WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: First Response Syphilis Anti-TP Card Test
WHO reference number: PQDx 0471-010-00

First Response Syphilis Anti-TP Card Test with product codes **PI08FRC25, PI08FRC50**, **PI08FRC100, and PI08FRC25-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 13 January 2021.

Summary of WHO Prequalification Assessment for First Response Syphilis Anti-TP Card Test

	Date	Outcome
Prequalification listing	13 January 2021	listed
Dossier assessment	28 May 2020	MR
Site inspection(s) of the quality management system	3 May 2019 and 7 January 2021	MR
Product performance evaluation	Quarter 4 2020	MR

MR: Meets Requirement

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the 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.

Public report amendment	Summary of amendment	Date of report amendment
2.0	Introduction of new pack size with auto safety lancet, PI08FRC25-SA, in addition to the existing prequalified catalogues of PI08FRC25, PI08FRC50, and PI08FRC100.	22 September 2023

Intended use

According to the claim of intended use from Premier Medical Corporation Private Limited, "*First Response Syphilis Anti-TP 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 of all classes specific to Treponema pallidum in human serum, plasma or venous or*

capillary whole blood. The test can be used as an aid in the diagnosis of Syphilis infection. The product can be used for symptomatic, asymptomatic, and pregnant women populations. The test kit is not automated and does not require any additional instruments. The test is intended to be used at Point of care and/or laboratory settings. Reactive specimens should be confirmed by supplemental testing."

Assay description

According to the claim of assay description from Premier Medical Corporation Private Limited, "*When the test specimen and assay buffer are added to the specimen well, they flow along the colloidal gold-coupling antibody-Treponema pallidum antigen complex present at the conjugate pad. If Treponema pallidum specific antibodies i.e IgG or IgM are present in the sample, they will further form a complex of colloidal gold-coupling antibody-Treponema pallidum antigen-anti-treponema pallidum antibody which will migrate through the nitrocellulose membrane, when it encounters at test band (which contains Treponema pallidum antigen) the complex binds with antigen at test line forming colloidal gold-coupling antibody-Treponema pallidum- anti-Treponema pallidum antibody-antigen complex giving reactive result. This will make the purple-colored line visible at the test band. While in the case of non-reactive specimen, there is no antibody-Treponema pallidum-anti-Treponema pallidum antibody-antigen complex to bind with the test band and the purple coloured line will not appear. At the control line, the goat-anti mouse IgG will bind with the colloidal gold-coupling antibody-Treponema pallidum complex forming a purple colored line. Here the coupling antibody used in the colloidal gold conjugation with Treponema pallidum antigen is a mouse IgG. Thus irrespective of the reactive or non-reactive result the control line will appear."*

Test kit contents

Component	**25 tests (product code PI08FRC25)**	**50 tests (product code PI08FRC50)**	**100 tests (product code PI08FRC100)**	**25 tests (product code PI08FRC25-SA)**
Test device pouch containing: 1 test device, 1 desiccant	25	50	100	25
Specimen transfer device	25	50	100	25
Assay buffer bottle	1 of 2.5 ml	2 of 2.5 ml	4 of 2.5 ml	1 of 2.5 ml
Sterile twist lancets	25	50	100	\
Auto Safety lancets	\	\	\	25
Alcohol swabs	25	50	100	25
Instructions for use	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.
- Sterile gauze pad and tissue paper.
- Permanent marker pen and timer.
- Extra sterile twist lancets, alcohol swabs and specimen transfer device, if needed.
- Sharp disposable box and biohazardous waste container.
- Venipuncture blood collection kit (if whole blood is collected by venipuncture).

Storage

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

Shelf-life upon manufacture

24 months.

Warnings/limitations

Please refer to the current version of the manufacturer's instructions for use attached to this public report.

Prioritization for Prequalification

Based on the established eligibility criteria, the First Response Syphilis Anti-TP Card Test was given priority for WHO prequalification assessment.

Dossier assessment

Premier Medical Corporation Private Limited submitted a product dossier for the First Response Syphilis Anti-TP Card Test, 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 28 May 2020.

Manufacturing site inspection

An inspection of Premier Medical Corporation Private Limited located at A1-302, GIDC, Sarigam 396 155, Valsad, Gujarat, India and 32-35A, Shree Ganesh Industrial Estate, Kachigam, Nani Daman,

Daman 396215, India was conducted from 12-14 March 2018. A desk assessment of the same sites was performed between 17-24 December 2020. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for *in vitro* diagnostic products and gives a summary of the inspection findings.

To note:
At the time of inspection in 2018, this product had not been submitted to WHO and was, therefore, not verified. The information and compliance with ISO 13485 found during the onsite inspection and desk assessment will be used in lieu of an onsite inspection for this product. This product will be verified at the next inspection. Due to the COVID-19 pandemic and the corresponding national and international travel restriction, some inspection dates may be impacted.
Information on the most current inspection can be found at:

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

All published WHOPIRs are with the agreement of the manufacturer.

The manufacturer's responses to the nonconformities found during the inspection and desk assessment were accepted on 3 May 2019 and 7 January 2021.

Product performance evaluation

First Response Syphilis Anti-TP Card Test (Premier Medical Corporation Private Limited) was evaluated by the National Serology Reference Laboratory (NRL), Australia, on behalf of WHO in the fourth quarter of 2020, according to protocol PQDx_326, version 2.0.

Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 570 plasma/serum specimens was used. The specimens were characterized using the following reference assays: LIAISON Treponema Screen CLIA (DiaSorin S.p.A), followed by SERODIA TP-PA (Fujirebio) for detection of anti-TP antibodies; and BD Macro-Vue RPR Card Tests (Becton Dickinson) for detection of non-TP antibodies.

The clinical performance characteristics of the First Response Syphilis Anti-TP Card Test for the detection of anti-TP antibodies were as follows:

Clinical performance characteristics in comparison with an agreed reference standard	
Sensitivity % (N=270)	99.6% (95% CI: 98.0-100)
Specificity % (N= 300)	100% (95%CI: 98.4-100)
Invalid rate % (N= 570)	0
Inter-reader variability % (N= 570)	0

Analytical performance evaluation

Analytical performance characteristics	
Sensitivity during seroconversion on 1 seroconversion panel in comparison with a benchmark assay (LIASION Treponema Screen CLIA)	Of a total of 9 specimens, 5 were detected by the assay under evaluation versus 4 specimens detected by the benchmark assay.
Analytical sensitivity on a performance panel (AccuSet Syphilis Performance Panel 0820-0300)	20 of the 20 specimens were correctly classified.
Analytical sensitivity on WHO reference preparation panel (NIBSC code 05/132)	The lowest concentration detected by the assay was 0.006 IU/mL.
Lot to lot variation on a dilution panel	Lot to lot variation was within +/- 1 two-fold dilutions for all 10-dilution series.

