A Rapid test for Detection of Antibodies to HIV in Human Serum/Plasma.

QUADRO™ HIV 1-2 Ab

Meril Diagnostics

For in vitro diagnostic use
Read this pack insert thoroughly before use

INTENDED USE:

QUADRO™ HIV 1-2 Ab is a Single use, rapid, flow-through *in-vitro* qualitative assay for the detection of antibodies to HIV-1 and HIV-2 in Human Serum, Plasma specimens. The test is intended for used by trained personnel in Medical facility and Clinical laboratory as a screening test for HIV antibody detection.

INTRODUCTION:

Acquired Immunodeficiency Syndrome (AIDS) is caused by two types of Human Immunodeficiency Virus, HIV-1 and HIV-2. Transmission of infection is mainly by exposure to certain infected body fluids e.g., blood and blood products, genital secretion and by transplacental route. Infection by HIV-1 has been reported worldwide, HIV-2 infection has been occurring mainly in West Africa and some European countries. Both these viruses show substantial antigenic cross reactivity in their core proteins, but the envelope glycoproteins are least cross reactive.

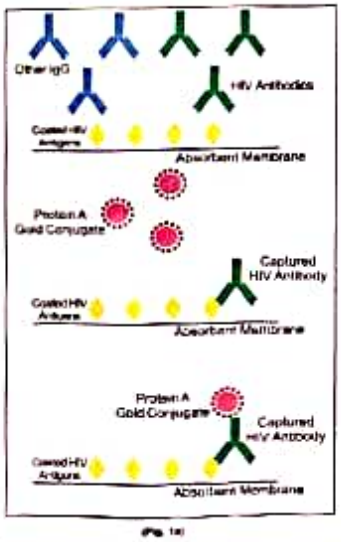
HIV Test kits are classified in terms of generations by WHO as

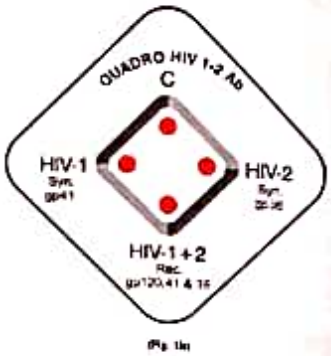
1) 1st Generation Assays: Using Viral Lysate Antigens
2) 2nd Generation Assays: Using Recombinant or Synthetic peptides
3) 3rd Generation Assays: Detects IgM, IgG and IgA antibodies together.
4) 4th Generation Assay: Detects p24 antigen and Antibodies to HIV together.

QUADRO™ HIV 1-2 Ab is designed to detect antibodies to envelope glycoproteins of HIV-1 & HIV-2 by using unique combination of synthetic and recombinant antigens in the same test device differentially. Consequently potential infectious samples of serum and plasma can be identified.

PRINCIPLE:

The QUADRO™ HIV 1 - 2 Ab is a HIV rapid screening test kit contains a nitrocellulose membrane which is pre coated with synthetic peptides of highly immunodominant regions of HIV-1 (gp41) and HIV-2 (gp36) on test region "HIV-1" and "HIV-2" respectively along with a uniquely formulated Recombinant proteins of HIV 1-2 (immunogenic portion of gp120, gp41 & gp36) on the test region of HIV 1-2. As the test sample pass through the membrane, HIV Antibodies if present in the sample reacts with the antigens coated on the membrane at their respective region. Protein A Gold conjugate binds with the Fc region of the ant bodies bound on the membrane and forms a pinkish red colored dot. (Fig. 1a & 1b)





REAGENTS AND MATERIALS PROVIDED:

Each kit contains:

COMPONENTS	DESCRIPTION	PREPARATION
1. QUADRO™ HIV 1-2 Ab Devices	Individual pouch	Cut open the pouch. Bring to R.T before use
2. Assay buffer	Buffer containing chemicals & stabilizers	Bring to R.T before use
3. Gold conjugate	Protein-A colloidal Gold conjugate with stabilizers	Bring to R.T before use
4. Plastic dropper	Plastic dropper for sample addition	Always Ensure to use new dropper

STORAGE AND STABILITY:

The sealed pouches in the test kit to be stored between 2-8°C till the duration of the shelf life as indicated on the pouch. DO NOT FREEZE.

PACK SIZES:

HD4RPD-01 - 10 Test pack
HD4RPD-02 - 25 Test pack
HD4RPD-03 - 50 Test pack
HD4RPD-04 - 100 Test pack

MATERIAL REQUIRED BUT NOT PROVIDED:

Hand Gloves, Syringes, Blood collection tubes, Bio-hazard container, Absorbant cotton balls for blood collection, 0.1% Hypochlorite.

