

**WHO Prequalification of In Vitro Diagnostics
PUBLIC ASSESSMENT 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, I20FRC100, and I20FRC25-SA, 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 the WHO Prequalification Assessment for the First Response
HIV1+2/Syphilis Combo Card Test**

	Date	Outcome
Prequalification listing	24 June 2019	listed
Dossier assessment	2 May 2019	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 and change request reference, where applicable.	Date of report amendment
2.0	A new specimen transfer device with a 20 µl marking line was introduced to make it more user-friendly.	6 February 2020
3.0	Introduction of new pack size with auto safety lancet, with product code I20FRC25-SA.	9 August 2023
4.0	Addition of performance evaluation results for HIV subtypes.	27 March 2024
5.0	Introducing new suppliers for Alcohol Swab and Twist Lancet resulted in the change of labels.	12 March 2025
6.0	1. Change in the regulatory certification and labelling of the supplier for sterile lancet "Shandong Lianfa Medical Plastic Products Co., Ltd." 2. Change in the label of the alcohol swab supplied by Medtrue Enterprises Co., Limited.	17 September 2025

	3. Additional supplier for Sterile auto safety lancet, resulting in an additional Sterile auto safety lancet label in the WHOPAR (PQC-IVD-2025-0016).	
7.0	Changes to the assay buffer bottle supplied with the IVD for the First Response product line. There are no changes to the assay buffer itself (PQC-IVD-2025-0015).	12 January 2026

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 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 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 by supplemental testing with ELISA, Western Blot or TPHA.”*

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)	25 tests (product code I20FRC25-SA)
Test device pouch containing: 1 test device, 1 desiccant	25	30	50	60	100	25
Specimen transfer device	25	30	50	60	100	25
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	1 of 2.5 ml
Sterile twist lancets	25	30	50	60	100	\
Auto Safety Lancet	\	\	\	\	\	25
Alcohol swabs	25	30	50	60	100	25
Instructions for use	1	1	1	1	2	1

Items required but not provided

- New pair of disposable gloves and face mask for each test conducted/specimen collected by fingerstick.
- Permanent Marker pen and timer
- Extra lancets and 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)
- Sterile gauze pad and tissue paper.

Storage

The test kit must be stored between 4 and 30 °C.

Shelf-life upon manufacture¹

24 months.

Dossier assessment

Premier Medical Corporation Private Limited submitted a product dossier for the 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 the First Response HIV1+2/Syphilis Combo Card Test meets WHO prequalification requirements.

Manufacturing site inspection

The inspection of the manufacturing site(s) was conducted to assess whether the manufacturer's quality management system (QMS) and manufacturing practices are in alignment with:

(i) applicable international standards, such as ISO 13485 (Medical devices – Quality

¹ The assigned device shelf-life is based on stability data generated from the date of manufacture. The finished goods shelf-life, calculated from the date of packaging completion, may be shorter depending on the time elapsed between manufacture and final packaging of the device.

management systems – Requirements for regulatory purposes);
 (ii) the manufacturer's own documented procedures and quality requirements; and
 (iii) other relevant international standards and guidelines applicable to in vitro diagnostic (IVD) medical devices. The WHO's Public Inspection Reports are accessible at:

<https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports>

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, sodium citrate or ACD). A volume of 20 µL of specimen is needed to perform the assay. This type of assay requires nosophisticated 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) on 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
Analytical sensitivity on WHO reference preparation panels	All 6 HIV subtypes/groups in the 1 st International Reference Panel for anti-HIV (NIBSC code 02/210) were detected	The 1 st International Standard for human syphilitic plasma IgG (NIBSC code 05/122) was detected
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, sodium citrate, or ACD), 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	The 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 review

The labelling submitted for the First Response HIV 1+ 2/Syphilis Combo card Test was reviewed by WHO staff and external technical experts appointed by WHO. The review evaluated the labelling for clarity and consistency with the information submitted in the product dossier, alignment with international guidance and standards, and suitability for the intended users and settings in WHO Member States, including low- and middle-income countries.

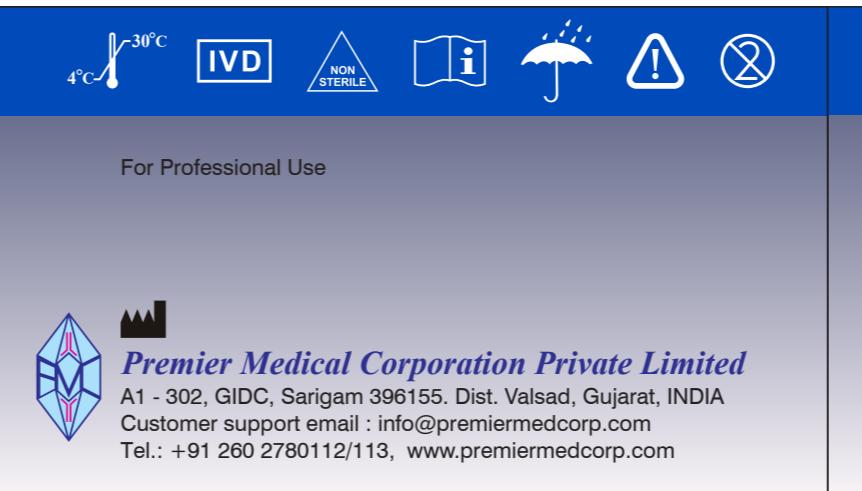
The table below provides traceability of the labelling documents reviewed during the assessment, including document titles, version numbers, approval dates, and control identifiers.

Controlled Labelling References

Document Type	Document Title	Version / Revision	Date Approved	Controlled Document No.
Outer box artwork	Carton – 25 Tests	AC	2020-12	FM-QA-40
	Carton – 30 Tests	AA	2025-12	FM-QA-40
	Carton – 50 Tests	AB	2024-03	FM-QA-40
	Carton – 60 Tests	AA	2025-12	FM-QA-40
	Carton – 100 Tests	AB	2020-12	FM-QA-40
	Carton – 50 Tests Inner	AA	2020-12	FM-QA-40
	Carton – 25 Tests with Sterile Safety Lancet	AB	2021-12	FM-QA-40
Pouch / Device label	Aluminium Pouch	01	2024-08-23	FM-QA-40
Reagent bottle labels	Assay buffer bottle label DBS-035	01	2024-08-23	FM-QA-40
Accessory labeling (e.g., pipettes, buffer caps)	Alcohol Swab (Medtrue)	02	2025-11-25	FM-QA-40
	Alcohol Swab (Phoenix)	01	2024-08-23	FM-QA-40
	Sterile Twist Lancet – (LAN-007)	01	2024-08-23	FM-QA-40
	Sterile Twist Lancet – (LAN-008)	01	2024-08-23	FM-QA-40
	Sterile Safety Lancet – (LAN-014)	01	2025-02-10	FM-QA-40
	Specimen Transfer Device (25 Nos.) (STD-008)	01	2024-08-23	FM-QA-40

	Specimen Transfer Device (30 Nos.) (STD-009)	01	2024-08-23	FM-QA-40
Instructions for Use (IFU)	(S)I20-INS-007	AB	2020-02-17	FM-QA-40
	(S)I20-INS-011	AB	2021-12-15	FM-QA-40

Labels



Carton – 25 Tests

For *in vitro* Diagn

Carton – 30 T

HIV 1+2 / SYPHILIS Combo (Whole Blood/Serum/Plas)

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Mfg. Lic. No.: MFG/MD/2018/000064

LOT

1

□

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.

