Performance Characteristics:

1. Limit detection: Values less than 6 IU/mL give non-reproducible results.

2. Measurement range:

Linear range up to 200 IU/ml, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in saline (10 parts serum sample + 40 parts normal saline ex: 10µl serum sample+40 µl saline) and retested again and the results should be multiplied by 5. The linearity limit and measurement range depends on the sample to reagent/ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

3. Prozone effect: No prozone effect was detected upon 800 IU/ml.

4. Precision:

	Intra-assay (n=10)		Inter-assay (n=10)	
Mean (IU/mL)	14.9	45.8	14.9	45.8
SD	0.96	1.32	1.2	2.54
CV	6.5	2.90	8.0	5.60

5. Accuracy: Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 86 samples ranging from 1 to 180 IU/mL of RF were assayed. The correlation coefficient (r) was 0.95 and the regression equation y = 0.797x - 1.075.

The results of the performance characteristics depend on the analyzer used.

Interferences :

Hemoglobin (10 g/l), bilirrubin (20 mg/dl) and lipemia (10 g/l) do not interfere.

Notes :

1. Multipoint calibration gives more accurate results than one point calibration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Bibliography :

1. Frederick Wolfe et al. Arthritis and Rheumatism 1991; 34: 951- 960.
2. Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1-21.
3. Robert H Shmerling et al. The American Journal of Medicine 1991; 91: 528 – 534.
4. Vladimir Mulé et al. Scand J Rheumatology 1972; 1: 181 – 187.
5. Paul R et al. Clin Chem 1979; 25/11: 1909 – 1914.
6. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

Standard Testing Protocol
Product : RF Turbilatex T2
Rev.No.2
Effective Date: 28-10-2021

Manufactured in India by :
Proton Biologicals India Pvt. Ltd.
#302, 3rd Floor, Bommasandra, Jigani Link Road,
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Customer Care: 080-27825012/13

An ISO 13485 Certified Company

RF Estimation Kit

GenX RF (Rheumatoid Factors) Turbilatex Method

Proton
Best in Class

Intended Use: Kit for the quantitative determination of Rheumatoid Factors (RF) in human serum and plasma

IVD

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
PBRAF (T2) - 50	50 ml	Two Liquid Reagents with Liquid Calibrator
PBRAF (T2) - 25	25 ml	

PRODUCT FEATURES:

- Two liquid reagents (Turbilatex and Diluent).
- Liquid Calibrator provided.
- No Calibration graph required
- Wave Length 578 nm (546-620nm)
- Linearity: 200 IU/ml
- No Prozone effect was detected upon 800 IU/ml.
- No interference from Hemoglobin(10g/l),Bilirubin in (20 mg/dl) and Lipemia (10 g/l)
- Can be used on semi and fully auto analyzers

GenX RF (Rheumatoid Factors) Turbilatex Method

Proton
Best In Class

Principle of the Method :

The RF-Turbilatex is a quantitative turbidimetric test for the measurement of RF in human serum or plasma. Latex particles coated with human gamma globulin are agglutinated when mixed with samples containing RF. The agglutination causes an absorbance change, dependent upon the RF contents of sample that can be quantified by comparison from a calibrator of known RF concentration.

Clinical Significance:

Rheumatoid factors are a group of antibodies directed to determinants in the Fc portion of the immunoglobulin G molecule. Although rheumatoid factors are found in a number of rheumatoid disorders, such as systemic lupus erythematosus (SLE) and Sjögren's syndrome, as well as in non rheumatic conditions, its central role in clinic lies its utility as an aid in the diagnosis of rheumatoid arthritis (RA). A study of the "American College of Rheumatology" shows that the 80.4% of RA patients were RF positive.

Reagents:

Diluent (R1)	Tris buffer 20 mmol/L, pH 8.2. Sodium azide 0.95 g/L
Latex (R2)	Latex particles coated with human gammaglobulin, pH 7.4. Sodium azide 0.95g/L
RF-CAL	Liquid Calibrator. Human serum. {Lot Specific }.

Calibration :

Use RF Calibrator

The sensitivity of the assay and the target value of the calibrator have been standardized against the International Reference WHO. 64/2 (Rheumatoid Arthritis Serum)

RF Calibrator:

Calibrator is Liquid Stable and does not need any reconstitution.
Calibrator is stable till the expiry date mentioned on the label

STORAGE AND STABILITY:

All the components of the kit are stable until the expiration date on the labels when stored tightly closed at 2-8°C an d contaminations are prevented during their use. Do not use reagents over the expiration date.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the labels when stored at 2-8°C and the contamination is prevented during their use. Do not freeze the latex and diluent.

