TECHNICAL SPECIFICATION.

Product Name

FIRST RESPONSE® HIV 1+2 / SYPHILIS COMBO CARD TEST

Manufacturer: Premier Medical Corporation Private Limited

Unit: A1-302, GIDC, Sarigam-396155. Dist. Valsad, Gujarat, INDIA.

An ISO 13485 & EN ISO 13485 Certified company

Digitally signed by Chirtoacă Iurie
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Introduction of the IVD device:

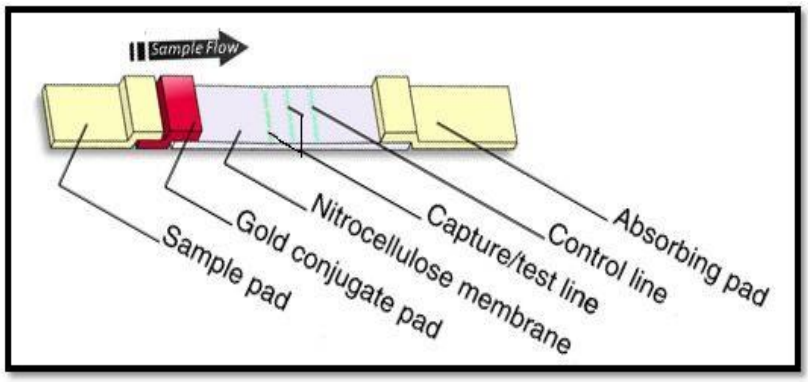
First Response® HIV 1+2 / Syphilis Combo Card Test is intended for use by healthcare professionals and trained user. It is a rapid, qualitative, screening, in vitro diagnostic test for detection of antibodies specific to HIV (type 1 & 2) and *Treponema pallidum* in human serum, plasma or whole blood. The test is used as an aid in the diagnostics of HIV and/or Syphilis. The test kit is not automated and does not require any additional instrument. Reactive specimens should be confirmed further with suppplimental/ confirmatory tests.

HIV (Human Immunodeficiency Virus) is recognized as the etiologic agent of Acquired Immune Deficiency Syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected blood, certain bodily fluids or tissues, and from mother to fetus or child during the prenatal period. The clinical diagnosis of HIV has been done by detection of HIV 1 and HIV 2 antibodies in human plasma, serum, or venous/capillary whole blood by immunoassay. Researchers have constructed HIV-1 and HIV-2 genes for the expression of recombinant antigens in bacterial systems such as *E. coli* and focused on HIV-1 and HIV-2 proteins, which are definitely immunogenic. The major immunoreactive antigens of these proteins have been reported to have HIV-1 gp41, p24, and HIV-2 gp36 based on western blot analysis.

Syphilis is a venereal disease caused by the spirochete bacterium *Treponema pallidum*. It is ordinarily transmitted by sexual contact. It can also be transmitted congenitally by trans-placental passage of *Treponema pallidum* to the fetus and by blood transfusion. Researchers have constructed P47, P45, P17, P15 genes for the expression of recombinant antigens in bacterial systems such as *E. coli*, which are potential immunogen.

First Response® HIV 1+2/Syphilis Combo Card Test is a 3rd generation HIV/Syphilis immunoassay. The design of 3rd generation assays allows the detection of HIV specific IgG as well as IgM, which may occur early in infection.

A single test strip composition:



Intended use:

First Response® HIV 1+2 / Syphilis Combo Card Test is intended for use by healthcare professionals and trained user. It is a rapid, qualitative, screening, in vitro diagnostic test for detection of antibodies specific to HIV (type 1 & 2) and *Treponema pallidum* in human serum,

plasma or whole blood. The test kit is not automated and does not require any additional instrument. Reactive specimens should be confirmed further with supplemental/ confirmatory tests.