: AA, 2025-12

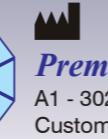
I20FRC30

Anti HIV 1+2 / SYPHILIS Combo Card Test

FIRST RESPONSE

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

For Professional Use



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

REF I20FRC3
 30 Tests/**k**

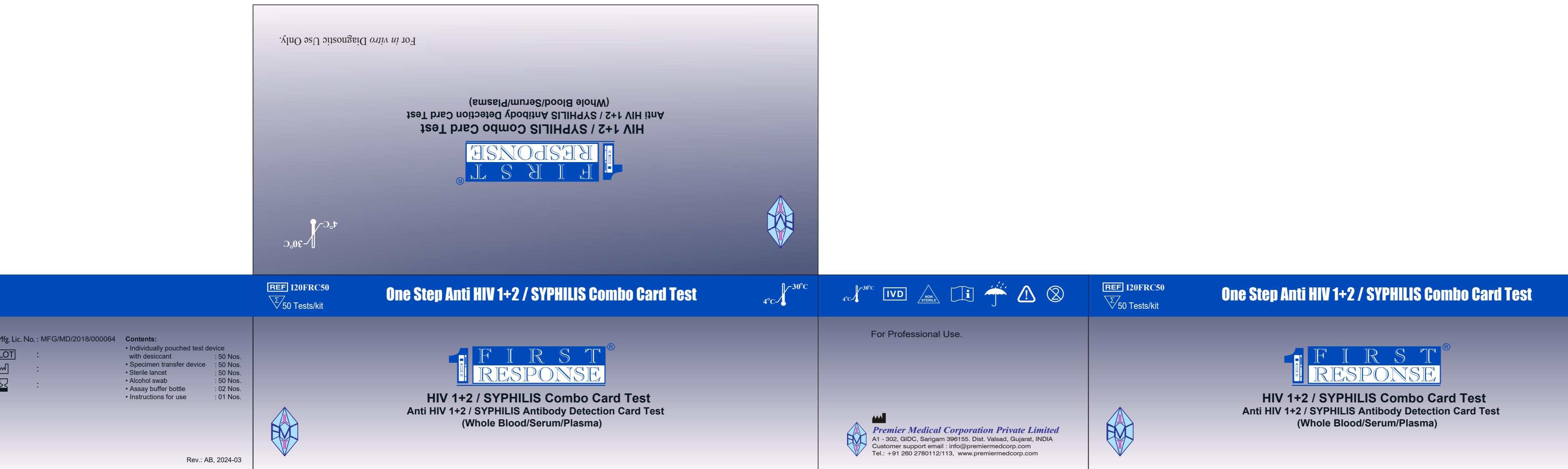
One Step Anti HIV 1 + 2 / SYPHILIS Comb

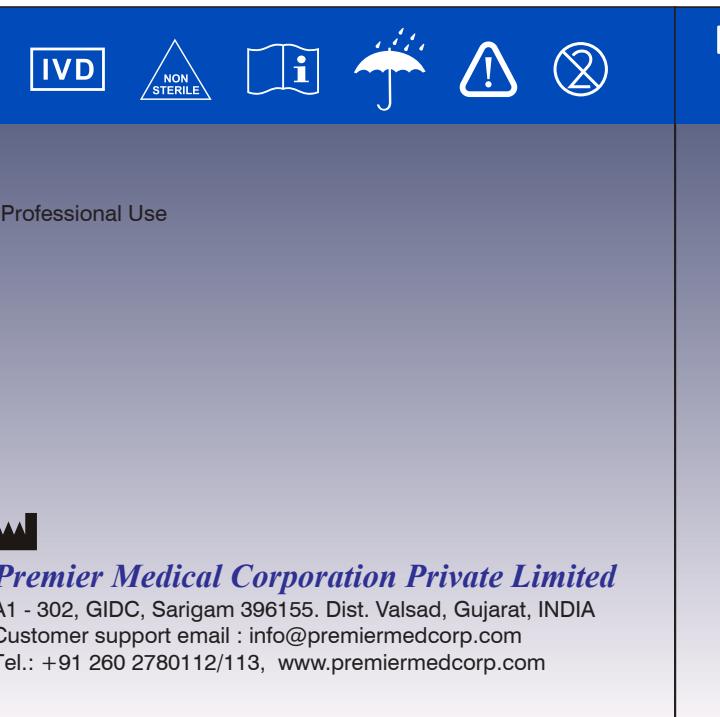
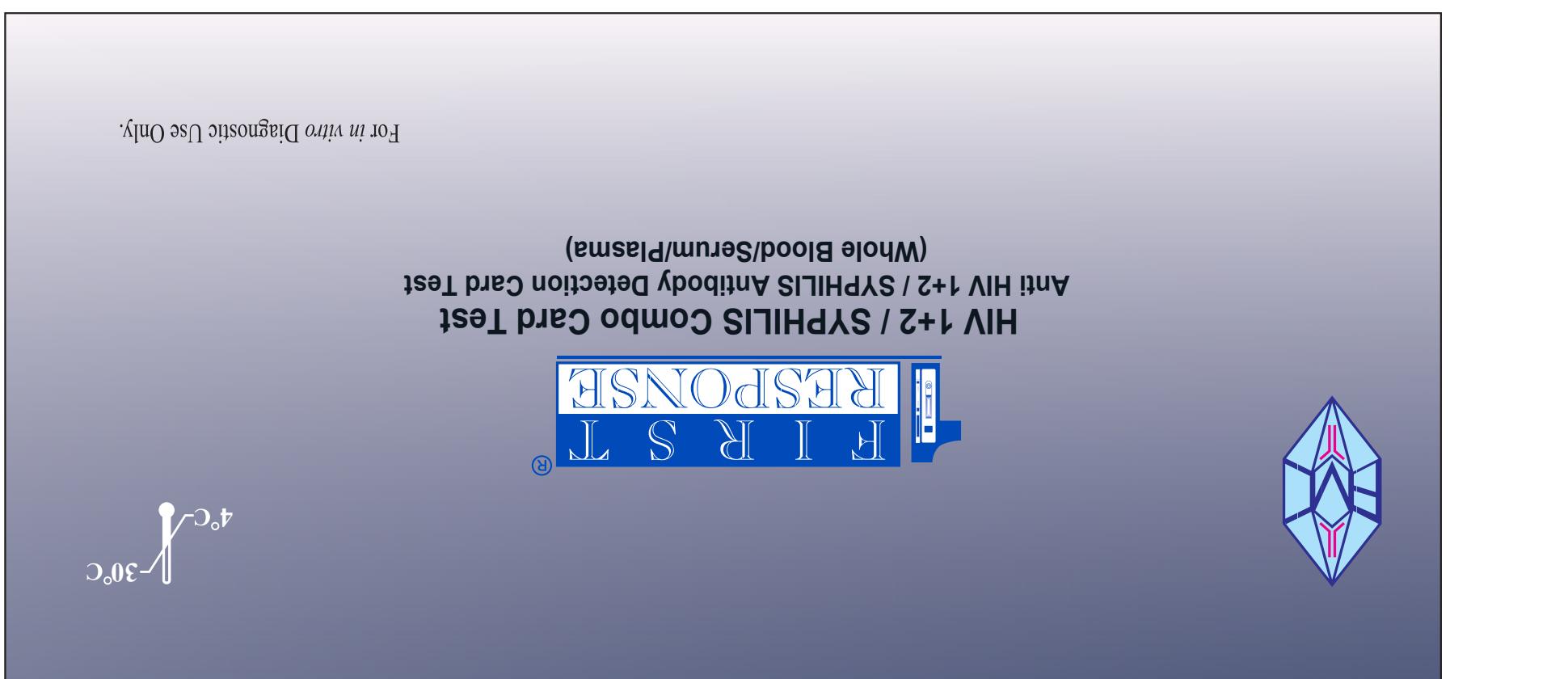
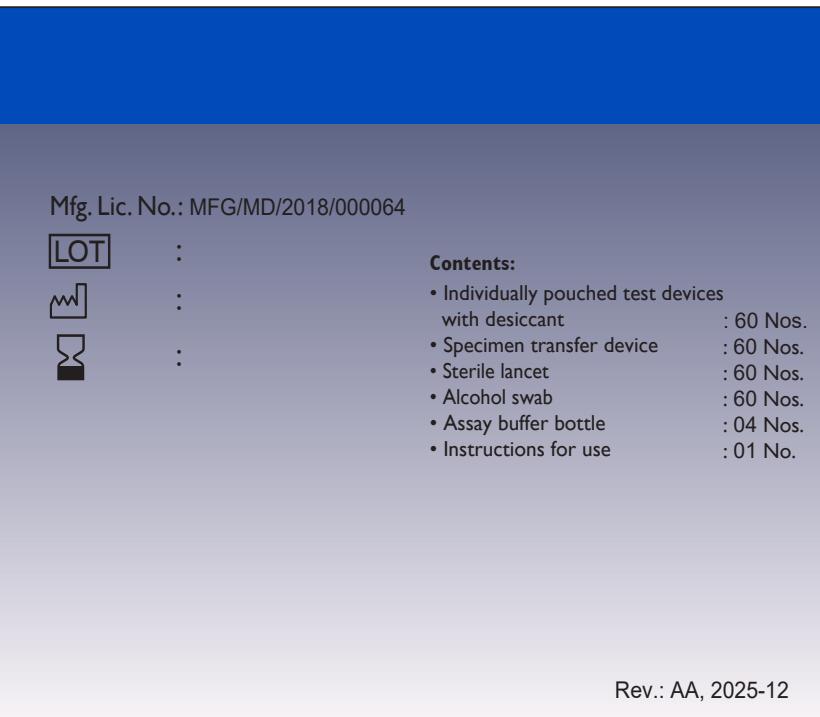
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HIV 1+2 / SYPHILIS Combo Card