Operational characteristics and ease of use

This assay does not require laboratory equipment and can be performed in laboratories with limited facilities or non-laboratory settings.

The assay was found easy to use by the operators performing the evaluation.

Key operational characteristics	
Number of steps*	2 steps in total No steps with precision pipetting are required. The Specimen is added to the device using the Specimen transfer device supplied with the kit.
Time to result	20 minutes
Endpoint stability (interval)	5 minutes (the test can be read between 20-25 minutes after addition of diluent)
Internal QC	Yes. Reagent addition control

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

Based on these results, the performance evaluation for the First Response Syphilis Anti-TP Card Test meets the WHO prequalification requirements.

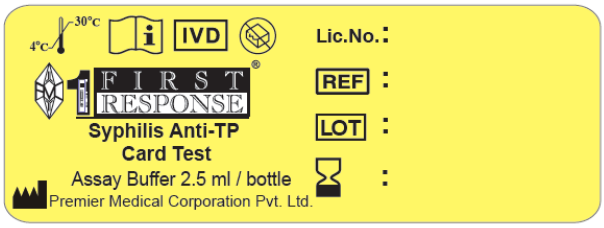
Labelling

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

1. Labels:

1.1 Test Pouch

1.2 Assay buffer bottle label



1.3 Alcohol swab(in product codes PI08FRC25, PI08FRC50, PI08FRC100 and PI08FRC25-SA)

1.4 Sterile lancet (in product codes PI08FRC25, PI08FRC50, and PI08FRC100)

1.5 Safety Lancets (in product code PI08FRC25-SA)

1.5 Test kit Carton: 25 test/pack (PI08FRC25)

Product Name : F.R Syphilis Anti-TP Card Test
Pack Size : 25 Tests / bulk
Language: English

For *in vitro* Diagnostic Use Only.

Syphilis Anti-TP Card Test
Rapid Syphilis Card Test
Whole Blood / Serum / Plasma

FIRST RESPONSE

4°C 30°C

REF PI08FRC25 | 25 Tests/kit | **One Step Syphilis Anti-TP Card Test** | 4°C 30°C

Mfg. Lic. No. :
LOT :
:
:

Contents:

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

REV.: AA , 2019-04

FIRST RESPONSE

Syphilis Anti-TP Card Test
Rapid Syphilis 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 PI08FRC25 | 25 Tests/kit | **One Step Syphilis Anti-TP Card Test** | 4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test
Rapid Syphilis Card Test
Whole Blood / Serum / Plasma

1.7 Test kit Carton 50 test/pack (PI08FRC50)

Product Name : F.R Syphilis Anti-TP Card Test
Pack Size : 50 Tests / bulk
Language: English

Mfg. Lic. No. :
LOT :
:
:

Contents:

• Individually pouched test devices with desiccant	: 50 Nos.
• Specimen transfer device	: 50 Nos.
• Sterile twist lancet	: 50 Nos.
• Alcohol swab	: 50 Nos.
• Assay buffer bottle	: 02 Nos.
• Instructions for use	: 01 No.

REV: AA , 2019-04

For *in vitro* Diagnostic Use Only.

Syphilis Anti-TP Card Test
Rapid Syphilis Card Test
Whole Blood / Serum / Plasma

4°C 30°C

REF PI08FRC50
50 Tests/kit

One Step Syphilis Anti-TP Card Test

4°C 30°C

Syphilis Anti-TP Card Test
Rapid Syphilis 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 PI08FRC50
50 Tests/kit

One Step Syphilis Anti-TP Card Test

4°C 30°C

Syphilis Anti-TP Card Test
Rapid Syphilis Card Test
Whole Blood / Serum / Plasma

1.8 Test kit Carton 100 test/pack (PI08FRC100)

Product Name : F.R Syphilis Anti-TP Card Test
Pack Size : 100 Tests / bulk
Language: English

Mfg. Lic. No. :

LOT :

:

:

Contents:

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

REV.: AA , 2019-04

REF PI08FRC100

100 Tests/kit

One Step Syphilis Anti-TP Card Test

4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

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 PI08FRC100

100 Tests/kit

One Step Syphilis Anti-TP Card Test

4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

For *in vitro* Diagnostic Use Only.

1.9 Inner carton for 100 tests/ pack: 50 Tests per carton X 2 numbers on outer carton)

Product Name : F.R Syphilis Anti-TP Card Test
Pack Size : 50 Tests - Inner Carton
Language: English

For *in vitro* Diagnostic Use Only.

FIRST RESPONSE®

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

Σ 50 Tests/kit

One Step Syphilis Anti-TP Card Test

Mfg. Lic. No. :

LOT :

:

:

Contents:

- Individually pouched test device with desiccant : 50 Nos.
- Instructions for use : 01 No.

REV.: AA , 2018-02

FIRST RESPONSE®

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

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

Σ 50 Tests/kit

One Step Syphilis Anti-TP Card Test

4°C 30°C

FIRST RESPONSE®

Syphilis Anti-TP Card Test

Rapid Syphilis Card Test

Whole Blood / Serum / Plasma

1.10 Test kit Carton: 25 test/pack (PI08FRC25-SA)

Part No. :(S)PI08-CAR-016 Rev.: AB, 2021-12
Product Name : F.R SYPHILIS Anti-TP Card Test
Pack Size : 25 Tests / bulk (Auto Lancet)
Dimension:165 (L) X 110 (W) X 80 (H) MM & GSM: 450
Language: English

For *in vitro* Diagnostic Use Only.

Syphilis Anti-TP Card Test
Rapid Syphilis Card Test
Whole Blood / Serum / Plasma

FIRST RESPONSE

4°C 30°C

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

LOT :

:

:

Contents:

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

Rev.: AB, 2021-12

REF PI08FRC25-SA
25 Tests/kit
One Step Syphilis Anti-TP Card Test
4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test
Rapid Syphilis 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 PI08FRC25-SA
25 Tests/kit
One Step Syphilis Anti-TP Card Test
4°C 30°C

FIRST RESPONSE

Syphilis Anti-TP Card Test
Rapid Syphilis 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.

