WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: First Response HIV1+2/Syphilis Combo Card Test
WHO reference number: PQDx 0364-010-00

First Response HIV1+2/Syphilis Combo Card Test with product codes **I20FRC25, I20FRC30, I20FRC50, I20FRC60, and I20FRC100**, manufactured by **Premier Medical Corporation Private Limited, Rest of World regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 24 June 2019.

Summary of WHO prequalification assessment for First Response HIV1+2/Syphilis Combo Card Test

	Date	Outcome
Prequalification listing	24-Jun-2019	listed
Dossier assessment	02-May-2019	MR
Site inspection(s) of quality management system	12-Mar-2018	MR
Product performance evaluation	Q1 of 2018	MR

MR: Meets Requirements

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	A new specimen transfer device having (20 µl marking line) is introduced to make it more user friendly".	19-Feb-2020

Intended use

According to the claim of intended use from Premier Medical Corporation Private Limited *"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 the detection of antibodies (IgG & IgM) specific to HIV (type 1 & 2) and Treponema pallidum in human serum, plasma or venous and capillary whole blood. The test can be used as an aid in diagnosis of HIV and/or Syphilis. The product is intended to be used for*

symptomatic, asymptomatic as well as pregnant women population. The test kit is not automated and does not require any additional instrument. Reactive specimens should be confirmed further with ELISA, Western Blot or TPHA ''.

Assay description

According to the claim of assay description from Premier Medical Corporation Private Limited "*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. The nitrocellulose membrane is coated with cocktail of recombinant antigen 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" and control reagent coated at control line "C". When a serum or plasma or whole blood specimen is applied to the specimen well of test device, the cocktail of recombinant HIV 1+2 (gp41 & gp36) antigen - colloidal gold conjugate (CGC) & recombinant Treponema pallidum antigens colloidal gold conjugate, will react with HIV and/or Syphilis specific antibodies, if present in the specimen. The antibody-CGC antigen complex and assay buffer move along the membrane chromatographically to the test regions and form a visible line as the antigen-antibody-CGC antigen 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 Syphilis 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 1+2 line"

Test kit contents

Component	**25 tests (product code I20FRC25)**	**30 tests (product code I20FRC30)**	**50 tests (product code I20FRC50)**	**60 tests (product code I20FRC60)**	**100 tests (product code I20FRC100)**
Test device pouch containing: 1 test device, 1 desiccant	25	30	50	60	100
Specimen transfer device	25	30	50	60	100
Assay buffer bottle	1 of 2.5 ml	1 of 2.5 ml	2 of 2.5 ml	4 of 2.5 ml	4 of 2.5 ml
Sterile twist lancets	25	30	50	60	100
Alcohol swabs	25	30	50	60	100
Instructions for use	1	1	1	1	2

Items required but not provided

- New pair of disposable gloves and face mask.
- Permanent Marker pen and timer
- Extra lancets and alcohol swabs, if needed
- Sharp disposable box and biohazardous waste container
- Venipuncture blood collection kit (if whole blood is collected by venipuncture
- Sterile gauze pads

Storage

The test kit should be stored at 4-30 °C.

Shelf-life upon manufacture

24 months.

Warnings/limitations

Refer to current version of manufacturer's instructions for use.

Prioritization for prequalification

Based on the established eligibility criteria, First Response HIV 1+2 / Syphilis Combo Card Test was given priority for WHO prequalification assessment.

Dossier assessment

Premier Medical Corporation Private Limited submitted a product dossier for First Response HIV1+2/Syphilis Combo Card Test as per the "*Instructions for compilation of a product dossier*" (PQDx_018 version 3). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 02 May 2019.

Based on the product dossier screening and assessment findings, the product dossier for First Response HIV1+2/Syphilis Combo Card Test meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture Premier Medical Corporation Private Limited, Sarigam, Gujarat, India of First Response HIV1+2/Syphilis Combo Card Test in March 2018 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 version 4). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 30 August 2018.

Based on the site inspection and corrective action plan review, the quality management system for First Response HIV1+2/Syphilis Combo Card Test meets WHO prequalification requirements.

Product performance evaluation

First Response HIV 1+ 2/Syphilis Combo card test (Premier Medical Corporation Private Limited) is a single use, rapid, qualitative lateral flow immunochromatography assay for the detection of HIV-1/2 and syphilis antibodies in human serum/plasma, whole blood (finger stick, EDTA, heparin or sodium citrate). A volume of 20 µL of specimen is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be performed in laboratories with limited facilities and non-laboratory settings. Reading of the results can be done visually i.e. subjectively read.

First Response HIV 1+ 2/Syphilis Combo card test (Premier Medical Corporation Private Limited) was evaluated by WHO in the first quarter of 2018 at the Institute of Tropical Medicine, Belgium, using serum/plasma specimens.

In this limited evaluation on a panel of 400 clinically-derived specimens, compared to the reference assays (HIV reference algorithm: Vironostika HIV Ag/Ab [bioMérieux] EIA and Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics] EIA or Genscreen HIV-1/2 Version 2 [Bio-Rad]; followed by INNO-LIA HIV I/II Score [Fujirebio Inc.]; Syphilis reference algorithm: Vitros Syphilis TPA Assay [Ortho Clinical Diagnostics], followed by SERODIA-TP.PA [Fujirebio Inc.]), the following results were obtained:

Performance characteristics in comparison with an agreed reference standard				
	HIV-1/2		Syphilis	
	Initial (95% CI)	Final (95% CI)	Initial (95% CI)	Final (95% CI)
Sensitivity % (N=200)	100% (98.2% - 100%)	100% (98.2% - 100%)	99.0% (96.4% - 99.9)	99.0% (96.4% - 99.9%)
Specificity % (N=200)	99.0% (96.4% - 99.9%)	99.5% (97.2% - 100%)	99.0% (96.4% - 99.9%)	100% (98.2% - 100%)
Invalid rate	0%			
Inter-reader variability	0.5%		0.3%	