PRECAUTIONS:

1. For in-vitro diagnostics use only
2. Allow all reagents and sample(s) to attain room temperature (18°C to 30°C) before use.
3. Do not use the kit contents beyond the expiry date.
4. Do not touch the nitrocellulose part of the device. Finger print or scratch on nitrocellulose membrane may give erroneous results.
5. Test devices and reagents of different lot must not be mixed and used.
6. Perform the test by using kit's reagents. Performing the test with any other reagents may give erroneous results.
7. Follow the assay procedure and storage instructions strictly. Deviation will lead to erroneous results.
8. Do not use haemolysed or turbid or hazy specimen for testing.
9. Use sufficient volume of sample for testing and add entire 40µl or one drop of the patient sample at once on the reaction membrane.
10. Do not pipette reagents by mouth and do not smoke, eat or drink while handling specimens and performing a test.
11. Do not re-use the Test devices or droppers which lead to aberrant results.

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12. Avoid contact of reagents with eyes and skin.
13. Wear protection clothing such as laboratory coats and disposable gloves and eye protection when specimens are assayed. Avoid re-using gloves or use of washed gloves.
14. Handle sample(s) and used materials as if it is capable of transmitting infection.
15. Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. Remnants of sample(s), used materials, pipette tips etc. should be disposed in suitable biohazard container. Materials should be autoclaved at 121°C for 30 minutes or dipped in 0.1% hypochlorite solution for 30 minutes prior to disposal.
16. Clean up spills thoroughly using an appropriate disinfectant.
17. Sodium Azide is present at 0.1% in all assay reagents, which can react with lead and copper plumbing to form highly explosive metal azides. If needed to be discarded into a drain, flush a large amount of water to prevent azide build up.

SPECIMEN COLLECTION AND PREPARATION:

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

PLASMA:

1. Collect blood specimen into a collection tube containing EDTA or Citrate or Heparin.
2. Separate the plasma by centrifugation, 1500 RPM for 10 minutes.
3. Carefully withdraw the plasma into new pre-labelled tube.

SERUM:

1. Collect the blood specimen into a collection tube containing no anticoagulants.
2. Allow the blood to clot.
3. Separate the serum by centrifugation, 1500 RPM for 10 minutes.
4. Carefully withdraw the serum into a new pre-labelled tube. Test the specimens as soon as possible after collection.

Stored separated serum/plasma specimens at 2-8°C up to 3 days can be used for testing. Serum/Plasma specimens should be frozen at -20°C for longer storage. If the sample is frozen, completely thaw the sample prior to testing. Avoid repeated freezing and thawing of specimens.

TEST PROCEDURE:

Bring the specimen and test components to room temperature.

IT IS IMPORTANT TO ENSURE SEQUENTIAL ADDITION OF REAGENTS AS RECOMMENDED AND ALSO ALLOW THE REAGENTS TO SOAK COMPLETELY BEFORE ADDITION OF CONSEQUENT REAGENT.

WHILE ADDITION OF REAGENTS OR SAMPLES ENSURE TO HOLD THE DROPPER OR REAGENTS VERTICALLY ALLOWING FULL DROP OF REAGENT OR SAMPLE FALL OVER DEVICE MEMBRANE EQUALLY. FIG (2)

Fig 2

MIX THE SPECIMEN PROPERLY PRIOR TO ASSAY.

1. When ready to test, open the pouch at the notch and remove device. Place the Test device on a clean, flat surface. Label the patient/sample identity details legibly with a marker.
2. Add **3 drops** of Assay buffer onto the untested device and allow it to absorb completely (Fig 3).

Fig 3

3. Add **1 drop** or 40 µl of patient serum/plasma with a dropper or micro pipette. Ensure quick and complete addition of the sample at a single instance to allow proper exposure of the sample on the membrane (Fig. 4).

Fig 4

4. Allow the sample to absorb in and add **3 drops** of assay buffer onto the membrane to wash any non specific binding over the membrane and allow it to absorb completely (Fig 5).

Fig 5

5. Add **2 drops** of Gold Conjugate and allow it to absorb completely. The gold conjugate binds specifically with the Fc portions of the patient antibodies captured on the membrane (Fig 6).

Fig 6

6. Add **3 drops** of the assay buffer to allow proper washing of the unbound gold conjugate from the membrane and allow it to absorb completely (Fig 7).

Fig 7

Read the results as per the illustration shown below immediately and discard the tested device as potentially infectious.

INTERPRETATION OF RESULTS:

NEGATIVE RESULT:

If only the Control (C) pinkish red dot is developed, the test indicates that no detectable HIV antibodies are present in the specimen. The result is non-reactive for Anti-HIV (Fig 8).