Precision

a) Within-run precision was determined by using 15 replicates of 9 different specimens containing different concentrations of antibodies. Within-run, precision was observed as 100%.

b) Between-run, precision was determined by using the 9 different specimens containing different concentrations of antibody in 5 different replicates with 3 different lots of test devices. Between run, precision was observed as 100%.

External Evaluation Report

Place of Evaluation	Year of Testing	Sensitivity	Specificity
Virus Research Institute, Uganda	2018	100% (95.38%-100%)	100% (95.38%-100%)
Zimbabwe (Pregnant women whole blood specimen)	2019	100% (95.97%-100%)	100% (97.65%-100%)

Limitations & Interferences

1) The assay procedure and interpretation of assay result sections must be followed closely. Failure to follow the procedure may lead to inaccurate test results.
2) First Response® Syphilis Anti-TP Card Test is designed to detect antibodies specific to *Treponema pallidum* in human serum, plasma, and whole blood. Other body fluids or pooled specimens may not give accurate results.
3) First Response® Syphilis Anti-TP Card Test is limited to the qualitative detection of antibodies specific to *Treponema pallidum* in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
4) Haemolytic specimen may give reddish background even after end of test interpretation time.
5) High lipaemic specimens/ turbid specimens must be centrifuged and use clear supernatant for testing.
6) Interpret the purple colored faint line as a reactive line. Repeat the test in case of very faint test line or if have any doubt for test line.
7) A non-reactive result for an individual subject indicates the absence of detectable antibodies specific to *Treponema pallidum*. However, a non-reactive result can occur if the quantity of the antibodies specific to *Treponema pallidum* in the specimen is below the detection limits of the assay.
8) Heparin, EDTA, sodium citrate, and ACD anticoagulants have been validated for use with this test.
9) False negative results may occur as a result of a very high antibody titre in a specimen". In such instances "Contact the manufacturer (or distributor) for further instruction.
10) Although a reactive result may indicate infection with *Treponema pallidum*, a diagnosis of Syphilis can only be made on clinical grounds, if an individual meets the case definition for Syphilis established by the Centers for Disease Control[9].
11) Immunochromatographic testing alone cannot be used to diagnose *Treponema pallidum* infection even if the antibodies against *Treponema pallidum* are present in a patient specimen. A negative result at any time does not preclude the possibility of *Treponema pallidum* infection.

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use		Contains sufficient for < n > tests
	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) Tramont EC. *Treponema pallidum* (syphilis). In: Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. New York: Churchill Livingston 2005:2768-85.
2) Hook EW III Stephens J. Ennis DM. Azithromycin compared with penicillin G benzathine for treatment of incubating syphilis. AnnIntern Med 1999 Sept 21; 131(6):434-437.
3) Johns DR, Tierney M, Felsenstein D. Alteration in the natural history of neurosyphilis by concurrent infection with the human immunodeficiency virus. NEngl J Med 1987; 316:1569-72.
4) 2016, WHO guidelines for the treatment of *Treponema pallidum* (syphilis).
5) https://www.cdc.gov/std/syphilis/stdfact-syphilis-detailed.htm
6) Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," Statistics in Medicine, 17, 857-872 (1998).
7) http://vassarstats.net/clin1.html#def , Richard Lowry.
8) TGS-5: Designing Instruction for use for in vitro diagnostic medical devices.
9) Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention: https://www.cdc.gov/std/tg2015/syphilis.htm

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
A1-302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA.
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)PI08-INS-001, Rev.: AC, Date: 2021-01-12 ENGLISH
Note : Instructions for use will be printed in local language of the country using the test, if required.

FIRST RESPONSE® SYPHILIS ANTI-TP CARD TEST

Rapid Syphilis card test for the detection of antibodies to *Treponema pallidum* in human whole blood/serum/plasma

REF PI08FRC25, PI08FRC50 & PI08FRC100

4°C 30°C IVD

Intended use

First Response® Syphilis Anti-TP 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 of all classes specific to *Treponema pallidum* in human serum, plasma or Venous or capillary whole blood. The test can be used as an aid in the diagnosis of Syphilis infection. The product can be used for symptomatic, asymptomatic, and pregnant women populations. The test kit is not automated and does not require any additional instruments. The test is intended to be used at Point of care and/or laboratory settings. Reactive specimens should be confirmed by supplemental testing.

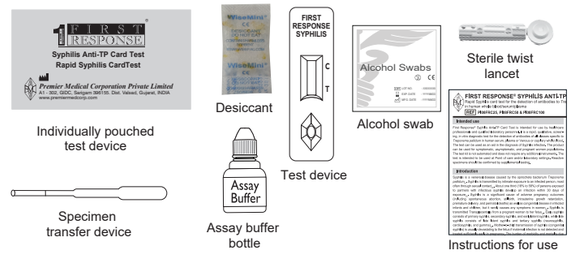
Introduction

Syphilis is a venereal disease caused by the spirochete bacterium *Treponema pallidum*[1]. Syphilis is transmitted by intimate exposure to an infected person, most often through sexual contact[2]. About one third (18% to 58%) of persons exposed to partners with infectious syphilis develop an infection within 30 days of exposure[2]. Syphilis is a significant cause of adverse pregnancy outcomes (including spontaneous abortion, stillbirth, intrauterine growth retardation, premature delivery, and perinatal deaths) as well as congenital disease in infected infants and children, but it rarely causes any symptoms in women[3]. Syphilis is transmitted Transplacentally from a pregnant woman to her fetus[4]. Early syphilis consists of primary syphilis, secondary syphilis, and early latent syphilis, while late syphilis consists of late latent syphilis and tertiary syphilis (neurosyphilis, cardiosyphilis, and gumma)[4]. Mother-to-child transmission of syphilis (congenital syphilis) is usually devastating to the fetus if maternal infection is not detected and treated sufficiently early in pregnancy. The burden of morbidity and mortality due to congenital syphilis is high. In 2012, an estimated 350 000 adverse pregnancy outcomes worldwide were attributed to syphilis, including 143 000 early fetal deaths/stillbirths, 62 000 neonatal deaths, 44 000 preterm/low-birth-weight babies, and 102 000 infected infants. Most untreated primary and secondary syphilis infections in pregnancy result in severe adverse pregnancy outcomes. Latent (asymptomatic) syphilis infections in pregnancy also cause serious adverse pregnancy outcomes in more than half of cases. Mother-to-child transmission of syphilis is declining globally due to increased efforts to screen and treat pregnant women for syphilis[4].