SAMPLES: Fresh serum is the only specimen

Stable for 7 days at 2-8°C or 3 months at –20°C.
The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE:Fixed Time (One Point Calibration)

Pipette into test tubes labeled Calibrator (C) and Test (T).

	Control (C)	Test (T)
Reagent	400 µl	400 µl
Diluent	20 µl	20 µl
RF Calibrator	100 µl	100 µl
Latex		

Reaction temperature : 37°C
Mix well and read absorbances of Calibrator (C) and Test (T) against distilled water at 578 nm (546-620 nm) as follows:

Initial absorbance A1 -exactly after 5 sec.
Final absorbance A2 - exactly 120 sec. after A1
Determine ΔA for Calibrator (C) and Test (T)

CALCULATIONS :

$$\text{RF Conc.: (IU/mL)} = \frac{\text{(A2-A1) Sample}}{\text{(A2-A1) Calibrator}} \times \textit{On the Label (Lot Specific)}$$

SYSTEM PARAMETERS :

Reaction Type	:	*Fixed Time / Initial Rate / Two Point Kinetic*
Reaction Direction	:	Increasing
Sample Volume	:	20 µl
Reagent Volume	:	400 µl Diluent + 100 µl Turbi Latex
Wave Length	:	578 nm (546-620 nm)
Calibrator Conc.	:	**On the Label (Lot Specific)**
Flow Cell Temp.	:	37°C
Linearity	:	200 (One Point Calibration)
Zero setting with	:	Distilled Water
Units	:	IU/mL
Delay	:	5 sec.
Interval	:	120 sec

Reference Values:

Normal values will be between 0 - 30 IU/ml. Each laboratory should establish its own reference range.
If the Rheumatoid Factors are completely absent some times the absorbances and graph may go below zero i.e base line resulting in to Negative Results. In such Cases Laboratory Technician may report the results as,
"Less than 0.0 IU/ml - Negative for Rheumatoid Factors"

Performance Characteristics

1. Linearity limit: Up to 150 mg/L, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in saline (10 parts serum sample + 40 parts saline ex: 10µl serum sample+40 µl saline) and retested again and the results should be multiplied by 5.The linearity limit depends on the sample / reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection limit: Values less than 2 mg/L give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 800 mg/L.
4. Precision:

	Intra-assay (n=10)			Inter-assay (n=10)		
Mean (IU/mL)	8.6	16.9	50.5	8.6	16.8	50.5
SD	0.56	0.61	0.97	0.74	1.11	3.2
CV	6.5	3.6	1.9	7.7	6.6	6.3

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 65 samples ranging from 1 to 150 mg/l of CRP were assayed. The correlation coefficient (r) was 0.98 and the regression equation $y=0.892x+0.282$.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Bilirrubin (20 mg/dl), lipemia (10 g/l) and rheumatoid factors (300 IU/ml) do not interfere. Hemoglobin (≥ 5 g/l), interferes.

NOTES

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Lars-Olof Hanson et al. Current Opinion in Infect Diseases 1997; 10: 196-201.
2. Chetana Vaishnavi. Immunology and Infectious Diseases 1996; 6: 139 – 144.

Standard Testing Protocol
Product: GenX CRP-Turbi (T2)
Rev. No.2,
Effective Date: 28-10-2021

Manufactured in India by :
Proton Biologicals India Pvt. Ltd.
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customercare@protonbiologicals.com | www.protonbiologicals.com
Customer Care: 080-27825012/13

An ISO 13485 Certified Company

CRP Estimation Kit - 2nd Generation

GenX CRP (C-Reactive Protein)

Turbilatex Method

Proton
Best In Class

Intended Use: Kit for the quantitative determination of C - Reactive Protein (CRP) in human Serum.

IVD

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
PBCRPT2 - 50	50 ml	R1- 40ml+R2-10ml Calibrator-1 Vial

PRODUCT FEATURES

- Two liquid reagents (Turbilatex and Diluent)
- **Highly Accuracy for Lower and Higher values**
- **Linearity upto 150 mg/L**
- Liquid Calibrator provided
- Greater detectability of Pediatric CRP Values
- No Prozone effect was detected upon 150 mg/L
- Bilirubin (20 mg/dl), lipemia (10 g/l) and rheumatoid factors (300 IU/ml) do not interfere. Hemoglobin (≥ 5 g/l), interferes.
- Can be used on semi and fully auto analyzers

GenX CRP (C-Reactive Protein) Turbilatex Method

Proton
Best in Class

PRINCIPLE OF THE METHOD

CRP-Turbilatex is a quantitative turbidimetric test for the measurement of C- reactive protein (CRP) in human serum

Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

CLINICAL SIGNIFICANCE

CRP is an acute-phase protein present in normal serum, which increases significantly after most forms of tissue injuries, bacterial and virus infections, inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP concentration can rise up to 300 mg/L in 12-24 hours.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/L, pH 8.2. Sodium azide 0.95 g/L.
Latex (R2)	Latex particles coated with goat IgG anti-human CRP, pH 7.3 Sodium azide 0.95 g/L.
CRP-CAL	Calibrator C-Reactive protein (Concentration Lot Specific, Verify on the labels)

CALIBRATION

The sensitivity of the assay and the target value of the calibrator have been standardized against the Reference Material ERM-DA 472/IFCC.