In addition, analytical performance characteristics were assessed using commercially available panels and the following results were obtained:

Additional performance characteristics		
	HIV-1/2	Syphilis
Sensitivity during seroconversion in comparison with a benchmark assay	Seroconversion sensitivity index of 0, therefore detection is 0 specimens earlier/later than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus) in average on 8 seroconversion panels	Seroconversion sensitivity index of -1, therefore detection is 1 specimen earlier than the benchmark assay (Vitros Syphilis TPA Assay) on one seroconversion panel
Analytical sensitivity on a mixed titer panel in comparison with an agreed reference standard	25 of 25 specimens were correctly classified.	17 of 17 specimens were correctly classified.
Lot to lot variation on a dilution panel	Acceptable	Acceptable – except for one 2-dilution difference in one of 10 dilution panels

Key operational characteristics	
Validated specimen types	Serum, plasma (EDTA, heparin or sodium citrate), venous whole blood, capillary whole blood
Number of steps	3 without precision required
Time to result	15 minutes
Endpoint stability	10 minutes (do not interpret after 25 minutes after addition of buffer)
Internal QC	Yes, control line on the test device
In-use stability of reagents	Assay buffer (opened & unopened) & the unopened test device are stable until the expiry date printed on the label when stored at 4-30°C.

Labelling

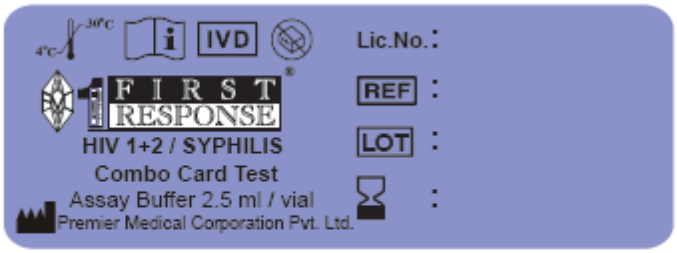
1. **Labels**
2. **Instructions for use**

1. Labels

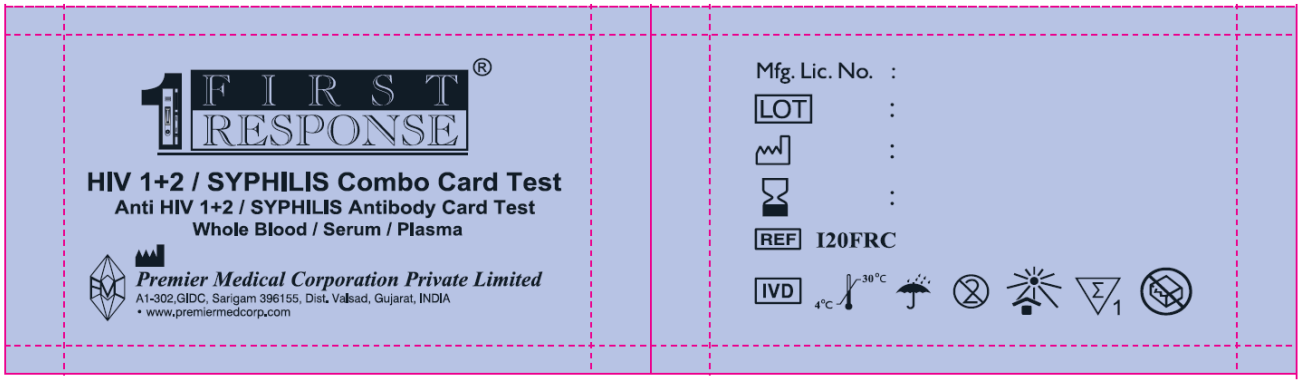
1.1 Sterile safety lancet label

1.2 Alcohol swab labels

1.3 Assay buffer label

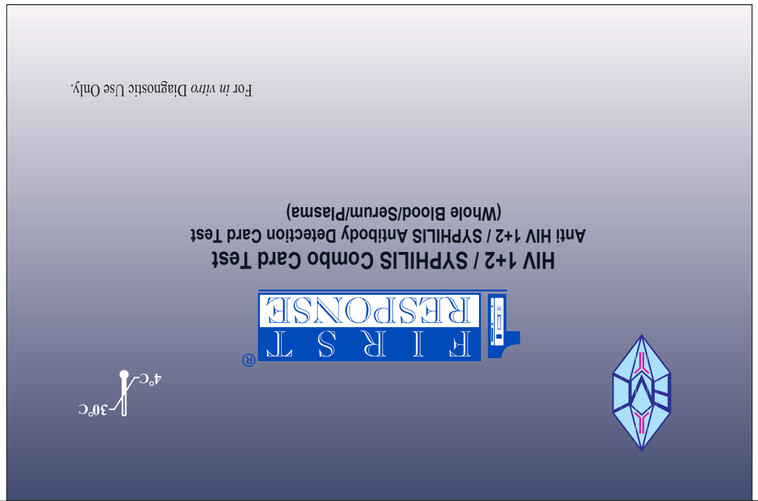


1.4 Aluminum pouch labels



1.5 Outside box labels

Product : First Response HIV 1+2 / SYPHILIS Combo Card Test
Pack size: 25 Tests



Mfg. Lic. No. :

LOT :

:

:

Contents:

• Individually pouched test devices with desiccant	: 25 Nos.
• Specimen transfer device	: 25 Nos.
• Sterile lancet	: 25 Nos.
• Alcohol swab	: 25 Nos.
• Assay buffer bottle	: 01 No.
• Instructions for use	: 01 No.

REF I20FRC25
Σ 25 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C 30°C

FIRST RESPONSE

HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

4°C 30°C IVD NON STERILE

For Professional Use

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF I20FRC25
Σ 25 Tests/kit
One Step Anti HIV 1+2 / SYPHILIS Combo Card Test
4°C 30°C
FIRST RESPONSE
HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

Product : First Response HIV 1+2 / SYPHILIS Combo Card Test
Pack size: 30 Tests

For *in vitro* Diagnostic Use Only.

HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

FIRST RESPONSE

4°C – 30°C

Mfg. Lic. No. :

LOT :

:

:

Contents:

• Individually pouched test devices with desiccant	: 30 Nos.
• Specimen transfer device	: 30 Nos.
• Sterile lancet	: 30 Nos.
• Alcohol swab	: 30 Nos.
• Assay buffer bottle	: 01 No.
• Instructions for use	: 01 No.

REF 120FRC30
Σ 30 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C – 30°C

FIRST RESPONSE

HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

4°C – 30°C | IVD | NON STERILE

For Professional Use

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF 120FRC30
Σ 30 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

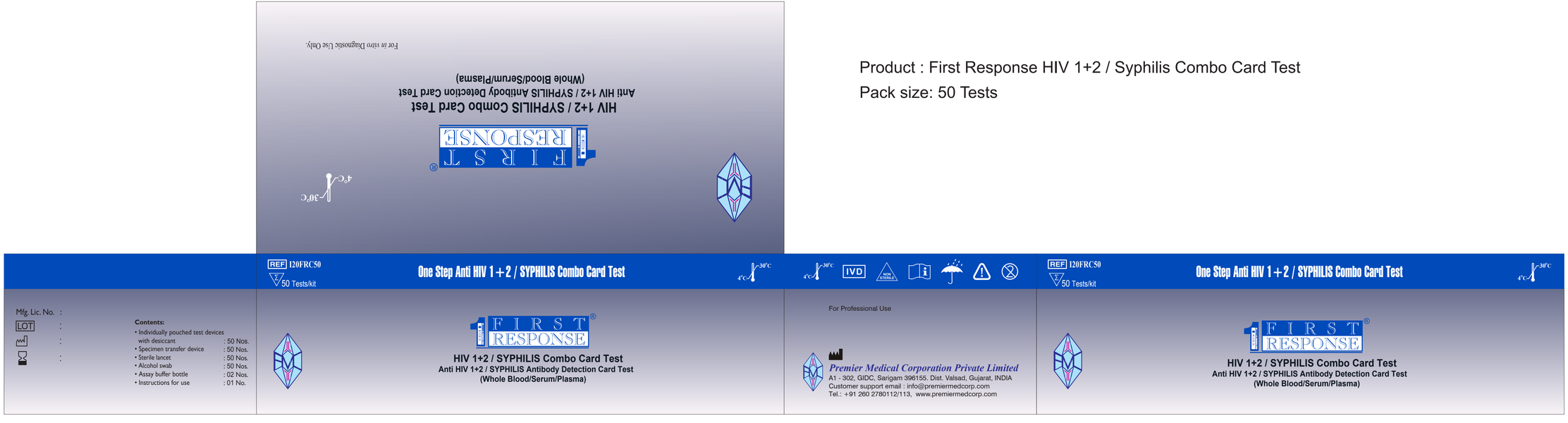
4°C – 30°C

FIRST RESPONSE

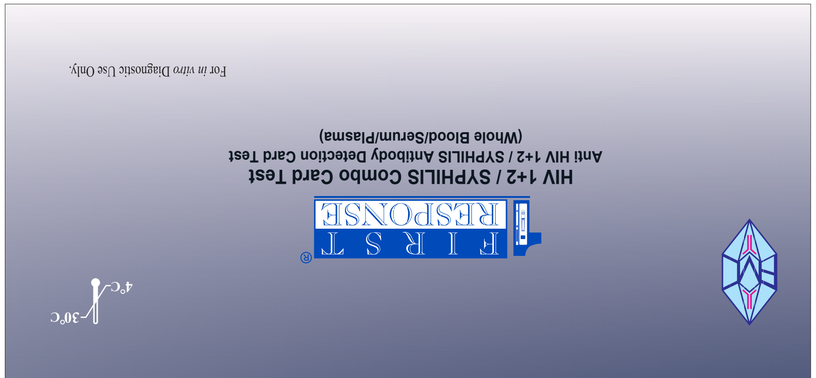
HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

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

Pack size: 50 Tests



Product : First Response HIV 1+2 / SYPHILIS Combo Card Test
Pack size: 60 Tests



Mfg. Lic. No. :
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Contents:

• Individually pouched test devices with desiccant	: 60 Nos.
• Specimen transfer device	: 60 Nos.
• Sterile lancet	: 60 Nos.
• Alcohol swab	: 60 Nos.
• Assay buffer bottle	: 04 Nos.
• Instructions for use	: 01 Nos.

REF 120FRC60
60 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C-30°C

FIRST RESPONSE

HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

4°C-30°C IVD NON STERILE

For Professional Use

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF 120FRC60
60 Tests/kit
One Step Anti HIV 1+2 / SYPHILIS Combo Card Test
4°C-30°C
FIRST RESPONSE
HIV 1+2 / SYPHILIS Combo Card Test
Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test
(Whole Blood/Serum/Plasma)

Mfg. Lic. No. :

LOT :

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Contents:

• Individually pouched test devices with desiccant	: 100 Nos.
• Specimen transfer device	: 100 Nos.
• Sterile lancet	: 100 Nos.
• Alcohol swab	: 100 Nos.
• Assay buffer bottle	: 04 Nos.
• Instructions for use	: 02 Nos.

REF 120FRC100

100 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C 30°C

FIRST RESPONSE®

HIV 1+2 / SYPHILIS Combo Card Test

Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test

(Whole Blood/Serum/Plasma)

REF 120FRC100

100 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C 30°C

For *in vitro* Diagnostic Use Only.