Assay Principle

When the test specimen and assay buffer are added to the specimen well, they flow along the colloidal gold-coupling antibody-Treponema pallidum antigen complex present at the conjugate pad". If *Treponema pallidum* specific antibodies i.e IgG or IgM are present in the sample, they will further form a complex of colloidal gold-coupling antibody-*Treponema pallidum* antigen-anti-*treponema pallidum* antibody which will migrate through the nitrocellulose membrane, when it encounters at test band (which contains *Treponema pallidum* antigen) the complex binds with antigen at test line forming colloidal gold-coupling antibody-*Treponema pallidum*- anti-*Treponema pallidum* antibody-antigen complex giving reactive result. This will make the purple-colored line visible at the test band. While in the case of non-reactive specimen, there is no antibody-Treponema pallidum-anti-Treponema pallidum antibody-antigen complex to bind with the test band and the purple coloured line will not appear. At the control line, the goat-anti mouse IgG will bind with the colloidal gold-coupling antibody-*Treponema pallidum* complex forming a purple colored line. Here the coupling antibody used in the colloidal gold conjugation with *Treponema pallidum* antigen is a mouse IgG. Thus irrespective of the reactive or non-reactive result the control line will appear.

Materials Provided



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

Materials provided	PI08FRC25	PI08FRC50	PI08FRC100
Test device pouch containing: 1 test device, 1 desiccant	25 Nos.	50 Nos.	100 Nos.
Specimen transfer device	25 Nos.	50 Nos.	100 Nos.
Assay buffer bottle (2.5 ml)	1 No.	2 Nos.	4 Nos.
Sterile twist lancets	25 Nos.	50 Nos.	100 Nos.
Alcohol swabs	25 Nos.	50 Nos.	100 Nos.
Instructions for use	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 device, 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® Syphilis Anti-TP 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 temperatures above 30°C and in humid conditions.
4) Assay buffer (opened and unopened) and 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 a desiccant color has changed from orange to green, do not use the test device.
6) The 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 device 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

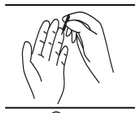
1) For in vitro diagnostic use only.
2) Read the instructions carefully before performing the test, any deviation will invalidate the test results.
3) Apply standard biosafety precautions for handling and disposal of potentially infective materials including human biological specimens irrespective of disease state.
4) Do not drink the assay buffer. It contains (0.02%) sodium azide as a preservative. Fatal if swallowed, in contact with skin or if inhaled. May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed. When disposed of through sink, flush with a large quantity of water. Sodium azide has the potential to react with metals commonly found in the plumbing infrastructure, such as copper and lead, to form insoluble metallic azides – a highly explosive and shock sensitive compound.
5) Devices and assay buffer of a different lot must not be used.
6) Do not use the test device if the pouch is not intact.
7) Do not use the sterile twist lancet, if the lock is not intact.
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 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, it 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.
16) Do not mix and interchange different specimens.

Specimen Collection

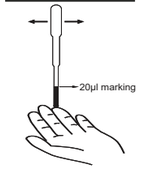
1) **Venous blood collection:** Collect the whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium citrate, ACD or in Plain tube by venipuncture.
2) **Plasma collection:** Collect the whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium 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 whole blood.
- Wipe the complete fingertip with the alcohol swab provided and wait until the fingertip is dried completely.

- Verify the seal (vertical side or side lock) before detaching the cap. Lock confirms the 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 whole blood using sterile gauze. Without pressing too hard, gently squeeze fingertip once again to obtain second drop of whole 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.

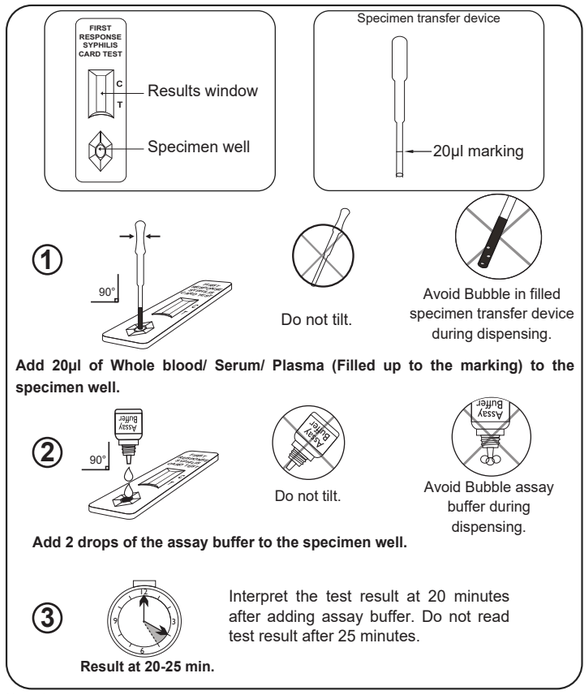
Note : Sterile twist lancet is for single use only. Do not share used sterile twist lancets with another person. Dispose of used sterile twist lancets in sharp box and alcohol swab in biohazard waste container immediately after use.
Do not use expired sterile twist lancet. Use of any expired sterile twist lancet may cause infections at the punctured skin due to 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 specimens 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 specimens 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 72 hours (3 days). 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.
5) Haemolysed specimens and lipaemic specimens showed poor background clearance, hence not recommended for testing. The lipaemic specimens can be used for testing after centrifugation. Such specimens must be centrifuged at 5000 g for 10 minutes and use the 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 the 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. Take out the specimen transfer device from the plastic bag provided inside the kit and follow the specimen collection process.
4) 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) Specimen transfer device should be held perpendicular to the specimen well of the testing device. Gently squeeze the bulb of the specimen transfer device to add 20 µl of 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) Add two drops of the assay buffer to the specimen well. Start the timer once the buffer has been added.
   Note:Avoid the assay buffer nozzle to come in contact with the specimen well to avoid contamination. The assay buffer bottle should be held perpendicular to the testing device to avoid dispensing excess of buffer.
8) Observe for development of purple colored lines in the results window. Interpret test results at 20 minutes after adding assay buffer to the specimen well. Do not interpret the test result after 25 minutes.