Fig 8

POSITIVE RESULT:

a) If Control, HIV-1 & HIV-1+2 pinkish red dots are developed, the test indicates for the presence of HIV-1 antibodies in the specimen: the result is reactive for Anti-HIV-1 (Fig. 9).

Fig 9

b) If Control, HIV-2 & HIV 1+2 pinkish red dots are developed, the test indicates for the presence of HIV-2 antibodies in the specimen: the result is reactive for Anti-HIV-2 (Fig. 10).

Fig 10

c) If Control, HIV-1, HIV-2 & HIV 1+2 pinkish red dots are developed, the test indicates for the presence of antibodies to HIV-1 and HIV-2 in the specimen, the result is reactive for Anti-HIV-1 and Anti-HIV-2 simultaneously (Fig. 11).

Fig 11

d) If Control, HIV 1+2 pinkish red dots are developed, the test indicates for the presence of antibodies to either of HIV-1 or HIV-2, and interpret the results after confirming with other supplemental tests (Fig 12).

Fig 12

INVALID RESULT:

If no Control dot is developed, the assay is invalid regardless of color development on 'Test' dots as indicated below. Repeat the assay with a new device (Fig 13).

Fig 13

(In case if the issue persist after repetition, kindly contact our customer service cell at 1800 266 3745)

LIMITATIONS OF THE TEST:

1. This kit is designed for primary screening of HIV infection.
2. As with all diagnostic tests, the test result must always be co-related with clinical findings.
3. A negative result at any time does not preclude the possibility of exposure or infection.
4. Presence of heterophile antibodies in patient's sample with Auto immune disorders, Rheumatic diseases, Renal failure, Kidney dysfunction and autoimmune disorder may lead to false results need to be reconfirmed with confirmatory tests.
5. A negative result can occur if the quantity of the analyte of interest present in the specimen is below the detection limits of the assay or the analyte of interest that are detected are not present during the stage of disease in which a sample is collected.
6. All the reactive specimens should be tested for reproducibility of their results as per the Regulations followed using the ELISA, CLIA, PCR Test kits approved by the authorities.
7. Repeat the test in case of very faint dot or if have any doubt for test dot.
8. **Use caution while interpreting the results of the test in immunocompromised patients.**
9. HIV-1 & 2 shares their morphology to an extent above 60% and hence certain samples can exhibit cross reactivity between HIV 1 & 2. Appearance of HIV 1 & 2 spots may not be treated as co-infection of HIV 1 & 2 in all conditions and need to confirmed with confirmatory assays for the same.
10. Samples containing particulate matter and Hazy samples can block the membrane resulting in improper flow of reagent and high background colour.
11. **Use caution while interpreting the results of HIV status in Anti retroviral therapy (ART) patients and hyper gammaglobulinemia conditions.**
12. The manufacturer reserves their right to alter and modify the product design specifications without prior information to the buyer.

PERFORMANCE CHARACTERSTICS:

In-house study with a panel of 292 positive and 2652 negative samples whose results were earlier confirmed with commercially approved ELISA & Rapid Test kits was tested with QUADRO™ HIV 1-2 Ab. The results obtained are as follows.

Sensitivity: 100%

Specificity: 99.92%

		QUADRO™ HIV 1-2 Ab Results	
Licensed Test kit results		Positive	Negative
	HIV 1 Reactive (n=270)	270	0
	HIV-2 Reactive (n-20)	20	0
	HIV 1 & 2 Reactive (n=2)	2	0
	HIV Negative samples (n=2652)	2	2650

REPRODUCIBILITY:

Three studies were conducted to evaluate the reproducibility of QUADRO™ HIV 1-2 Ab. For each study a panel of 15 blind coded samples of 5 Reactive and 10 Non reactive samples were tested in triplicate at three different sites using three different lots of QUADRO™ HIV 1-2 Ab.

The overall reproducibility is 100%.

DISPOSAL:

Discard the Test device immediately after reading the results and before discarding it, add suitable disinfectant such as 0.1% hypochlorite on the device membrane and on all other items used for handling the specimen.

Put all items to be disposed in disposable bags and dispose off accordingly.

LIMITED EXPRESSION LIABILITY:

Meril Diagnostics Pvt. Ltd. hereby limits the warranty of the test kits to be used for in vitro Diagnostic assay with in the given limitations mentioned in this manual and its procedures and recommendations suggested are strictly followed. The manufacturer liability is limited to the either replacement of the product or refund of the purchase price of the product and in no case liable to claim any kind of amount greater than the purchase price of the product in respect of damages to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any consequential or economic loss incurred from the product or in its application accordingly.

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