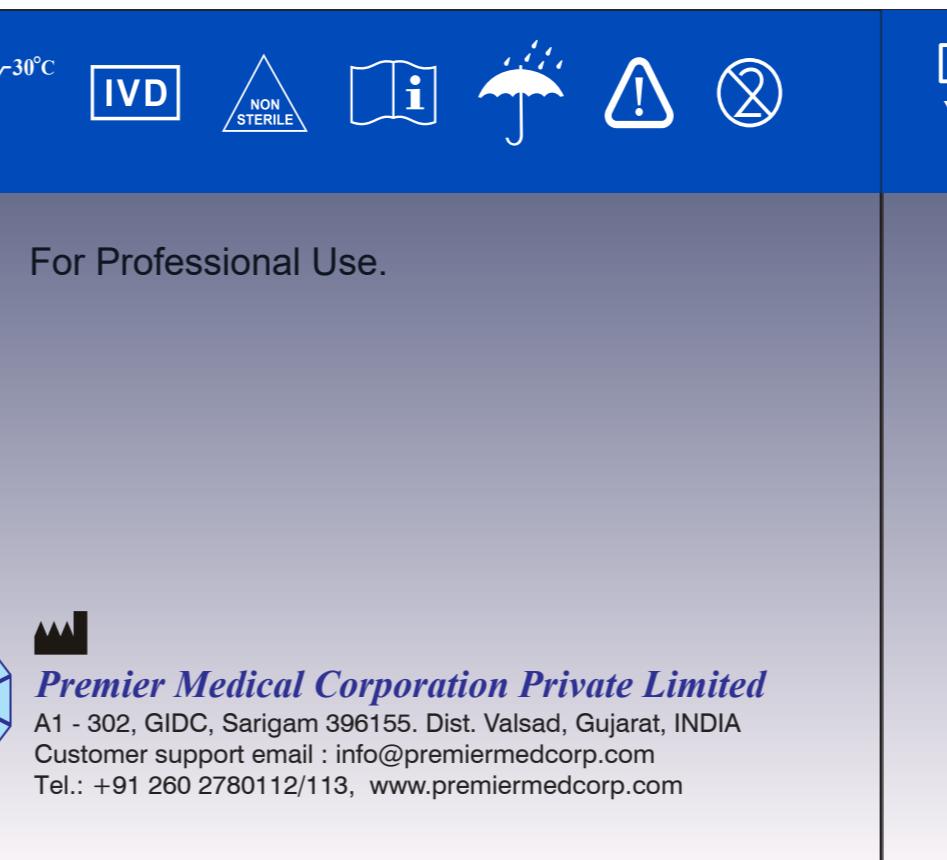
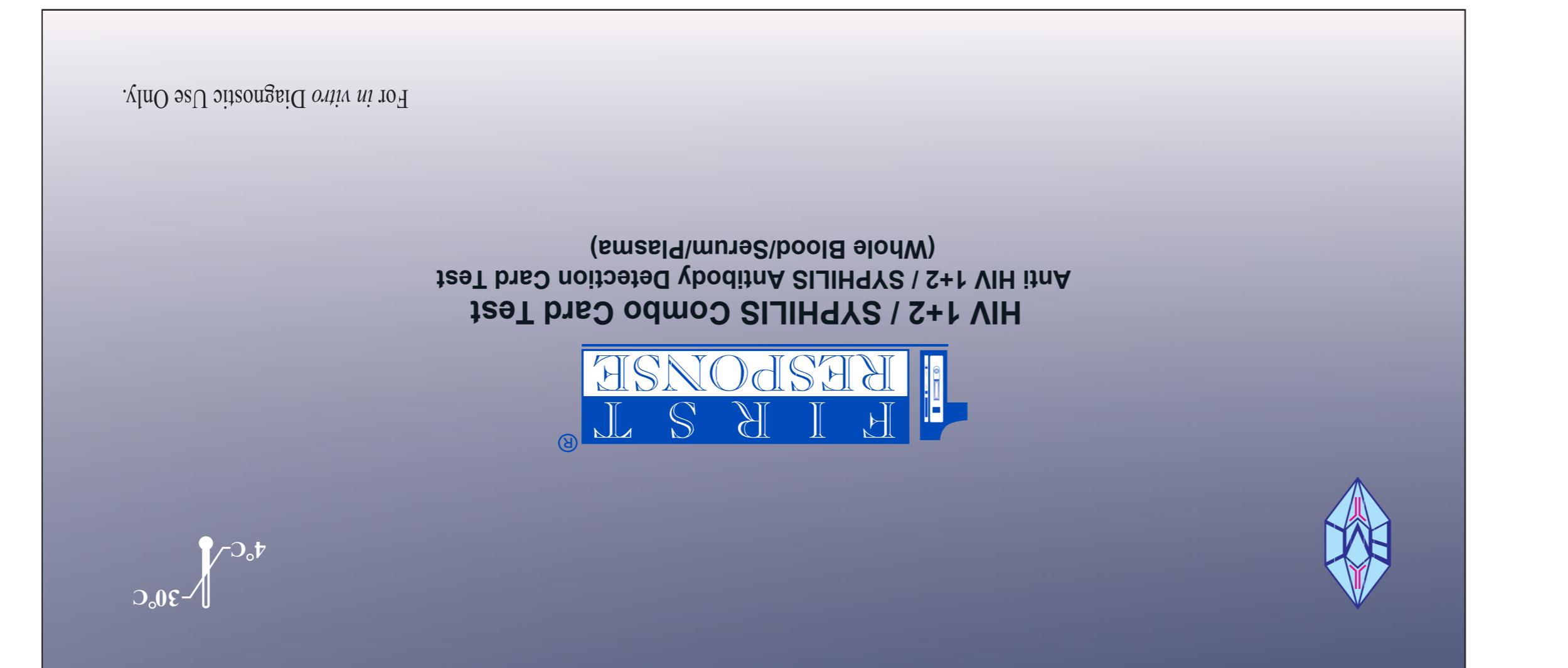
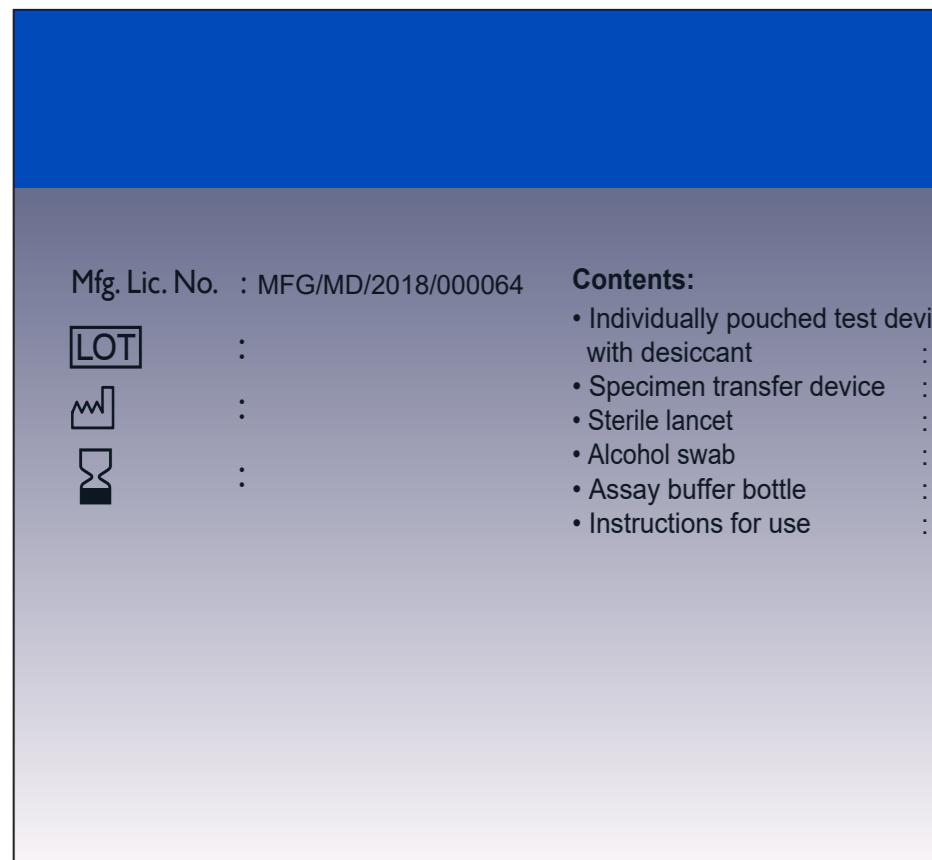
Anti HIV 1+2 / SYPHILIS Antibody Detection (Whole Blood/Serum/Plasma)

