24 antigen appears (after 2 weeks) in detectable levels, before anti-HIV (after 4 weeks) but becomes undetectable as the antibodies are formed and their level increases (seroconversion). 4th generation HIV immunoassays incorporate HIV p24 antigen along with HIV antibody detection which allows the identification of acute HIV infections by reducing the window period.



(fig.1)

INTENDED USE

HIV Ag & Ab Card (4th Generation) is a visual, rapid qualitative and sensitive solid phase immunochromatographic assay for the differential detection of HIV-1 p24 Antigen and Antibodies (IgM, IgG & IgA) to HIV-1& HIV-2 in Human Serum/ Plasma/ Whole blood (collected by venipuncture or Fingerprick). The test is a screening test for p24 antigen (HIV-1) and HIV antibodies (anti-HIV-1 & anti-HIV-2) and is for in vitro diagnostic use only. It is intended for screening of blood donors or other individuals at risk for HIV-1 & HIV-2 infection and for clinical diagnostic testing. It is a screening test for p24 antigen (HIV-1) and HIV antibodies (anti-HIV-1 & anti-HIV-2). The test is for in vitro diagnostic use only and is intended for screening of blood donors or others individuals at risk for HIV-1 and/or HIV-2 infection and for clinical diagnostic testing.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

HIV Ag & Ab (4th Generation) Test kit consists two devices; one device for detection of HIV-1 p24 antigen and second device for the detection of HIV-1 & HIV-2 antibodies in human serum/ plasma/whole blood.

HIV Antigen device contains two lines; "C" (Control Line) & "Ag" (HIV-1 p24 Antigen detection Test Line). Test line is coated with anti-p24 antibodies. When a sample is added to the HIV Antigen device followed by the addition of assay buffer, HIV-1 p24 antigen if present in the sample will bind to the anti-p24 gold colloid conjugate making antigen antibodies complex. This complex migrates along the membrane to the test region and forms the visible purple line at "Ag" region as antibody-antigen-antibody gold conjugate complex.

HIV Antibody test device contains two lines; "C" (Control line), & "Ab" (HIV-1 & HIV-2 Antibody detection Test Line). The test line is coated with HIV-1 & HIV-2 Antigens for the detection of HIV Antibodies. When a sample is added to the HIV Antibody device followed by the addition of assay buffer, anti-HIV-1 and/or anti-HIV-2 antibodies if present in the sample, bind to the respective HIV-1 and/or HIV-2 antigen gold colloid conjugate making antigen-antibodies complexes. These complexes migrate along the membrane to the test region and form the visible purple line at "Ab" region as antigen-antibody-antigen gold colloid conjugate complex.

The intensity of the test bands in the respective device will vary depending upon the amount of antigen/antibody present in the sample. The appearance of any pink/purple color line in a specific test region should be considered as reactive for that particular antigen and/or antibody. A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

KIT PRESENTATION & MATERIALS PROVIDED

HIV Ag & Ab Card (4th Generation) kit contains following components:

1.	HIV Ag & Ab Card	50 nos.
2.	Assay Buffer	2 vials (25 Tests each)
3.	Sample Dropper	1 pack of 50 nos.
4.	Instruction Manual	1 No.

Component available on request: Negative Control, HIV Antibody Positive Control and HIV Antigen Positive Control.

Optional material required, but not provided: Swab and Sterile Lancet.

KIT PRESENTATION

25 Test Pack | 50 Test Pack | 100 Test Pack

STORAGE AND STABILITY

HIV Ag & Ab Card (4th Generation) should be stored at 2-30ºC in the cool & dry area available. Expiry date on the kit indicates the date beyond which the kit should not be used. The kit should not be frozen & must be protected from exposure to humidity.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Consult Instructions for use
LOT	Lot Number Batch Number	2ºC 30ºC	Temperature Limit
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged	BIO	Contains biological Material of Animal Origin
2	Single use only		Keep away from sunlight
	Keep Dry	IN	Country of Manufacture