A general description of the principle of the assay method:

First Response® HIV 1+2 / Syphilis Combo Card Test is based on the principle of immunochromatography for qualitative detection of antibodies(IgG & IgM)specific for HIV-1&2 and/or Syphilis. This card test in which nitrocellulose membrane is precoated with recombinant antigens for HIV 1 (gp41) and HIV 2 (gp36) at test line HIV and Recombinant TP antigen (P47, P45, P17, P15) specific for Treponema pallidum at the test line Syp of the device with control line at "C". When a serum or plasma or whole blood specimen is applied to the Specimen well of plastic device, the cocktail of recombinant HIV-1+2 (gp41 & gp36) antigen-colloidal gold conjugate & recombinant Treponema pallidum antigens colloid gold conjugate.

The specimen and Assay diluents move along the membrane chromatographically to the test regions and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. If the specimen contains antibodies to Treponema pallidum, the colored line will appear in the test area at test line Syp, corresponding to Syp line. If the specimen contains antibodies to HIV 1 and/or 2, the colored line will appear in the test area at test line HIV, corresponding to HIV line. The presence of both test line indicates specimen contain antibodies to HIV as well as Treponema pallidum. The absence of the colored line at the test line regions indicates that the specimen is non reactive for HIV and Treponema pallidum, showing a negative result. Control line will appear irrespective of reactive or non reactive specimen. The control line will serve to validate test performance.

A description of the components of the assay.:

Nitrocellulose Membrane strips: Nitrocellulose membrane acts as immuno- chromatographic solid phase. The membrane is coated with three different lines: Test line HIV: contains (Membrane glycoprotein gp41 with O group sequence and membrane glycoprotein gp 36), Test Line Syphilis: contains Syphilis recombinant antigen (Treponema pallidum) and Control Line: Goat anti mouse IgG.

Conjugate releasing pad: It facilitates release of conjugate on addition of specimen and assay buffer. Specific recombinant chimeric protein of HIV-1 and HIV-2 along with Treponema pallidum antigen coupled separately with colloidal gold conjugate is dispensed and dried on conjugate realizing pads.

Specimen pad/ Blood separator pad: It facilitates slow release of assay buffer and specimen to make continuous flow of reactant material as well as antigen for specimen.

Absorbent pad: This helps background clearance as it absorbs excess liquid from the membrane. This aids in clear readability of the test results. Furthermore, it helps to prevent backflow of the

specimen within test time.

Plastic cassette for lateral flow assay: The cassette is designed taking into consideration the following:

- The cassette material does not affect the performance of the enclosed strip.
- The cassette prevents the movement of the strip during the test.
- There are separate windows for Specimen addition and Inspection of results.
- Prevents backflow of the specimen.

The final assembled device packed in aluminum pouch along with silica bag as desiccant.

Description of Critical Components and its functions:

- **Laminated and assemble nitrocellulose membrane:**

Test Line HIV: HIV-1& 2 glycoprotein GP41 and gp36 antigen- Capture line for the detection of HIV-1 and 2 specific antibodies in the human serum/ plasma/ whole blood.

Test Line Syp: Treponema Pallidum Antigen - Capture line for the detection of Treponema Pallidum specific antibodies in the human serum/ plasma/ whole blood.

- **Conjugate realizing pad:**

Conjugate: Cocktail HIV-1 and HIV-2 antigen coupled colloidal gold particles, *Treponema Palllidum* coupled colloidal gold particles- Development of color band at test bands if specimen contain specific antigen and at control band.

- **Assay buffer:** Buffer solution supplemented with detergent polymers, and preservatives-To flow specimens across antigens coated nitrocellulose membrane and to release conjugate gold particles from conjugate pad.