Carton - 50 Tests

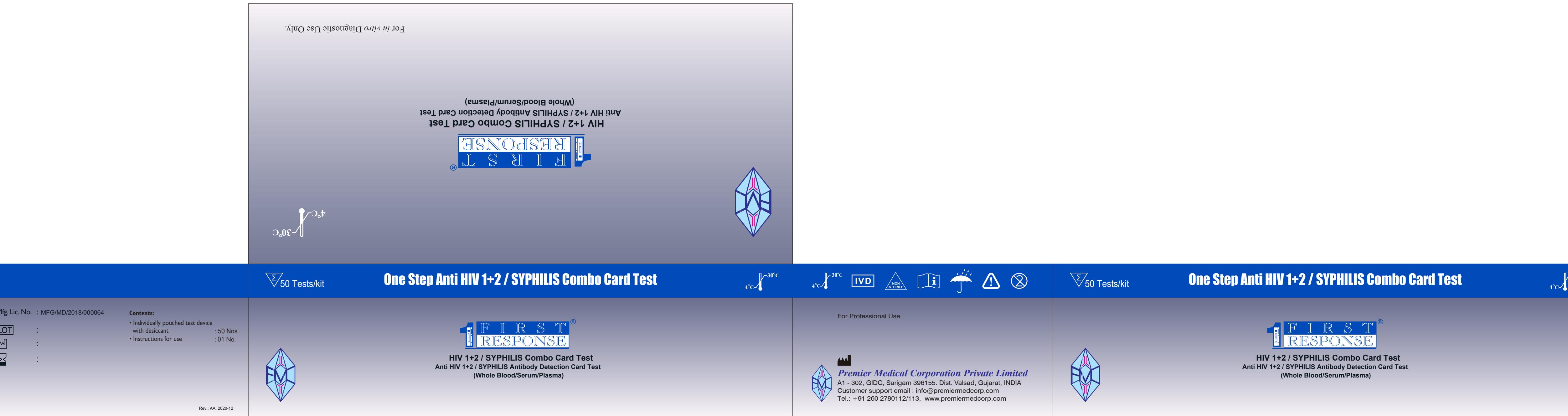


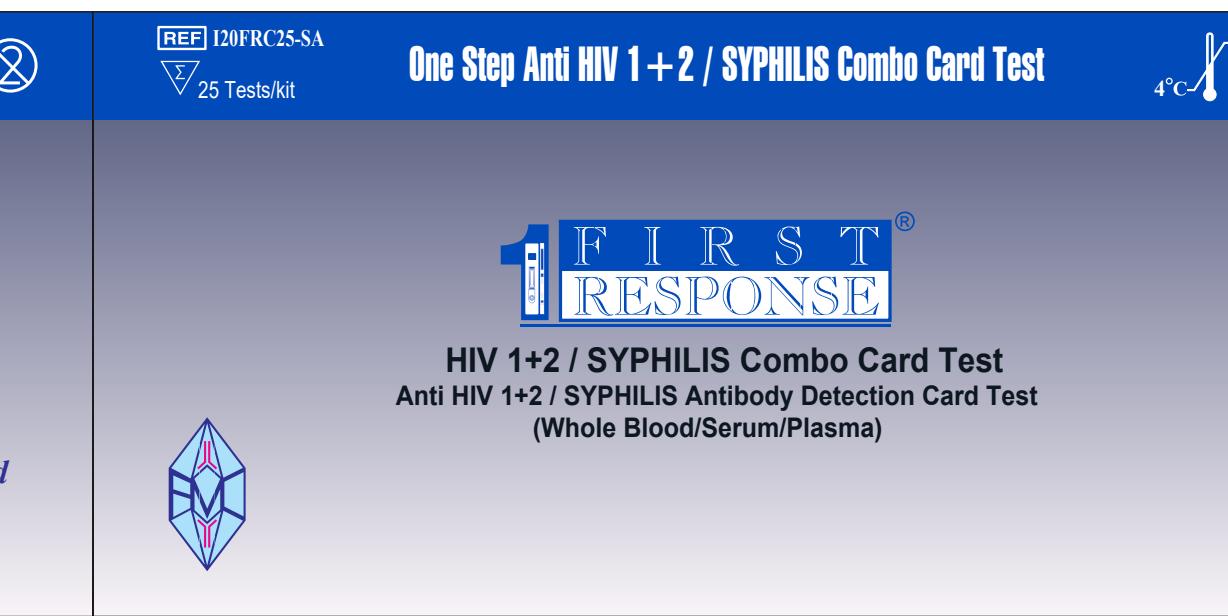
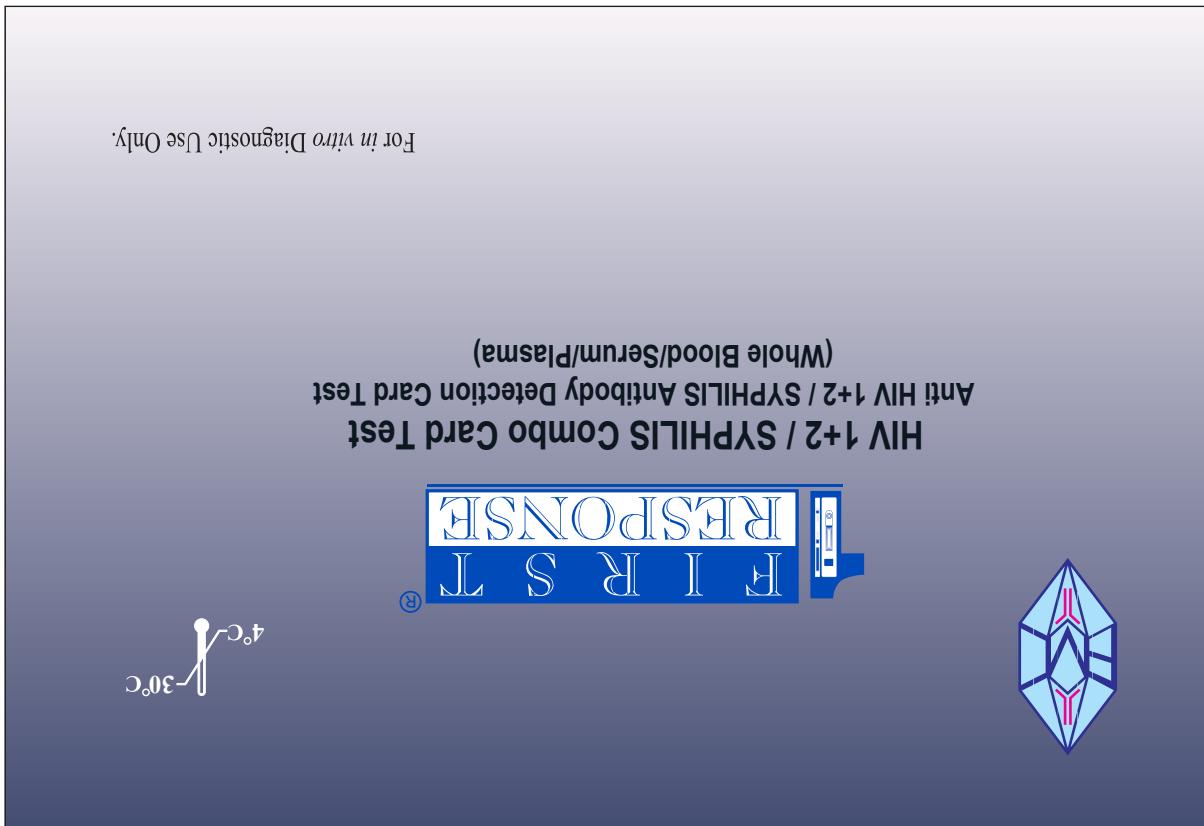
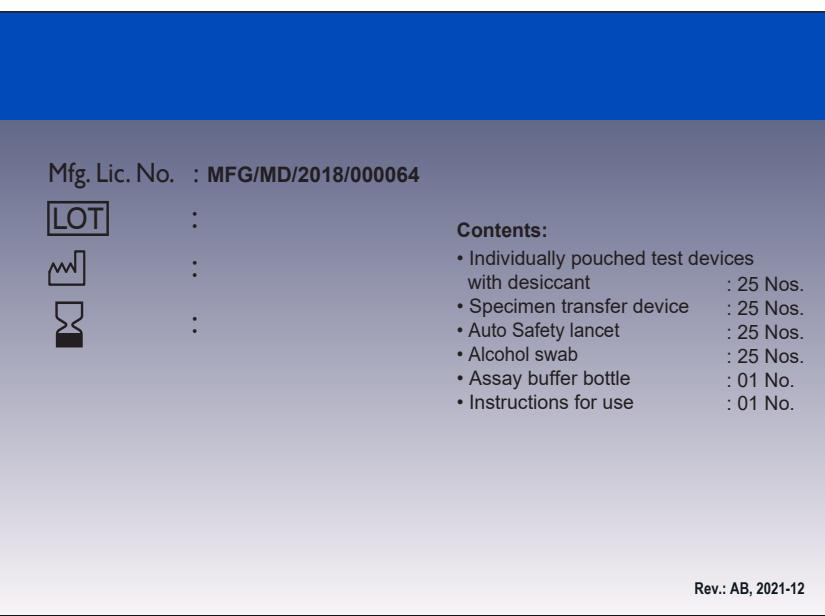