WARNING & PRECAUTIONS 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 and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Mark the test card with patient's name or identification number. Improper identification may lead to wrong result reporting.
4. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 minutes before disposal or any other suitable disinfectant or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures and state guidelines.
5. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
6. Assay Buffer contains Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds.
7. Do not open the foil pouch until it attains room temperature.
8. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit.
9. Do not perform the test in area with strong air flow i.e. under fan or strong air conditioning.
10. Bring kit to room temperature (20-30ºC) before use.
11. Do not use kit components beyond the expiration date, which is printed on the kit.
12. Use a separate Sample Dropper for each specimen.
13. Do not re-use the test kit.
14. Do not use the test kit if the pouch is damaged or the seal is broken.
15. Do not mix or use the assay buffer of different kits/lot.
16. Do not smoke, drink or eat while handling specimen.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

a) Collect the whole blood in a clean container without anticoagulants for serum or containing anticoagulants (EDTA, citrate or heparin) for whole blood/plasma samples by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8°C for not more than 3 days, otherwise false / erroneous results may be obtained.

b) Hemolyzed or clotted blood sample or sample with microbial contamination should not be used.

c) Serum/ plasma specimens, if not tested immediately, should be refrigerated at 2-8°C. For storage for more than 3 days, freeze the specimen at -20°C or below.

d) Repeated freezing and thawing of the specimen should be avoided.

e) Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged at 10,000 RPM for 15 minutes and the clear supernatant should only be used for testing.

f) The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START

1. Bring the required number of HIV Ag & Ab card (4th Generation) test device foil pouches and specimen to room temperature prior to testing.
2. The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial and orifice the closed nozzle, as illustrated below in Fig. iii & iv, before use:

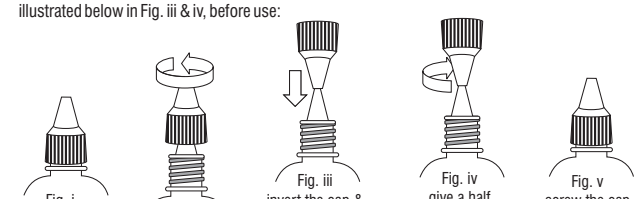


Fig. i close vial provided in the kit

Fig. ii open the cap

Fig. iii invert the cap & press on the closed nozzle

Fig. iv give a half turn twist to puncture the nozzle

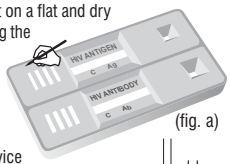
Fig. v screw the cap tightly after use

TEST PROCEDURE

1) Bring the complete kit and specimen to be tested to room temperature prior to testing. **RT 20-30°C**

2) Remove the test card from the foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test card from the foil pouch. **Do not use the card if desicant is pink in color.**

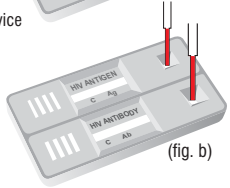
3) Label the test card with patient's name or identification number.



(fig. a)

4) The test card contains 2 devices - one HIV antigen detection device and one HIV antibody detection device placed in a single tray.

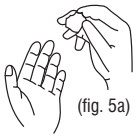
5) Add 1 drop (20 µl) of the sample (Serum / Plasma / Whole Blood) using sample dropper / micropipette into the sample wells of both the HIV Antigen and HIV Antibody device respectively.

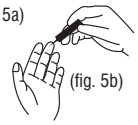


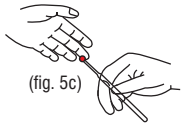
(fig. b)

OR

Whole blood sample collection from finger prick


(fig. 5a)


(fig. 5b)


(fig. 5c)

5a) Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).

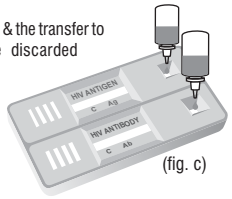
5b) Take the lancet and prick the side of the pulp (ball of the finger) with the lancet, perpendicular to the lines of the fingerprint.