FIRST RESPONSE®

HIV 1+2 / SYPHILIS Combo Card Test

Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test

(Whole Blood/Serum/Plasma)

4°C 30°C

4°C 30°C IVD NON STERILE

For Professional Use

Premier Medical Corporation Private Limited

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

Customer support email : info@premiermedcorp.com

Tel.: +91 260 2780112/113, www.premiermedcorp.com

Product : First Response HIV 1+2 / SYPHILIS Combo Card Test

Pack size: 100 Tests

REF 120FRC100

100 Tests/kit

One Step Anti HIV 1+2 / SYPHILIS Combo Card Test

4°C 30°C

FIRST RESPONSE®

HIV 1+2 / SYPHILIS Combo Card Test

Anti HIV 1+2 / SYPHILIS Antibody Detection Card Test

(Whole Blood/Serum/Plasma)

2. Instructions for use[1]

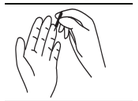
[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages

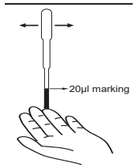
6) Do not use the test device if the pouch is not intact.
7) Do not use the sterile twist lancet if the seal is broken (Refer specimen collection section).
8) Do not use the test device if the desiccant color has changed from orange to green.
9) Do not smoke, eat or drink while handling specimens and performing a test.
10) Do not re-use the test device, alcohol swab, sterile twist lancet, and specimen transfer device as these are intended for single use only.
11) Perform the test by using kit assay buffer, any other buffer or fluid will invalidate the test results.
12) Do not allow the tip of assay buffer bottle to touch specimen well as it may contaminates the assay buffer.
13) Do not use the test device and assay buffer beyond the date of expiry.
14) Do not eat the desiccant.
15) Do not use any other specimen other than human Whole blood/Serum/Plasma. Do not mix and interchange different specimens.

Specimen Collection

1) **Venous blood collection:** Collect the Whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium citrate or ACD by venipuncture.
2) **Plasma collection:** Collect the Whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium citrate or ACD by venipuncture and centrifuge it at 3000 g for 10-15 minutes to obtain Plasma.
3) **Serum collection:** Collect Whole blood in the collection tubes without having any anticoagulants by venipuncture. Keep it in standing position for 30 minutes and centrifuge it at 3000 g for 10-15 minutes to obtain serum.
4) **Capillary whole blood specimen collection:**

Sidelock confirms integrity of lancet. Verify the seal before detaching the cap.





- Wear gloves and massage the fingertip gently. It will help to obtain a round drop of blood.
- Wipe the complete fingertip with the alcohol swab provided and wait until the fingertip dried completely.
- Verify the seal before detaching the cap. Sidelock confirms integrity of sterile twist lancet. Detach the protective cap of the sterile twist lancet. Squeeze the fingertip then prick the lateral side (avoid callus) of the fingertip with sterile twist lancet provided. Safely dispose of the used sterile twist lancet in sharps container immidiately after use..
- Wipe the first drop of the blood using sterile gauze. Without pressing too hard, gently squeeze fingertip once again to obtain the second drop of blood (~40-50 µl).
- Take the specimen transfer device provided and hold it vertically. Gently squeeze the bulb of specimen transfer device and immerse open end in the center of a blood drop and release the bulb slowly to draw up the blood up to the 20 µl marking line on the specimen transfer device.
- Do not use the specimen transfer device having no marking. After completion of specimen collection, take the sterile gauze and apply pressure to the wound site to stop the bleeding. The specimen transfer device is for single use only.

Note: Sterile twist lancet is for single use only. Do not share used sterile twist lancet with another person. Dispose of used sterile twist lancet in sharp box and alcohol swab in biohazard waste container immediately after use.

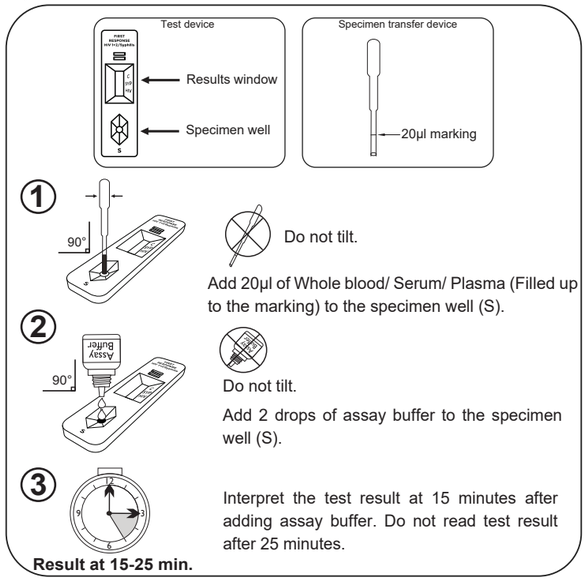
Do not use expired sterile twist lancet. Use of any expired sterile twist lancet may cause infections at the punctured skin due to the expiry of its sterility. Use new sterile twist lancet, alcohol swab and specimen transfer device and choose a different puncture site, if another finger pricking is required.

Specimen storage

1) Venous whole blood specimen should be used for testing immediately (within 1 hour) or shall be stored at 2-8°C for up to 72 hours (3 days). Do not use whole blood specimen stored for more than 3 days, it can cause a non-specific reaction. Do not freeze whole blood specimens.
   Note: Mix the whole blood specimens in the tube by inverting the tube 3 or 4 times before use.
2) If serum or plasma specimens are not immediately tested, then they should be refrigerated at 2-8°C. For storage period greater than 72 hours (3 days), freezing at ≤-20°C is recommended up to 4 months.
3) Venous whole blood, serum and plasma specimens stored at 2-8 °C must be brought to room temperature before use. Serum or plasma specimens stored at ≤-20°C must be thawed at 15 to 25°C. Avoid more than 2 freeze-thaw cycles.
4) Serum or plasma specimens containing precipitate may yield inconsistent test results. Such specimens must be centrifuged at 5000 g for 10 minutes and then use clear supernatants for testing.