Caution

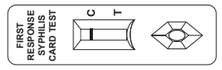
- Hold the specimen transfer device and assay buffer bottle vertically, else it may lead to inaccurate result. Exactly 1 drop of a specimen without having a bubble into droplet to be added, bubble formation during specimen addition may lead to an inaccurate result.
- Exactly 2 drops of assay buffer should be added. Adding more than 2 drops of assay buffer may cause over flooding or reverse migration phenomenon, which may lead to an inaccurate result of the test.
- Adding less than 2 drops of assay buffer or (formation of a bubble while the addition of assay buffer) may cause improper migration and poor background clearance which may lead to an inaccurate result of the test.
- Do not read the test result after 25 minutes. Reading the result after the 20-25 minutes window 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® Syphilis Anti-TP Card Test indicates that the active ingredients 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.

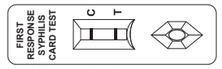
How to Interpret test results

Negative Results



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

Positive Results

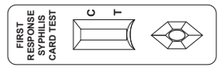


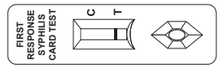
Syphilis Positive

If two purple colored lines appear, one at the control line 'C' and other at the test line 'T' as in the figure, then the specimen is reactive for antibodies to Syphilis.

Interpret the faint line as a reactive line.

Invalid Results





No presence of purple colored control line 'C' in the result window (irrespective of 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 new test device.

Performance characteristics

First Response® Syphilis Anti-TP Card Test has been tested using an in-house panel of positive and negative clinical specimens characterized by a commercially available reference(Agglutination/RDT) test kit. First Response® Syphilis Anti-TP Card Test showed 100% sensitivity and 100% specificity. First Response® Syphilis Anti-TP Card Test showed 100% agreement with reference assays.

Reference Method	Specimen details	First Response® Syphilis Anti TP Card Test		
		Syphilis Positive	Syphilis Negative	Total
Commercial Available Reference Test kit	Syphilis Positive Plasma specimens			
	Syphilis Positive Plasma Specimen	46	0	46
	Syphilis Negative Plasma specimens			
	Negative Plasma Specimen	0	395	395
	Total Plasma specimens	46	395	441
	Syphilis Positive Serum specimens			
	Syphilis Positive Serum Specimen	255	0	255
	Syphilis Negative Serum specimens			
	Negative Serum Specimen	0	3431	3431
	Total Serum specimens	255	3431	3686
	Syphilis Capillary Positive Whole blood specimens			
	Syphilis Capillary Positive Whole blood specimen	125	0	125
	Syphilis Capillary Negative Whole blood specimens			
	Syphilis Capillary Negative Whole Blood Specimen	0	269	269
	Total Capillary Whole blood specimens	125	269	394
	Syphilis Venous Positive Whole blood specimens			
	Syphilis Venous Positive Whole blood specimen	75	0	75
	Syphilis Venous Negative Whole blood specimens			
	Syphilis Venous Negative Whole Blood Specimen	0	185	185
	Total Venous Whole blood specimens	75	185	260

	Specimen details		First Response® Syphilis Anti TP Card Test			
	Test Marker	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Commercial Available Reference Test kit	**Plasma Specimens**					
	Syphilis	Sensitivity	46	00	46	(90.39%-100%)
		Specificity	00	395	395	(98.79%-100%)
	Serum Specimens					
	Syphilis	Sensitivity	255	00	255	(98.15%-100%)
		Specificity	00	3431	3431	(99.86%-100%)
	Capillary Whole blood Specimens					
	Syphilis	Sensitivity	125	00	125	(96.28%-100%)
		Specificity	00	269	269	(98.24%-100%)
	Venous Whole blood Specimens					
	Syphilis	Sensitivity	75	00	75	(93.92%-100%)
		Specificity	00	185	185	(97.46%-100%)

Seroconversion panel testing

The Analytical sensitivity of the First Response® Syphilis Anti-TP Card Test was carried out by testing commercially available seroconversion panel. The commercially available rapid lateral flow test was used as a reference kit for comparative performance study. Three seroconversion panel were tested, in-house. There was no difference between First Response Syphilis Anti-TP Card and the benchmark method".

Analytical Sensitivity - In - House Evaluation

Total Seroconversion/ performance panels	Total Specimens	First Response® Syphilis Anti-TP Card Test			Reference CE-marked rapid lateral flow test.		
		Positive	Negative	Detection Index**	Positive	Negative	Detection Index**
3	44	36	8	0.81	36	8	0.81

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

WHO International Standard for Syphilis were tested in First Response® Syphilis Anti-TP Card Test which shows 100% Sensitivity.

WHO International Standard	
1st IS for human syphilitic plasma IgG NIBSC code:05/122 0.3 IU/ml	First Response® Syphilis Anti-TP Card Test
	Positive

Cross reactivity study

First Response® Syphilis Anti-TP Card Test was tested with other diseases/conditions, which may give cross-reactivity with the test. The following 19 potential cross-reacting diseases/conditions did not affect the performance of First Response® Syphilis Anti-TP Card Test.

Specimen Details	Syphilis Negative	Syphilis Positive	Specimen Details	Syphilis Negative	Syphilis Positive
P. falciparum Malaria Positive	05	Not Tested	HIV-1 Positive	415	Not Tested
P.vivax Malaria Positive	05	Not Tested	HIV-2 Positive	91	Not Tested
Dengue NS1 Positive#	05	05	HSV 1/2 Positive#	13	10
Pregnant Woman ^	154	24	HTLV-I Ab Positive#	07	05
CMV Positive#	05	05	HTLV-II Ab Positive#	09	05
ANA Positive#	05	05	Rubella IgG & IgM Positive#	15	10
HAV Positive#	05	05	Thyroiditis specimens#	10	10
EBV Positive#	05	05	Anti-malarial drug medication#	03	03
HBV Positive#	103	05	Anti-TB drug medication#	03	03
HCV Positive#	103	05			

^ Note: Pregnant women specimens which is naturally appeared in syphilis positive specimens.
# Spiked Syphilis positive specimens.

Potential interference substances

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

Specimen Details	Syphilis Negative	Syphilis Positive	Specimen Details	Syphilis Negative	Syphilis Positive
Lipaemic specimen#	25	05	Low Hematocrit specimens	05	Not Tested
Icteric specimens#	05	05	Whole blood specimen in ACD anticoagulant ^	185	08
Haemolytic specimens	05	Not Tested	RF Ab Positive#	09	09
High Hematocrit specimens	05	Not Tested	dsDNA Antibody Positive Plasma#	01	05

^ Note: Naturally appeared Syphilis positive specimens.
# Spiked Syphilis positive specimens.