5c) Make sure a well formed drop of blood is present on the tip of the finger.

5d) Take the sample dropper/micropipette and collect 20 µl blood by dipping the tip of the sample dropper/ micropipette into the blood drop. **Add 1 drop of blood immediately in the sample well of the HIV Antigen device.**

5e) **Repeat the above step (5d) for HIV Antibody device.**

⚠ **Caution:** Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate. Sample dropper should be discarded immediately considering it to be biohazardous.



(fig. c)

6) **In case of blood samples, wait for 30 seconds (till the blood is completely absorbed).**

7) Add 1 drop Assay Buffer into the sample well of both device.

⚠ **Addition of incorrect no. of drops of sample/Assay Buffer may give invalid or false result.**

8) Allow reaction to occur for 20 minutes.

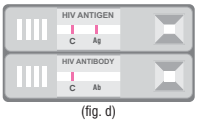
9) Read results at 20 minutes. Positive results may appear as early as 2-10 minutes. However negative results must be confirmed at 20 minutes. Do not read results after 30 minutes.

10) Discard the HIV Ag & Ab Card (4th Generation) immediately after reading results at 20 minutes considering it to be potentially infectious. Follow the local regulations for disposal of potential biohazardous material.

INTERPRETATION OF THE RESULTS

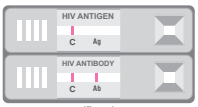
REACTIVE

HIV ANTIGEN REACTIVE: As shown in Fig. (d), appearance of purple colored line in test region "Ag" and red line in control region "C" of the HIV Antigen device and only one distinct red line in the control region "C" of the HIV Antibody device indicates that the sample is "REACTIVE" for HIV-1 p24 Antigen.



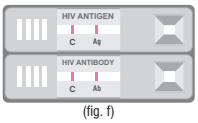
(fig. d)

HIV ANTIBODY REACTIVE: As shown in Fig. (e), appearance of only one distinct red line in the control region "C" of the HIV Antigen device and purple colored line in test region "Ab" and red line in control region "C" of the HIV Antibody device indicates that the sample is "REACTIVE" for HIV-1 and/or HIV-2 Antibodies.



(fig. e)

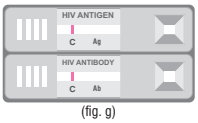
HIV ANTIGEN & ANTIBODY REACTIVE As shown in Fig. (f), appearance of purple colored line in test region "Ag" and red line in control region "C" of the HIV Antigen device and purple colored line in test region "Ab" and red line in control region "C" of the HIV Antibody device indicates that the sample is "REACTIVE" for both HIV-1 p24 Antigen and HIV-1 and/or HIV-2 Antibodies.



(fig. f)

NON-REACTIVE

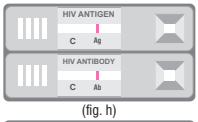
As shown in Fig. (g), appearance of only one distinct red line in the control region "C" of both the HIV Antigen and HIV Antibody device indicates that the sample is "NON REACTIVE" for both HIV-1 p24 Antigen and HIV-1 and/or HIV-2 Antibodies.



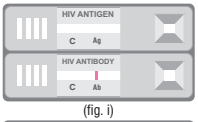
(fig. g)

INVALID

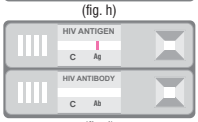
The test is invalid, if no control line appears (with or without the appearance of test lines) after the completion of test, either with clear background or with complete pinkish/ purplish background (fig. h to k).



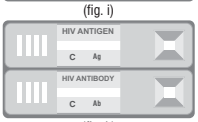
(fig. h)



(fig. i)



(fig. j)



(fig. k)

Repeat the test with a new card. Invalid test may be because of following reasons:

(a) Improper storage at temperature other than the recommended temperature.

(b) Wrong Procedure

(c) Long atmospheric exposure of the test device after opening the pouch.