Carton - 100 Tests



Carton – 50 Tests/kit





Carton – 25 Tests with Sterile Safety Lancet

Aluminium Pouch



HIV 1+2 / SYPHILIS Combo Card Test

Anti HIV 1+2 / SYPHILIS Antibody Card Test

Whole Blood / Serum / Plasma



Premier Medical Corporation Private Limited

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

• www.premiermedcorp.com

Mfg. Lic. No.: MFG/MD/2018/000064

LOT

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8

REF I20FRC

IVD



Assay Buffer Label





MEDTRUE®

Alcohol Swabs



CODE:MT59010102



LOT NO. : XXXXXX



MFG. DATE : YYYYMMDD



EXP. DATE : YYYYMMDD

For External Use Only

CONTAIN:One pad saturated
with 70% Isopropyl Alcohol.

DIRECTION:Cleaning the
required area.

Discard after single use.



MEDTRUE ENTERPRISE CO., LTD
Room No.301-302, Hongpujiezu Mansion
186-1 Jiangdongzhonglu Road, 210019 Nanjing, China



EC REP

RIOMAVIX SOCIEDAD LIMITADA
Calle de Almansa 55, 1D, Madrid 28039 Spain

TEAR HERE

PHX2006-NS



Alcohol Prep Pad

70% v/v Isopropyl Alcohol

For External Use Only

1 Pad

Medium

Discard Prep Pad After Single Use

Directions:

Apply topically as needed to cleanse
intended area



Phoenix Innovative Healthcare Manufacturers Pvt. Ltd.
EL-209, Shil Mahape Road, Electronic Zone,
MIDC, TTC Industrial Area, Mahape,
Navi Mumbai - 400 710 MH | India
Customer Care : 022-61075501
Email : customercare@phoenix-hs.com
NCE-MH/DRUGS/25-MH/101592



Advena Ltd.,
Tower Business Centre,
2nd Flr, Tower Street,
Swatar, BKR 4013, Malta



LOT XXXXXXXX

YYYY-MM
YYYY-MM

Rev.00

Blood Lancets (Sterile Lancet)

Model / Specification

I / 28G

UDI



LOT NO.

XXXXXXXXXX



MFG. DATE

YYYY-MM-DD



EXP. DATE

YYYY-MM-DD



QTY

25pcs



(01)06949517007109(11)YYMMDD
(17)YYMMDD(10)XXXXXXXXXX



STERILE

MD

STERILE R



-20°C



40°C



80%



0%

CE 0197

EC REP

Linkfar Healthcare GmbH

Niederrheinstraße 71, 40474 Düsseldorf, Germany

TEL: +49-21138530888



Shandong Lianfa Medical Plastic Products Co., Ltd.

No.1 Shuangshan Sanjian Road, Zhangqiu, Jinan City, 250200, Shandong P. R. China

Blood Lancets (Sterile Lancet)

Model / Specification

I / 28G

UDI



LOT NO.

XXXXXXXXXX



MFG. DATE

YYYY-MM-DD



EXP. DATE

YYYY-MM-DD

QTY

30pcs



(01)06949517007116(11)YYMMDD
(17)YYMMDD(10)XXXXXXXXXX



STERILE

MD

STERILE R



-20°C



40°C



0%



80%



0197

EC REP

Linkfar Healthcare GmbH

Niederrheinstraße 71, 40474 Düsseldorf, Germany

TEL: +49-21138530888



Shandong Lianfa Medical Plastic Products Co., Ltd.

No.1 Shuangshan Sanjian Road, Zhangqiu, Jinan City, 250200, Shandong P. R. China

STERILANCE™ Press Disposable Safety Lancet

Reorder No.: 05-052128

Spec: 21G / 2.8mm

Qty: 25 Pcs/Bag

Intended use:

The safety lancet is used for capillary blood collection.

Contraindications: Unknown.

Caution:

1. Do not use if lancet cap has been previously removed from lancet.
2. Check the use-by date on the packaging, and do not use the lancet beyond the use-by date.
3. The safety lancet is for disposable use and do not reuse the lancet.
4. Discard the used lancet into a suitable sharps container.