Test Procedure

1) Ensure that the test device & other components are at room temperature (15°C to 30°C) before starting the procedure.
2) Open the device pouch, take out the test device from aluminum pouch. Do not use the test device if the desiccant color has changed from orange to green.
3) Label the test device with the patient identification number. Place the test device on a flat, clean and dry surface.
4) Take out the specimen transfer device from plastic bag provided inside the kit. Gently squeeze the bulb of specimen transfer device and immerse the open end in the specimen and release the bulb slowly to draw up the serum/plasma/ capillary or venous whole blood up to 20µl marking line on the specimen transfer device.
5) Gently wipe away the excess specimen from the outer surface of the specimen transfer device with tissue paper before dispensing the specimen into the specimen well.
6) Gently squeeze the bulb of specimen transfer device to add 20 µl of venous or capillary whole blood/ serum/ plasma to the specimen well by gently touching the tips of the specimen transfer device to the sample pad.
   Caution: Dispose of used specimen transfer device and tissue paper as biohazard waste immediately after use
7) Hold the assay buffer bottle vertically and add two drops of assay buffer to the specimen well (S).
8) Observe for development of purple colored lines in the results window. Interpret test results at 15 minutes after adding assay buffer to the specimen well (S).
9) Do not interpret the test result after 25 minutes.



Caution

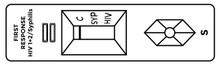
- Hold the specimen transfer device and assay buffer bottle vertically, else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more than 2 drops of assay buffer may cause over flooding or reverse migration phenomenon, which may lead to inaccurate results of the test.
- Adding less than 2 drops of assay buffer may cause improper migration and poor background clearance which may lead to inaccurate results of the test.
- Do not read the test result after 25 minutes. Reading the result after the 25 minutes may give inaccurate results. After recording the results, dispose of the used test device as biohazard waste.

Internal Quality Control

The visualization of the purple colored Control Line in First Response® HIV 1+2 / Syphilis Combo Card Test indicates that the active ingredient of the strips are functional and the migration is successful. The control line is a procedural control serves to demonstrate functional reagents and correct migration of fluid.

How to Interpret test results

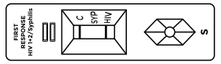
Negative results



If only a single purple colored line appears, at control line “C” as in the figure, then the specimen is non-reactive for antibodies to Syphilis and HIV.

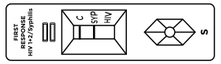
Positive results

HIV 1 and/or HIV 2



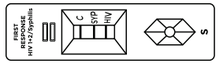
If two purple colored lines appear, one at the control line ‘C’ and other at the test line HIV “HIV” as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and non-reactive for antibodies to Syphilis.

Syphilis Positive



If two purple colored lines appear, one at the control line ‘C’ and other at the test line Syphilis ‘SYP’ as in the figure, then the specimen is reactive for antibodies to Syphilis and non-reactive for antibodies to HIV 1 and/or HIV 2.

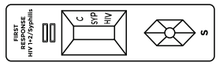
HIV 1 and/or HIV 2 and Syphilis Positive



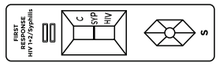
If three purple colored lines appear, one at the control line ‘C’, second at the test line Syphilis ‘SYP’ and third at the test line HIV “HIV” as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and Syphilis.

Note: Interprete faint lines as the reactive lines.

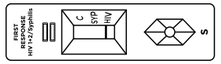
Invalid results



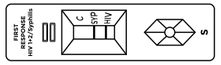
No presence of purple colored control line ‘C’ in the results window (irrespective of the presence of purple colored test lines) indicates an invalid result.



The directions may not be followed correctly or the test may have deteriorated.



The Invalid test results should be retested with a new test device.



Performance Characteristics

First Response® HIV 1+2 / Syphilis Combo Card Test has been tested using an in-house panel of Positive and Negative clinical specimens characterized by a commercial anti-HIV 1&2 ELISA kit and TPHA kit. First Response® HIV 1+2 / Syphilis Combo Card Test showed 100% sensitivity and 100% specificity. First Response® HIV 1+2 / Syphilis Combo Card Test showed 100% agreement with reference assays.

Reference Method	Specimen details	First Response® HIV 1+2/Syphilis Combo Card				
		HIV Positive	HIV Negative	Syphilis Positive	Syphilis Negative	Total
ELISA/ RDT Commercially available	HIV Positive and Syphilis Negative Plasma specimens					
	HIV 1 Positive Plasma Specimen	131	0	0	131	131
	HIV 2 Positive Plasma Specimen	6	0	0	6	6
	Syphilis Positive and HIV Negative Plasma specimens					
	Syphilis Positive plasma Specimen	0	46	46	0	46
	HIV and Syphilis Positive Plasma specimens					
	HIV and Syphilis Positive plasma Specimen	40	0	40	0	40
	HIV and Syphilis Negative Plasma specimens					
	Negative Plasma Specimen	0	370	0	370	370
	Total Plasma specimens	177	416	86	507	593
	HIV Positive and Syphilis Negative Serum specimens					
	HIV 1 Positive Serum Specimen	419	0	0	419	419
	HIV 2 Positive Serum Specimen	85	0	0	85	85
	Syphilis Positive and HIV Negative Serum specimens					
	Syphilis Positive Serum Specimen	0	101	101	0	101
	HIV and Syphilis Negative Serum specimens					
	Negative Serum Specimen	0	3455	0	3455	3455
	Total Serum specimens	504	3556	101	3959	4060
	HIV Positive and Syphilis Negative Whole blood specimens					
	HIV Positive Whole blood specimen	20	0	0	20	20
	Syphilis Positive and HIV Negative Whole blood specimens					
	Syphilis Positive Whole blood specimen	0	34	34	0	34
	HIV and Syphilis Positive Whole blood specimens					
	HIV and Syphilis Positive Whole blood Specimen	31	0	31	0	31
	HIV and Syphilis Negative Whole blood specimens					
	Negative Whole Blood Specimen	0	217	0	217	217
	Total Whole blood specimens	51	251	65	237	302