A description of the specimen collection:

[Collection by venipuncture]

1) Venous Blood collection: Collect the whole blood by venipuncture in to collection tube containing anticoagulants (EDTA, Heparin, ACD and Sodium Citrate anticoagulants have been validated for use with this test.)
2) Plasma collection: Collect the whole blood in collection tubes containing anticoagulants (such as EDTA, Heparin, ACD Or Sodium Citrate) by venipuncture. Centrifuge the tube at 3000 rpm for 10-15 min to obtain plasma(supernatant).
3) Serum collection: Collect whole blood in collection tubes without having any anticoagulants by venipuncture. Keep the tube in an upright position for 30 minutes and then centrifuge it at 3000 rpm for 10-15 minutes to obtain serum (supernatant).
4) Whole blood: specimens collected in appropriate anticoagulant may be used for testing

immediately or may be stored at 2-8 °C for up to 3 days. Do not freeze whole blood specimen.

5) If serum or plasma specimens are not immediately tested, they should be refrigerated at 2-8 °C. For storage periods Greater than three days, freezing at -20 °C is recommended. They should be brought to room temperature prior to use.

6) Serum or plasma specimens containing precipitate or high lipemia may yield inconsistent test results. Such specimens Must always be centrifuged prior to assaying.

Storage & Stability:

1) First Response® HIV 1+2 / Syphilis Combo Card Test kit should be stored at 4-30°C.
2) Do not freeze the kit or components.
3) The kit is sensitive to humidity and heat.
4) Assay buffer (opened & unopened) & the unopened test device are stable until the expiry date printed on the label, when stored at 4-30°C.
5) Perform the test immediately after removing the test device from the aluminium pouch.
6) The shelf life of the kit is as indicated on the outer package.

Performance Characteristics:

As per WHO Evaluation report the sensitivity and specificity of First Response® HIV 1+2 / Syphilis Combo Card Test is-
For HIV antibodies an initial sensitivity (95% CI) of 100% (98.2% - 100%) and an initial specificity (95% CI) of 99.0% (96.4% - 99.9%) compared to the reference assays. The final sensitivity (95% CI) was 100% (98.2% - 100%) and the final specificity (95% CI) was 99.5% (97.2% - 100%).

For the case of T pallidum an initial sensitivity (95% CI) of 99.0% (96.4% - 99.9) and an initial specificity (95% CI) of 99.0% (96.4% - 99.9%) compared to the reference assays. The final sensitivity (95% CI) was 99.0% (96.4% - 99.9%) and the final specificity (95% CI) was 100% (98.2% - 100%)

Operational Characteristics:

Temperature range:

- First Response® HIV 1+2 / Syphilis Combo Card Test is stable at temperature range of 4-30°C.
- Operating Temperature range is room temperature.
- Test result interpretation time 15 minutes.

Shelf Life:

First Response® HIV 1+2 / Syphilis Combo Card Test has shelf life of 24 months.

Certification:

- First Response® HIV 1+2 / Syphilis Combo Card Test is WHO pre-qualified product.
- First Response® HIV 1+2 / Syphilis Combo Card Test is manufactured by Premier Medical Corporation Pvt Ltd is ISO 13485 & EN ISO 13485 certified company.

General Kit components:

First Response® HIV 1+2 / Syphilis Combo Card Test contains:

- Test devices packed in aluminum pouch with desiccant,
- Specimen Transfer Device.
- Sterile lancets.
- Alcohol swabs.
- Assay buffer bottle.
- Instruction for use in English language.

Kit components based on pack size:

Kit contents	I20FRC25	I20FRC30	I20FRC50	I20FRC60	I20FRC100
Test device with desiccant	25 Nos	30 Nos	50 Nos	60 Nos	100 Nos
Specimen transfer device	25 Nos	30 Nos	50 Nos	60 Nos	100 Nos
Assay buffer bottle	01 No	01 Nos	02 Nos	04 Nos	04 Nos
Sterile Lancet	25 Nos	30 Nos	50 Nos	60 Nos	100 Nos
Alcohol swab	25 Nos	30 Nos	50 Nos	60 Nos	100 Nos
Instructions For Use	01 No	01 No	01 No	01 No	02 Nos