Potential interference drug substances

The details of potentially interfering drugs are mentioned in the following table. Each drug was spiked into syphilis positive specimens, and syphilis negative specimens to a final concentration of 250 µg/ml.
The following 26 potential interfering drug substances did not affect the performance of the First Response® Syphilis Anti-TP Card Test.

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

Precision

a) Within-run precision was determined by using 15 replicates of 9 different specimens containing different concentrations of antibodies. Within-run, precision was observed as 100%.

b) Between-run, precision was determined by using the 9 different specimens containing different concentrations of antibody in 5 different replicates with 3 different lots of test devices. Between run, precision was observed as 100%.

External Evaluation Report

Place of Evaluation	Year of Testing	Sensitivity	Specificity
Virus Research Institute, Uganda	2018	100% (95.38%-100%)	100% (95.38%-100%)
Zimbabwe (Pregnant women whole blood specimen)	2019	100% (95.97%-100%)	100% (97.65%-100%)

Limitations & Interferences

1) The assay procedure and interpretation of assay result sections must be followed closely. Failure to follow the procedure may lead to inaccurate test results.
2) First Response® Syphilis Anti-TP Card Test is designed to detect antibodies specific to *Treponema pallidum* in human serum, plasma, and whole blood. Other body fluids or pooled specimens may not give accurate results.
3) First Response® Syphilis Anti-TP Card Test is limited to the qualitative detection of antibodies specific to *Treponema pallidum* in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
4) Haemolytic specimen may give reddish background even after end of test interpretation time.
5) High lipaemic specimens/ turbid specimens must be centrifuged and use clear supernatant for testing.
6) Interpret the purple colored faint line as a reactive line. Repeat the test in case of very faint test line or if have any doubt for test line.
7) A non-reactive result for an individual subject indicates the absence of detectable antibodies specific to *Treponema pallidum*. However, a non-reactive result can occur if the quantity of the antibodies specific to *Treponema pallidum* in the specimen is below the detection limits of the assay.
8) Heparin, EDTA, sodium citrate, and ACD anticoagulants have been validated for use with this test.
9) False negative results may occur as a result of a very high antibody titre in a specimen". In such instances "Contact the manufacturer (or distributor) for further instruction.
10) Although a reactive result may indicate infection with *Treponema pallidum*, a diagnosis of Syphilis can only be made on clinical grounds, if an individual meets the case definition for Syphilis established by the Centers for Disease Control[9].
11) Immunochromatographic testing alone cannot be used to diagnose *Treponema pallidum* infection even if the antibodies against *Treponema pallidum* are present in a patient specimen. A negative result at any time does not preclude the possibility of *Treponema pallidum* infection.

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) Tramont EC. *Treponema pallidum* (syphilis). In: Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. New York: Churchill Livingston 2005:2768-85.
2) Hook EW III Stephens J. Ennis DM. Azithromycin compared with penicillin G benzathine for treatment of incubating syphilis. AnnIntern Med 1999 Sept 21; 131(6):434-437.
3) Johns DR, Tierney M, Felsenstein D. Alteration in the natural history of neurosyphilis by concurrent infection with the human immunodeficiency virus. NEngl J Med 1987; 316:1569-72.
4) 2016, WHO guidelines for the treatment of *Treponema pallidum* (syphilis).
5) https://www.cdc.gov/std/syphilis/stdfact-syphilis-detailed.htm
6) Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," Statistics in Medicine, 17, 857-872 (1998).
7) http://vassarstats.net/clin1.html#def , Richard Lowry.
8) TGS-5: Designing Instruction for use for in vitro diagnostic medical devices.
9) Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention: https://www.cdc.gov/std/tg2015/syphilis.htm

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
A1-302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA.
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)PI08-INS-006, Rev.: AB, Date: 2021-12-15 ENGLISH
Note : Instructions for use will be printed in local language of the country using the test, if required.

FIRST RESPONSE® SYPHILIS ANTI-TP CARD TEST

Rapid Syphilis card test for the detection of antibodies to *Treponema pallidum* in human whole blood/serum/plasma

REF PI08FRC25-SA

4°C 30°C IVD

Intended use

First Response® Syphilis Anti-TP 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 of all classes specific to *Treponema pallidum* in human serum, plasma or Venous or capillary whole blood. The test can be used as an aid in the diagnosis of Syphilis infection. The product can be used for symptomatic, asymptomatic, and pregnant women populations. The test kit is not automated and does not require any additional instruments. The test is intended to be used at Point of care and/or laboratory settings. Reactive specimens should be confirmed by supplemental testing.

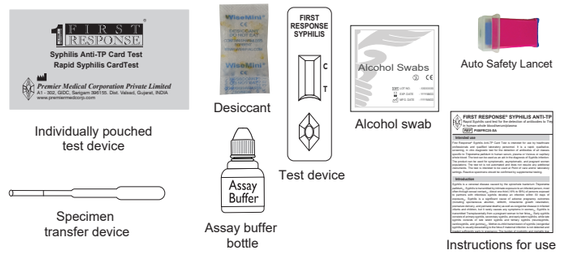
Introduction

Syphilis is a venereal disease caused by the spirochete bacterium *Treponema pallidum*[1]. Syphilis is transmitted by intimate exposure to an infected person, most often through sexual contact[2]. About one third (18% to 58%) of persons exposed to partners with infectious syphilis develop an infection within 30 days of exposure[2]. Syphilis is a significant cause of adverse pregnancy outcomes (including spontaneous abortion, stillbirth, intrauterine growth retardation, premature delivery, and perinatal deaths) as well as congenital disease in infected infants and children, but it rarely causes any symptoms in women[3]. Syphilis is transmitted Transplacentally from a pregnant woman to her fetus[4]. Early syphilis consists of primary syphilis, secondary syphilis, and early latent syphilis, while late syphilis consists of late latent syphilis and tertiary syphilis (neurosyphilis, cardiosyphilis, and gumma)[4]. Mother-to-child transmission of syphilis (congenital syphilis) is usually devastating to the fetus if maternal infection is not detected and treated sufficiently early in pregnancy. The burden of morbidity and mortality due to congenital syphilis is high. In 2012, an estimated 350 000 adverse pregnancy outcomes worldwide were attributed to syphilis, including 143 000 early fetal deaths/stillbirths, 62 000 neonatal deaths, 44 000 preterm/low-birth-weight babies, and 102 000 infected infants. Most untreated primary and secondary syphilis infections in pregnancy result in severe adverse pregnancy outcomes. Latent (asymptomatic) syphilis infections in pregnancy also cause serious adverse pregnancy outcomes in more than half of cases. Mother-to-child transmission of syphilis is declining globally due to increased efforts to screen and treat pregnant women for syphilis[4].