(d) Use of turbid/ lipemic/ haemolyzed sample.

In case of invalid result, serum/ plasma test samples should be centrifuged at 10,000 rpm for 15 minutes and retest using new Card.

LIMITATIONS AND INTERFERENCES

1. Any deviation from test procedure may lead to invalid/ erratic results.
2. Some samples show cross reactivity for HIV antibodies and/or p24 antigen. Following factors are found to cause false positive HIV antigen and/or antibody test results: Naturally occurring antibodies, Passive immunization, Leprosy, Renal Disorders, Mycobacterium avium, Herpes simplex, Hypergammaglobulinemia, Malignant neoplasms, 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.
3. This is only a screening test. All samples detected reactive must be confirmed by using HIV Western Blot and/or PCR. 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.
4. A negative result may occur if the concentration of antigen/antibody in a specimen is below the detection limit of the test or if the specimen was collected or transported improperly. Therefore a negative test result does not eliminate the possibility of HIV infection, and should be confirmed by ELISA and/or RT-PCR.
5. Positive test results do not rule out co-infections with other pathogens.

PERFORMANCE CHARACTERISTICS OF HIV Ag & Ab CARD (4th GENERATION)

Analytical Sensitivity: The sensitivity of the HIV Ag & Ab CARD (4th Generation) has been determined for HIV-1 p24 Antigen using WHO international standard: HIV-1 p24 antigen NIBSC Code No. 90/636 and it is equal to 100 IU/ml.

Sensitivity and Specificity studies were carried out on fresh as well as frozen samples from low risk as well as high risk groups. Performance of the test with reference to sensitivity and specificity has been determined by using clinical known negative and positive antibodies serum/plasma and whole blood samples and HIV p24 Antigen control.

The performance of the test was evaluated and compared with a licensed commercially available Elisa test. The results obtained are as follows:

Sample Type	No. of Samples Tested	Licensed Test	HIV Ag & Ab Card (4th Generation)
Negative	1200	1200	1199
HIV Positive	125	125	125

Sensitivity: **100%** Specificity: **99.91%**

Cross Reactivity: The cross reactivity of the kit has been tested using 60 samples from other diseases and results observed are as follows:

S. No.	Sample Type	No. of Samples	HIV Ag and Ab Card (4th Generation)	
			HIV Antigen card	HIV Antibody Card
1	HCV	6	NEGATIVE	NEGATIVE
2	HBV	8	NEGATIVE	NEGATIVE
3	Typhoid	6	NEGATIVE	NEGATIVE
4	Syphilis	3	NEGATIVE	NEGATIVE
5	Dengue NS1 Ag	4	NEGATIVE	NEGATIVE
6	Dengue Antibody	4	NEGATIVE	NEGATIVE
7	Scrub Typhus	2	NEGATIVE	NEGATIVE
8	CRP	3	NEGATIVE	NEGATIVE
9	Chikungunya	4	NEGATIVE	NEGATIVE
10	TB	3	NEGATIVE	NEGATIVE
11	Leptospira	4	NEGATIVE	NEGATIVE
12	Malaria P. falciparum	2	NEGATIVE	NEGATIVE
13	Malaria P. vivax	2	NEGATIVE	NEGATIVE
14	HAMA	4	NEGATIVE	NEGATIVE
15	RA	2	NEGATIVE	NEGATIVE
16	ASO	3	NEGATIVE	NEGATIVE

External Evaluation: The performance of HIV Ag & Ab Card has been evaluated by National Institute of Biologicals, India. The result obtained of 3 different lots are as follows:

Sensitivity: **100%** Specificity: **100%**

Precision: Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of ten samples - four negative, one HIV antigen (p24) positive, one HIV-2 positive and four HIV-1 Positive. The C.V. (%) of all the ten samples were within 10%.

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 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.

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in vitro diagnostic reagent, not for medicinal use

VER-01 R-00

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MN/AAI/117
Rev. Date: Jan.-24