Reference Method	Specimen details		First Response® HIV 1+2 / Syphilis Combo Card Test			
			Positive	Negative	Total Result	95% Confidence Interval
	Test Marker	Parameter				
ELISA/ RDT Commercially available	Plasma Specimens					
	HIV	Sensitivity	177	00	177	(97.35%-100%)
		Specificity	00	416	416	(98.85%-100%)
	Syphilis	Sensitivity	86	00	86	(94.67%-100%)
		Specificity	00	507	507	(99.06%-100%)
	Serum Specimens					
	HIV	Sensitivity	504	00	504	(99.05%-100%)
		Specificity	00	3556	3556	(99.86%-100%)
	Syphilis	Sensitivity	101	00	101	(95.43%-100%)
		Specificity	00	3959	3959	(99.87%-100%)
	Whole blood Specimens (Capillary and venous blood)					
	HIV	Sensitivity	51	00	51	(91.27%-100%)
		Specificity	00	251	251	(98.12%-100%)
	Syphilis	Sensitivity	65	00	65	(93.04%-100%)
		Specificity	00	237	237	(98.01%-100%)

Seroconversion Panel Testing

The Analytical sensitivity of the First Response® HIV 1+2 / Syphilis Combo Card Test was carried out by testing commercially available Seroconversion panel. The commercially available HIV/Syphilis combo rapid lateral flow test was used as a reference kit for comparative performance study. Twenty-two (22) seroconversion panel was tested, in-house.

Analytical Sensitivity - In - House Evaluation							
Total Seroconversion Panels	Total Specimens	First Response® HIV 1+2 / Syphilis Combo Card Test			Reference HIV/Syphilis Combo rapid lateral flow test.		
		Positive	Negative	Detection Index**	Positive	Negative	Detection Index**
22	130	36	94	0.27	35	95	0.26

**** Detection Index =** Total number of positive specimen by test kit / Total number of specimens.

Cross-Reactivity Study

First Response® HIV 1+2 / Syphilis Combo Card Test was tested with other diseases/conditions, which may give cross-reactivity with the test. The following 18 potential cross-reacting diseases/conditions did not affect the performance of the First Response® HIV 1+2 / Syphilis Combo Card Test.

Specimen details	HIV Negative	HIV Positive	Syphilis Negative	Syphilis Positive	Specimen details	HIV Negative	HIV Positive	Syphilis Negative	Syphilis Positive
P.falciparum Positive	05	Not Tested	05	Not Tested	HSV 1/2 Positive#	05	16	05	08
Pan Malaria Positive	05	Not Tested	05	Not Tested	HTLV- I Ab Positive#	07	08	07	04
Dengue NS1 Positive#	05	08	05	04	HTLV- II Ab Positive#	09	08	09	04
Pregnant Woman ^	320	02	321	01	HSV - IIgG Positive#	08	08	08	04
CMV Positive#	03	08	03	04	Rubella IgG & IgM Positive#	15	16	15	08
ANA Positive#	04	08	04	04	HBV Positive#	103	08	103	04
HAV Positive#	04	08	04	04	Chikungunya Positive#	Not tested	08	Not tested	04
EBV Positive#	02	08	02	04	Anti-malarial drug medication#	04	08	04	04
HCV Positive#	103	08	103	04	Anti-TB drug medication#	05	10	05	05

Note : ^ Naturally appeared HIV and Syphilis positive specimens.
Spiked HIV and Syphilis positive specimens.

Potential interference substances

The First Response® HIV 1+2 / Syphilis Combo Card Test was tested with potential interfering substances. The following 08 potential interfering substances did not affect the performance of First Response® HIV 1+2 / Syphilis Combo Card Test. However, Haemolysed specimens and lipaemic specimens showed poor background clearance, hence not recommended for testing. Lipaemic specimens can be used for the testing after centrifugation. Such specimens must be centrifuged at 5000 g for 10 minutes and use the supernatants for testing.

Specimen Details	HIV Negative	HIV Positive	Syphilis Negative	Syphilis Positive	Specimen Details	HIV Negative	HIV Positive	Syphilis Negative	Syphilis Positive
Lipaemic specimen**,#	25	08	25	04	Low Hematocrit specimens	05	Not tested	05	Not tested
Icteric specimens#	05	08	05	04	Whole blood specimen in ACD anticoagulant	182	Not tested	182	Not tested
Haemolytic specimens**	05	Not tested	05	Not tested	RF Ab Positive#	09	08	09	04
High Hematocrit specimens	05	Not tested	05	Not tested	dsDNA Antibody Positive Plasma#	01	08	01	04

Potential interference Drug substances

The details of interfering drug molecules are mentioned in the following table. Each interfering drug molecule substances were spiked at the final concentration of 250µg/ml in HIV 1, HIV 2 and Syphilis, positive as well as negative specimens, respectively. No false positive or false negative results were observed with any of drug molecules when tested with First Response® HIV 1+2 / Syphilis Combo Card Test.

Diclofenac	Naproxen IP	Acetaminophen	Rifampicin	Ibuprofen
Folic acid	Pantoprazole	Pyrazinamide	Metformin	Aspirin
Ecosprin	Hydrochlorothiazide	Cholecalciferol	Isoniazid	Ampicillin Sodium salt
Magnesium sulphate	Ascorbic Acid (Limec)	Ritonavir	Ferrous Ascorbate	Nevirapine
Daruvir	Cyclobenzaprine Hydrochloride			

Precision

The precision of the First Response® HIV 1+2 / Syphilis Combo Card Test was determined by using the 21 different specimens containing different concentrations of antibodies in 5 different replicates with 3 different lots of test devices. Between-run and within-run precision were observed 100%.