Symbolic interpretation:



Sterilized using
irradiation



European Authorized
Representative



Caution



Medical Device



European Authorized
Representative



Caution



Manufacturer

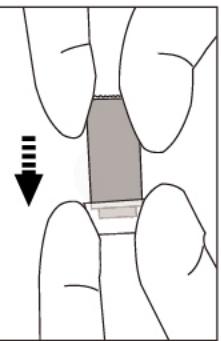


Notified Body

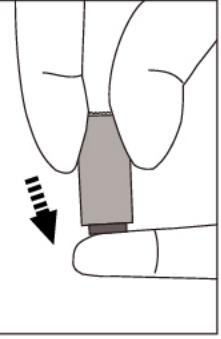


Use-by date

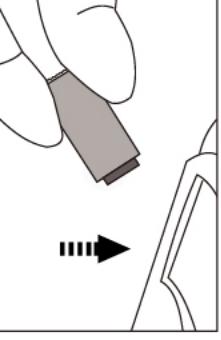
Instructions for Use:



1. Remove the protective cap.



2. Place the lancet firmly against the puncture site to activate. Do not remove the device until an audible click is heard.



3. Discard the used lancet into a suitable sharps container.



(01)16945630100007

(11)YYMMDD

(17)YYMMDD

(10)XXXXXX

Manufacturer:
Sterilance Medical (Suzhou) Inc .

No.168 PuTuoShan Road, New District,
215153 Suzhou, Jiangsu,
PEOPLE'S REPUBLIC OF CHINA
www.sterilance.com

European Authorized Representative:
Emergo Europe B.V.

Westervoortsedijk 60,
6827 AT Arnhem, The Netherlands



YYYY-MM-DD

YYYY-MM-DD

SPECIMEN TRANSFER DEVICE

20ul marking dropper

Qty : 25 pcs/pack



V2 Manufacturers

5/7 BIDC Estate Gorwa Vadodara 390016 India

Lot No : NNNNNNNN

Mfg. Date : DD/MM/YYYY

Exp. Date : DD/MM/YYYY



Single Use Only

SPECIMEN TRANSFER DEVICE

20ul marking dropper

Qty : 30 pcs/pack



V2 Manufacturers

5/7 BIDC Estate Gorwa Vadodara 390016 India

Lot No : NNNNNNNN

Mfg. Date : DD/MM/YYYY

Exp. Date : DD/MM/YYYY



Single Use Only

Instructions for use²

² 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:

- 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 immediately 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 prick 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

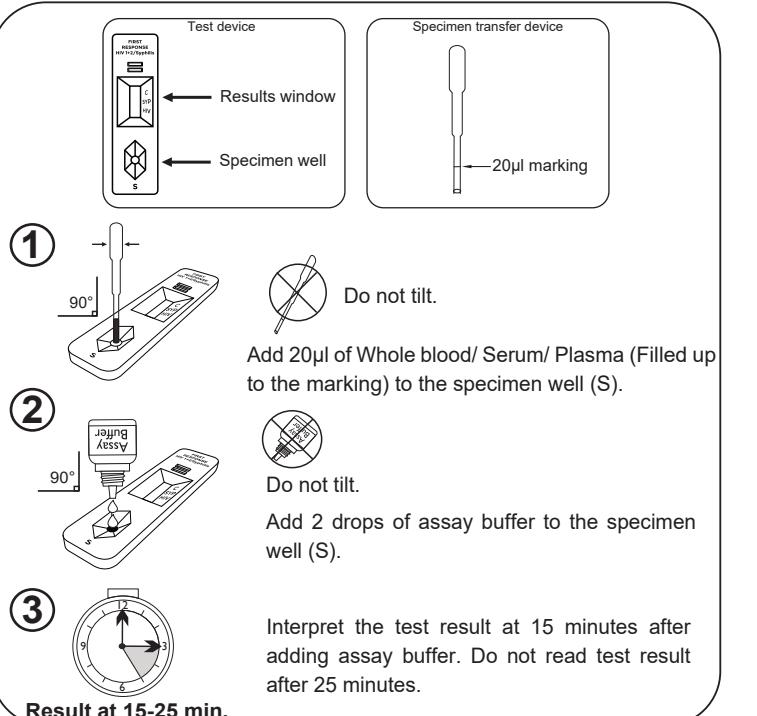
- 1) Ensure that the test device & other components are at room temperature (15°C to 30°C) before starting the procedure.
- 2) Open the device pouch, take out the test device from aluminum pouch. Do not use the test device if the desiccant color has changed from orange to green.
- 3) Label the test device 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

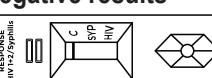
- Hold the specimen transfer device and assay buffer bottle vertically, else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more than 2 drops of assay buffer may cause over flooding or reverse migration phenomenon, which may lead to inaccurate results of 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. (2)

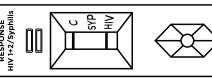
How to Interpret test results

Negative results



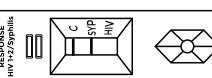
If only a single purple colored line appears, at control line "C" as in the figure, then the specimen is non-reactive for antibodies to Syphilis and HIV.

Positive results



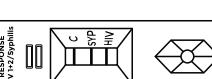
If two purple colored lines appear, one at the control line 'C' and other at the test line HIV "HIV" as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and non-reactive for antibodies to Syphilis.

HIV 1 and/or HIV 2



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.

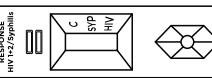
Syphilis Positive



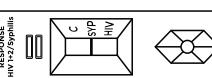
If three purple colored lines appear, one at the control line 'C', second at the test line Syphilis 'SYP' and third at the test line HIV "HIV" as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and Syphilis.

Note: Interpret faint lines as the reactive lines.

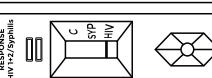
Invalid results



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
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	

ELISA/RDT Commercially available

Reference Method	Specimen details		First Response® HIV 1+2 / Syphilis Combo Card Test			
	Test Marker	Parameter	Positive	Negative	Total Result	95% Confidence Interval
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			

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		Product Code
	In vitro diagnostic medical device		Lot Number
	Store at 4-30 °C		
	Manufacturer		Date of manufacture (YYYY-MM)
	Caution		Expiration Date (YYYY-MM)
	Keep dry		Do not reuse
	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 Kinters, 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

Premier Medical Corporation Private Limited

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• ISO 13485 & EN ISO 13485 Certified Company

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

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



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.