Assay Principle

When the test specimen and assay buffer are added to the specimen well, they flow along the colloidal gold-coupling antibody-*Treponema pallidum* antigen complex present at the conjugate pad". If *Treponema pallidum* specific antibodies i.e IgG or IgM are present in the sample, they will further form a complex of colloidal gold-coupling antibody-*Treponema pallidum* antigen-anti-*Treponema pallidum* antibody which will migrate through the nitrocellulose membrane, when it encounters at test band (which contains *Treponema pallidum* antigen) the complex binds with antigen at test line forming colloidal gold-coupling antibody-*Treponema pallidum*- anti-*Treponema pallidum* antibody-antigen complex giving reactive result. This will make the purple-colored line visible at the test band. While in the case of non-reactive specimen, there is no antibody-T*reponema pallidum*-anti-*Treponema pallidum* antibody-antigen complex to bind with the test band and the purple coloured line will not appear. At the control line, the goat-anti mouse IgG will bind with the colloidal gold-coupling antibody-*Treponema pallidum* complex forming a purple colored line. Here the coupling antibody used in the colloidal gold conjugation with *Treponema pallidum* antigen is a mouse IgG. Thus irrespective of the reactive or non-reactive result the control line will appear.

Materials Provided



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

Materials provided	PI08FRC25-SA
Test device pouch containing: 1 test device, 1 desiccant	25 Nos.
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 device, 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® Syphilis Anti-TP 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 temperatures above 30°C and in humid conditions.
4) Assay buffer (opened and unopened) and 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 a desiccant color has changed from orange to green, do not use the test device.
6) The 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 device 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

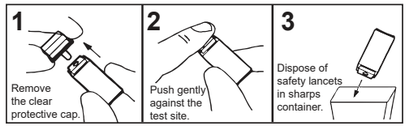
1) For in vitro diagnostic use only.
2) Read the instructions carefully before performing the test, any deviation will invalidate the test results.
3) Apply standard biosafety precautions for handling and disposal of potentially infective materials including human biological specimens irrespective of disease state.
4) Do not drink the assay buffer. It contains (0.02%) sodium azide as a preservative. Fatal if swallowed, in contact with skin or if inhaled. May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed. When disposed of through sink, flush with a large quantity of water. Sodium azide has the potential to react with metals commonly found in the plumbing infrastructure, such as copper and lead, to form insoluble metallic azides – a highly explosive and shock sensitive compound.
5) Devices and assay buffer of a different lot must not be used.
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.
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 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, it 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.
16) Do not mix and interchange different specimens.

Specimen Collection

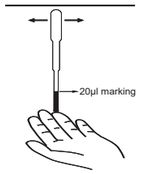
1) **Venous blood collection:** Collect the whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium citrate, ACD or in Plain tube by venipuncture.
2) **Plasma collection:** Collect the whole blood in the collection tubes containing anticoagulants like EDTA, Heparin, Sodium 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 whole blood.
- Wipe the complete fingertip with the alcohol swab provided and wait until the fingertip is 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.

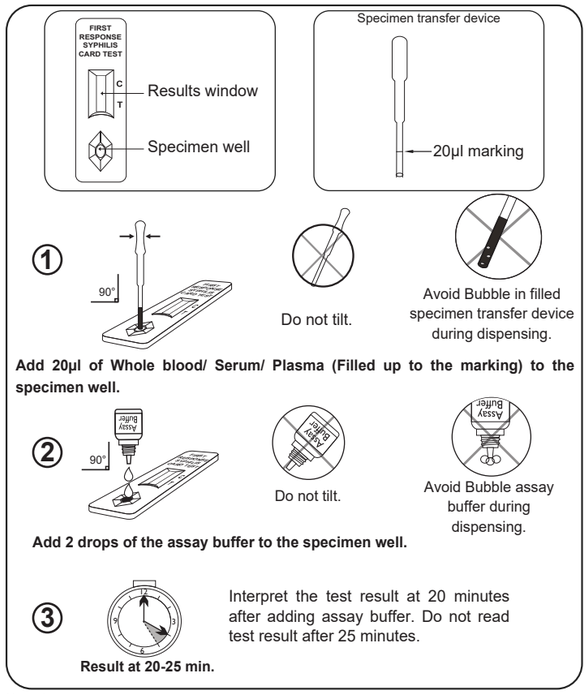
Note : Auto Safety lancet is for single use only. Do not share used auto safety lancet with another person. Dispose of used auto safety lancets in sharp box and alcohol swab in biohazard waste container immediately after use.
Do not use expired auto safety lancet. Use of any expired auto safety lancet may cause infections at the punctured skin due to expiry of its sterility. Use new auto safety 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 specimens 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 specimens 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 72 hours (3 days). 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.
5) Haemolysed specimens and lipaemic specimens showed poor background clearance, hence not recommended for testing. The lipaemic specimens can be used for testing after centrifugation. Such specimens must be centrifuged at 5000 g for 10 minutes and use the 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 the 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. Take out the specimen transfer device from the plastic bag provided inside the kit and follow the specimen collection process.
4) 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) Specimen transfer device should be held perpendicular to the specimen well of the testing device. Gently squeeze the bulb of the specimen transfer device to add 20 µl of 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) Add two drops of the assay buffer to the specimen well. Start the timer once the buffer has been added.
   Note:Avoid the assay buffer nozzle to come in contact with the specimen well to avoid contamination. The assay buffer bottle should be held perpendicular to the testing device to avoid dispensing excess of buffer.
8) Observe for development of purple colored lines in the results window. Interpret test results at 20 minutes after adding assay buffer to the specimen well. Do not interpret the test result after 25 minutes.



Caution

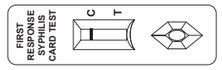
- Hold the specimen transfer device and assay buffer bottle vertically, else it may lead to inaccurate result. Exactly 1 drop of a specimen without having a bubble into droplet to be added, bubble formation during specimen addition may lead to an inaccurate result.
- Exactly 2 drops of assay buffer should be added. Adding more than 2 drops of assay buffer may cause over flooding or reverse migration phenomenon, which may lead to an inaccurate result of the test.
- Adding less than 2 drops of assay buffer or (formation of a bubble while the addition of assay buffer) may cause improper migration and poor background clearance which may lead to an inaccurate result of the test.
- Do not read the test result after 25 minutes. Reading the result after the 20-25 minutes window 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® Syphilis Anti-TP Card Test indicates that the active ingredients 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.