External Evaluation Report

Place of Evaluation	Year	Sensitivity		Specificity	
		Syphilis	HIV	Syphilis	HIV
Zimbabwe (Plasma)	2015	100% (92.94%-100%)	100% (95.60%-100%)	100% (98.00%-100%)	100% (97.59%-100%)
Ghana (Serum/Plasma)	2017	100% (94.29%-100%)	100% (94.29%-100%)	100% (96.88%-100%)	100% (96.88%-100%)
WHO evaluation (Serum/Plasma)	2018	99.0% (96.4% - 99.9%)	100% (98.2% - 100%)	100% (98.2% - 100%)	99.5% (97.2% - 100%)
Ghana (Capillary vs Venus whole blood specimen)	2018	100% (87.35%-100%)	100% (96.19%-100%)	100% (97.71%-100%)	100% (96.07%-100%)
Zimbabwe (Pregnant women whole blood specimen)	2019	100% (87.01%-100%)	100% (96.55%-100%)	100% (98.06%-100%)	100% (96.69%-100%)

Limitations

1) Do not use anti-coagulants other than heparin, EDTA, and sodium citrate.
2) Do not use the haemolysed specimen. A haemolysed specimen may give reddish background even after the end of test time.
3) Interpret a faint line as a positive line. Repeat the test in case of a very faint test line or if have any doubt for the test line.
4) Although a positive result may indicate an infection of HIV 1 and/or HIV 2 or Syphilis (*Treponema pallidum*), a diagnosis of diseases can only be made on clinical grounds. This test should not be used as the sole criteria for the diagnosis of HIV/ *Treponema pallidum*.
5) For confirmation, further analysis of the specimens should be performed, such as ELISA, or western blot analysis for HIV and TPHA for Syphilis. As with all diagnostic tests, results must be interpreted together with other clinical information available to the physician.
6) False negative results may arise because of hook effect due to a very high titer of antibody in a specimen. Repeat the test by using 1:10 dilution of the same specimen (01 portion) in respective non-reactive specimen matrix (09 portions).
7) A non-reactive result does not eliminate the possibility of infection with HIV1/2 and/or *Treponema pallidum*. The specimen may contain a low level of antibodies that cannot be detected by First Response® HIV 1+2 / Syphilis Combo Card Test. If a test result is non-reactive and clinical symptoms persists, additional testing using other reference method is recommended and/or retested for HIV antibodies after more than 21 days since the original testing.
8) Some HIV infected persons on antiretroviral medication may produce false negative results when tested with rapid diagnostic tests.

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use		Contains sufficient for < n > tests
NON STERILE	Non Sterile	REF	Product Code
IVD	In vitro diagnostic medical device	LOT	Lot Number
4°C 30°C	Store at 4-30 °C		Manufacturer
	Caution		Date of manufacture (YYYY-MM)
	Keep dry		Expiration Date (YYYY-MM)
	Do not reuse		Do not use if test device pouch is damaged
	Keep away from sunlight		

References:

1) Hook EW et al. 2002. A randomized, comparative pilot study of azithro mycin versus benzathine penicillin G for treatment of early Syphilis. Sexually Transmitted Diseases. 8: 486-90.
2) Universal Access Report, Scaling up priority HIV/AIDS interventions in the health sector, Progress report 2010.
3) UNAIDS, 2013. Report on the global AIDS epidemic “GLOBAL REPORT”.
4) Kieffer M. 2005. Mortality of infants born to HIV-infected mothers in Africa. The Lancet, 365(9454):120-121.
5) WHO, 2007. The global elimination of congenital Syphilis: rationale and strategy for action.
6) WHO, 2011. Sexually transmitted infections. Geneva: World Health Organization.
7) Aledort JE et al. 2006. Reducing the burden of sexually transmitted infections in resource-limited settings: the role of improved diagnostics. Nature, 444: 59-72.
8) Peeling RW, 2009. Utilization of rapid tests for sexually transmitted infections: promises and challenges. Infectious Diseases Journal, 3: 156-163.
9) Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," Statistics in Medicine, 17, 857-872 (1998).
10) Wilson, E. B. "Probable Inference, the Law of Succession, and Statistical Inference," Journal of the American Statistical Association, 22, 209-212 (1927).
11) TGS-5: Designing Instruction for use for in vitro diagnostic medical devices.
12) A Short guide on methods: Measuring the impact of national PMTCT programmes (2012 July).
13) http://vassarstats.net/clin1.html#def , Richard Lowry.
14) Mwumvaneza Mutagoma, Eric Remera, Dieudonné Sebuhoro, Steve Kanters, David J. Riedel, and Sabin Nsanzimana, “The Prevalence of Syphilis Infection and Its Associated Factors in the General Population of Rwanda: A National Household-Based Survey,” Journal of Sexually Transmitted Diseases, vol. 2016, Article ID 4980417, 8 pages, 2016. https://doi.org/10.1155/2016/4980417.

Product Disclaimer & Warnings

Every warnings and precaution should be taken into consideration before using the test. Failure to consider “Precaution, Warning, and Limitations” may not ensure the diagnostic ability and accuracy of this product. The test result may accordingly be affected by environmental factors and/or user error outside of the control of the Manufacturer and Distributor.

A definitive clinical diagnosis should not be based on the results of a single test, but it should be made by the physician after all clinical and laboratory findings have been evaluated.

“In no event shall our company or its distributor is liable for any direct, indirect, punitive, unforeseen, incidental, special consequential damages, to property or life, whatsoever arising out of or connected with an incorrect diagnosis, whether positive or negative, in the use or misuse of this product”.In the event of performance changes or product malfunction, please contact manufacturer.

Manufactured by
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Customer support E-mail : info@premiermedcorp.com
Tel.: +91 2602780112/113 • Website : www.premiermedcorp.com

• ISO 13485 & EN ISO 13485 Certified Company

Part No.(S)I20-INS-007, Rev.: AB, Date:2020-02-17 ENGLISH

Note : Instructions for use will be printed in local language of the country using the test, if required.