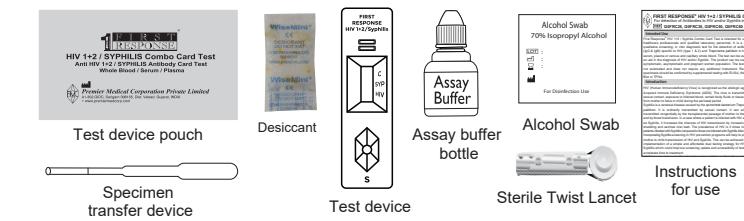
WHO has reported a significantly high number of HIV and Syphilis co-infection in mother to child transmission (MTCT) in Africa. Therefore, the WHO has announced in June 2012 that Prevention of Mother to Child Transmission (PMTCT) should not be considered alone for HIV but considered for HIV and/or Syphilis 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



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

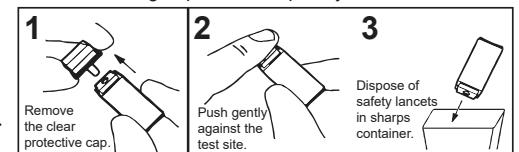
- 6) Do not use the test device if the pouch is not intact.
- 7) Do not use the auto safety lancet if the lancet found uncapped (Refer specimen collection section).
- 8) Do not use the test device if the desiccant color has changed from orange to green.
- 9) Do not smoke, eat or drink while handling specimens and performing a test.
- 10) Do not re-use the test device, alcohol swab, auto safety 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 contaminate 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:

- 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.



Auto Safety Lancet

(Sterile Pressure Activated Lancet)

Instructions for use →

- Do not use the auto safety lancet if the auto safety lancet found uncapped. Detach the protective cap of the auto safety lancet provided. Squeeze the fingertip then push gently at the lateral side (avoid callus) of the fingertip as shown in above figure. Safely dispose of the used auto safety lancet in sharps container immediately after use.

- Wipe the first drop of the blood using sterile gauze. Without pressing too hard, gently squeeze fingertip once again to obtain 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: Auto safety lancet is for single use only. Do not share used auto safety lancet with another person. Dispose of used auto safety lancet in sharp box and alcohol swab in biohazard waste container immediately after use.

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

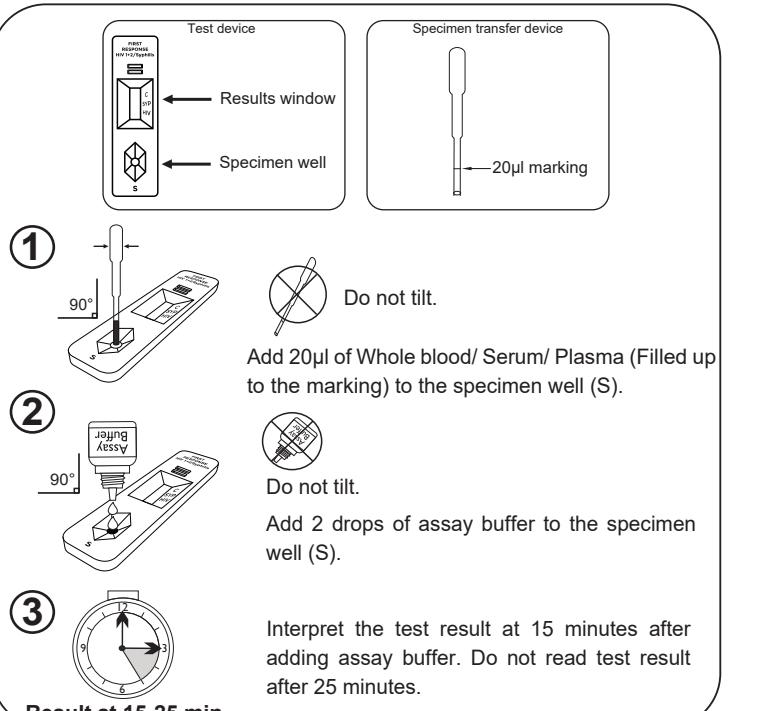
- 4) Serum or plasma specimens containing precipitate may yield inconsistent test results. Such specimens must be centrifuged at 5000 g for 10 minutes and then use clear supernatants for testing.

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

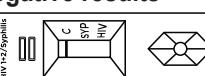
- Hold the specimen transfer device and assay buffer bottle vertically, else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more than 2 drops of assay buffer may cause over flooding or reverse migration phenomenon, which may lead to inaccurate results of 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. (2)

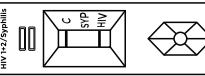
How to Interpret test results

Negative results



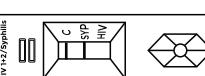
If only a single purple colored line appears, at control line "C" as in the figure, then the specimen is non-reactive for antibodies to Syphilis and HIV.

Positive results



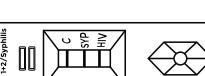
If two purple colored lines appear, one at the control line 'C' and other at the test line HIV "HIV" as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and non-reactive for antibodies to Syphilis.

HIV 1 and/or HIV 2



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.

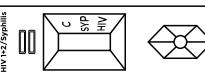
Syphilis Positive



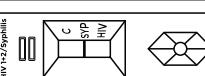
If three purple colored lines appear, one at the control line 'C', second at the test line Syphilis 'SYP' and third at the test line HIV "HIV" as in the figure, then the specimen is reactive for antibodies to HIV 1 and/or HIV 2 and Syphilis.

Note: Interpret faint lines as the reactive lines.

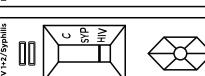
Invalid results



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
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	

ELISA/RDT Commercially available

Reference Method	Specimen details	First Response® HIV 1+2 / Syphilis Combo Card Test			
		Positive	Negative	Total Result	95% Confidence Interval
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)					

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		Product Code
	In vitro diagnostic medical device		Lot Number
	Store at 4-30 °C		
	Caution		Manufacturer
	Date of manufacture (YYYY-MM)		Expiration Date (YYYY-MM)
	Do not reuse		Do not use if test device pouch is damaged
	Keep dry		

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.
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- 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 Kinters, 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 be 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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• ISO 13485 & EN ISO 13485 Certified Company

Part No.(S)I20-INS-011, Rev.: AB, Date:2021-12-15



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-SA



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.

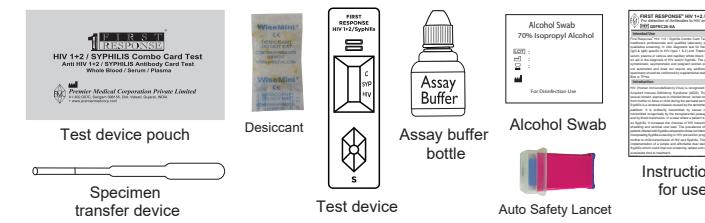
WHO has reported a significantly high number of HIV and Syphilis co-infection in mother to child transmission (MTCT) in Africa. Therefore, the WHO has announced in June 2012 that Prevention of Mother to Child Transmission (PMTCT) should not be considered alone for HIV but considered for HIV and/or Syphilis 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



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

Materials provided	I20FRC25-SA
Test device pouch containing:	1 test device, 1 desiccant
Specimen transfer device	25 Nos.
Assay buffer bottle (2.5 ml)	1 No.
Auto Safety lancets	25 Nos.
Alcohol swabs	25 Nos.
Instructions for use	1 No.

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 auto safety 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 auto safety 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.