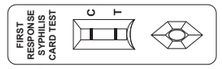
How to Interpret test results

Negative Results



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

Positive Results

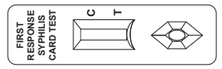


Syphilis Positive

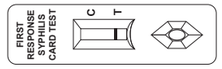
If two purple colored lines appear, one at the control line 'C' and other at the test line 'T' as in the figure, then the specimen is reactive for antibodies to Syphilis.

Interpret the faint line as a reactive line.

Invalid Results



No presence of purple colored control line 'C' in the result window (irrespective of 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 new test device.

Performance characteristics

First Response® Syphilis Anti-TP Card Test has been tested using an in-house panel of positive and negative clinical specimens characterized by a commercially available reference(Agglutination/RDT) test kit. First Response® Syphilis Anti-TP Card Test showed 100% sensitivity and 100% specificity. First Response® Syphilis Anti-TP Card Test showed 100% agreement with reference assays.

Reference Method	Specimen details	First Response® Syphilis Anti TP Card Test		
		Syphilis Positive	Syphilis Negative	Total
Commercial Available Reference Test kit	Syphilis Positive Plasma specimens			
	Syphilis Positive Plasma Specimen	46	0	46
	Syphilis Negative Plasma specimens			
	Negative Plasma Specimen	0	395	395
	Total Plasma specimens	46	395	441
	Syphilis Positive Serum specimens			
	Syphilis Positive Serum Specimen	255	0	255
	Syphilis Negative Serum specimens			
	Negative Serum Specimen	0	3431	3431
	Total Serum specimens	255	3431	3686
	Syphilis Capillary Positive Whole blood specimens			
	Syphilis Capillary Positive Whole blood specimen	125	0	125
	Syphilis Capillary Negative Whole blood specimens			
	Syphilis Capillary Negative Whole Blood Specimen	0	269	269
	Total Capillary Whole blood specimens	125	269	394
	Syphilis Venous Positive Whole blood specimens			
	Syphilis Venous Positive Whole blood specimen	75	0	75
	Syphilis Venous Negative Whole blood specimens			
	Syphilis Venous Negative Whole Blood Specimen	0	185	185
	Total Venous Whole blood specimens	75	185	260

	Specimen details		First Response® Syphilis Anti TP Card Test			
	Test Marker	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Commercial Available Reference Test kit	**Plasma Specimens**					
	Syphilis	Sensitivity	46	00	46	(90.39%-100%)
		Specificity	00	395	395	(98.79%-100%)
	Serum Specimens					
	Syphilis	Sensitivity	255	00	255	(98.15%-100%)
		Specificity	00	3431	3431	(99.86%-100%)
	Capillary Whole blood Specimens					
	Syphilis	Sensitivity	125	00	125	(96.28%-100%)
		Specificity	00	269	269	(98.24%-100%)
	Venous Whole blood Specimens					
	Syphilis	Sensitivity	75	00	75	(93.92%-100%)
		Specificity	00	185	185	(97.46%-100%)

Seroconversion panel testing

The Analytical sensitivity of the First Response® Syphilis Anti-TP Card Test was carried out by testing commercially available seroconversion panel. The commercially available rapid lateral flow test was used as a reference kit for comparative performance study. Three seroconversion panel were tested, in-house. There was no difference between First Response Syphilis Anti-TP Card and the benchmark method".

Analytical Sensitivity - In - House Evaluation							
Total Seroconversion/ performance panels	Total Specimens	First Response® Syphilis Anti-TP Card Test			Reference CE-marked rapid lateral flow test.		
		Positive	Negative	Detection Index**	Positive	Negative	Detection Index**
3	44	36	8	0.81	36	8	0.81

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

WHO International Standard for Syphilis were tested in First Response® Syphilis Anti-TP Card Test which shows 100% Sensitivity.

WHO International Standard	
1st IS for human syphilitic plasma IgG NIBSC code:05/122 0.3 IU/ml	First Response® Syphilis Anti-TP Card Test
	Positive

Cross reactivity study

First Response® Syphilis Anti-TP Card Test was tested with other diseases/conditions, which may give cross-reactivity with the test. The following 19 potential cross-reacting diseases/conditions did not affect the performance of First Response® Syphilis Anti-TP Card Test.

Specimen Details	Syphilis Negative	Syphilis Positive	Specimen Details	Syphilis Negative	Syphilis Positive
P. falciparum Malaria Positive	05	Not Tested	HIV-1 Positive	415	Not Tested
P.vivax Malaria Positive	05	Not Tested	HIV-2 Positive	91	Not Tested
Dengue NS1 Positive#	05	05	HSV 1/2 Positive#	13	10
Pregnant Woman ^	154	24	HTLV-I Ab Positive#	07	05
CMV Positive#	05	05	HTLV-II Ab Positive#	09	05
ANA Positive#	05	05	Rubella IgG & IgM Positive#	15	10
HAV Positive#	05	05	Thyroiditis specimens#	10	10
EBV Positive#	05	05	Anti-malarial drug medication#	03	03
HBV Positive#	103	05	Anti-TB drug medication#	03	03
HCV Positive#	103	05			

^ Note: Pregnant women specimens which is naturally appeared in syphilis positive specimens.
# Spiked Syphilis positive specimens.

Potential interference substances

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

Specimen Details	Syphilis Negative	Syphilis Positive	Specimen Details	Syphilis Negative	Syphilis Positive
Lipaemic specimen#	25	05	Low Hematocrit specimens	05	Not Tested
Icteric specimens#	05	05	Whole blood specimen in ACD anticoagulant ^	185	08
Haemolytic specimens	05	Not Tested	RF Ab Positive#	09	09
High Hematocrit specimens	05	Not Tested	dsDNA Antibody Positive Plasma#	01	05

^ Note: Naturally appeared Syphilis positive specimens.
# Spiked Syphilis positive specimens.

Potential interference drug substances

The details of potentially interfering drugs are mentioned in the following table. Each drug was spiked into syphilis positive specimens, and syphilis negative specimens to a final concentration of 250 µg/ml.
The following 26 potential interfering drug substances did not affect the performance of the First Response® Syphilis Anti-TP Card Test.

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