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

Rapid immunochromatographic Card Test for detection of Antibodies to HIV and/or Syphilis in human whole blood/ serum/ plasma

REF I20FRC25, I20FRC30, I20FRC50, I20FRC60 & I20FRC100

4°C 30°C IVD

Intended Use

First Response® HIV 1+2 / Syphilis Combo Card Test is intended for use by healthcare professionals and qualified laboratory personnel. It is a rapid, qualitative screening, in vitro diagnostic test for the detection of antibodies (IgG & IgM) specific to HIV (type 1 & 2) and *Treponema pallidum* in human serum, plasma or venous and capillary whole blood. The test can be used as an aid in the diagnosis of HIV and/or Syphilis. The product can be used for symptomatic, asymptomatic and pregnant women population. The test kit is not automated and does not require any additional instrument. Reactive specimens should be confirmed by supplemental testing with ELISA, Western Blot or TPHA.

Introduction

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 body fluids or tissues, and from mother to fetus or child during the perinatal period.

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 the transplacental passage of mother to the fetus and by blood transfusion. In a case where a patient is infected with HIV as well as Syphilis, it increases the chances of HIV transmission by increasing viral shedding and seminal viral load. The prevalence of HIV is 3 times more in patients infected with Syphilis compared to those not infected with Syphilis infection(14). Incorporating Syphilis screening in HIV prevention programs will help to prevent mother to child transmission of HIV and Syphilis. This can be achieved by the implementation of a simple and affordable dual testing strategy for HIV and Syphilis which could improve screening uptake and accessibility of testing to accelerate time to treatment.

WHO has reported a significantly high number of HIV and Syphilis co-infection in mother to child transmission (MTCT) in Africa. Therefore, the WHO has announced in June 2012 that Prevention of Mother to Child Transmission (PMTCT) should not be considered alone for HIV but considered for HIV and/or Syphilis both, with a vision to eliminate new HIV infections to children by 2015(12). To achieve this vision each pregnant woman should be tested for Syphilis and HIV both rather than HIV only. Development of a single test device containing HIV and Syphilis antigens will solve the issue defined above and will also be a useful step in achieving WHO’s ambitious goal.

Assay Principle

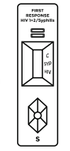
First Response® HIV 1+2 / Syphilis Combo Card Test is based on the principle of immunochromatography for the qualitative detection of antibodies(IgG & IgM) specific for HIV 1&2 and/or Syphilis. The nitrocellulose membrane is coated with a cocktail of recombinant antigen for HIV 1 (gp41) and HIV 2 (gp36) at test line “HIV” and Recombinant TP antigen (P47, P45, P17, P15) specific for Treponema pallidum at the test line “Syp” and control reagent coated at the control line “C”. When serum or plasma or whole blood specimen is applied to the specimen well of the test device, the cocktail of recombinant HIV 1+2 (gp41 & gp36) antigen - colloidal gold conjugate (CGC) & recombinant Treponema pallidum antigens colloidal gold conjugate will react with HIV and/or Syphilis specific antibodies, if present in the specimen. The antibody-CGC antigen complex and assay buffer move along the membrane chromatographically to the test regions and form a visible purple colored line as the antigen-antibody-CGC antigen complex forms with a high degree of sensitivity and specificity. If the specimen contains antibodies to Treponema pallidum, the purple colored line will appear in the test area at test line “Syp”, corresponding to the Syphilis line. If the specimen contains antibodies to HIV 1 and/or 2, the purple colored line will appear in the test area at test line “HIV”, corresponding to HIV 1+2 line.

The presence of both test lines indicates that the specimen contains antibodies to HIV as well as Treponema pallidum. The absence of the purple colored line at both test line regions indicates that the specimen is non-reactive for HIV and Treponema pallidum, showing a negative result. The purple colored Control line will appear irrespective of a reactive or non-reactive specimen. The control line is a procedural control, serves to demonstrate functional reagents and correct migration of fluid.

Materials Provided

Test device pouch

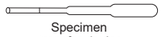

Desiccant



Test device

Assay buffer bottle

Alcohol Swab


Specimen transfer device


Sterile Twist Lancet


Instructions for use

Note: Materials provided other than assay buffer bottle are for single use only.

Materials provided	I20FRC25	I20FRC30	I20FRC50	I20FRC60	I20FRC100
Test device pouch containing: 1 test device, 1 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 (2.5 ml)	1 No.	1 No.	2 Nos.	4 Nos.	4 Nos.
Sterile twist lancets	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Alcohol swabs	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Instructions for use	1 No.	1 No.	1 No.	1 No.	2 Nos.

Materials Required but Not Provided

- New pair of disposable gloves and face mask for each test conducted/specimen collected by Fingerstick.
- Sterile gauze pad and tissue paper.
- Permanent marker pen and timer.
- Extra sterile twist lancets, alcohol swabs, and specimen transfer devices, if needed.
- Sharp disposable box and biohazardous waste container.
- Venipuncture blood collection kit (if whole blood is collected by venipuncture).

Storage and 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. Do not store the kit at the temperature above 30°C and in humid conditions.
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. If the desiccant color has changed from orange to green, do not use the test device.
6) Test device is stable until the printed expiry date on the pouch/external secondary packaging.

Precautions

1) Wear protective gloves and face mask while handling specimens.
2) Dispose of used gloves as biohazard waste. Wash hands thoroughly afterward.
3) Avoid splashing or aerosol formation.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Decontaminate and dispose of all used specimens, test devices, alcohol swabs, and specimen transfer devices as infectious waste, in a biohazardous waste container. Dispose of used sterile twist lancets in a sharps box and face mask in a waste container.

Warnings

1) For in vitro diagnostic use only.
2) Read the instructions carefully before performing the test, any deviation will invalidate the test results.
3) Apply standard biosafety precautions for handling and disposal of potentially infective materials including human biological specimens irrespective of the disease state.
4) Do not drink the assay buffer. It contains sodium azide as a preservative which may be toxic if ingested. When disposed of through sink, flush with a large quantity of water.
5) Devices and assay buffer of a different lot must not be used.