PREPARATION

Working reagent: Swirl the latex vial gently before use. Prepare the necessary amount as follow:

8 mL Diluent + 2 mL Latex Reagent

CRP Calibrator: Calibrator is Liquid Stable and does not need any reconstitution. Calibrator is stable till the expiry date mentioned on the label

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the labels when stored at 2-8°C and the contaminations is prevented during their use.

SAMPLES

Fresh serum. Stable 7 days at 2-8°C or 3 months at –20°C.

The samples with presence of fibrin should be centrifuged before testing.

Do not use highly hemolized or lipemic samples.

PROCEDURE: Fixed Time

Pipette into test tubes labeled Calibrator (C) and Test (T).

Reagent	(Calibrator)	(Test Sample)
Working Reagent	0.5 ml	0.5 ml
CRP Calibrator	5 µl	-
Sample	-	5 µl

Reaction temperature : 37°C

Mix well and read absorbances of Calibrator (C) and Test (T) against distilled water at 578 nm (546-600 nm) as follows:

Initial absorbance A1 -exactly after 5 sec.
Final absorbance A2 - exactly 120 sec. after A1
Determine ΔA for Calibrator (C) and Test (T)

CALCULATIONS :

$$\text{CRP Conc.: (mg/L)} = \frac{\text{(A2-A1) Sample}}{\text{(A2-A1) Calibrator}} \times \text{Calibrator Concentration}$$

SYSTEM PARAMETERS :

Reaction Type	:	Fixed Time / Initial Rate / Two Point Kinetic
Reaction Direction	:	Increasing
Sample Volume	:	5.0 µl (0.005 mL)
Working Reagent Volume	:	0.5 ml
Wave Length	:	578nm (546-600 nm)
Calibrator Conc.	:	Concentration Lot Specific, Verify on the labels
Flow Cell Temp.	:	37°C
Linearity	:	150
Zero setting with	:	Distilled Water
Units	:	mg/L
Delay	:	5 sec
Interval	:	120 sec

QUALITY CONTROL

Control Sera are recommended to monitor the performance of manual and automated assay procedures.

Each laboratory should establish its own Quality Control scheme

REFERENCE VALUES

Normal values Adults up to 6 mg/L.

New Borns up to 3 weeks <4.1 mg/L

Infants and Children <2.8 mg/L

Each laboratory should establish its own reference range.

For *in vitro* diagnostic use only

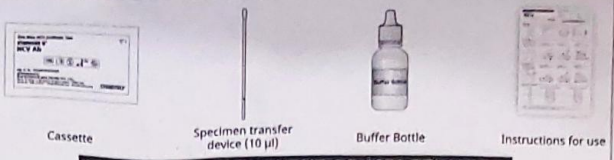
STANDARD Q®
HCV Ab

STANDARD Q HCV Ab Rapid Test

STANDARD®

PLEASE READ COMPLETE KIT INSERT CAREFULLY BEFORE YOU PERFORM THE TEST

[Materials Provided]

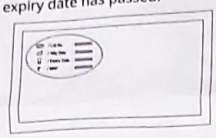


DO NOT USE COMPONENT OF ANY OTHER KIT

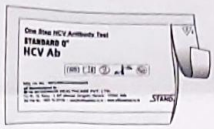
[Preparation]

1 Carefully read the instruction for using the STANDARD Q HCV Ab Test.

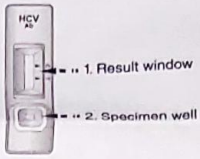
2 Look at the expiry date at the back of the cassette package. Use another lot, if expiry date has passed.



3 Open the cassette package & check for the cassette & silica gel.



<Cassette Packaging>



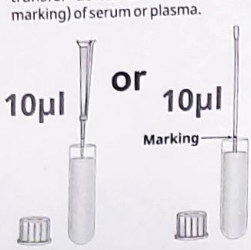
<Cassette>

<Silica gel>

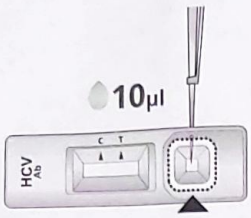
[Test Procedure]

1. For Serum or Plasma specimen

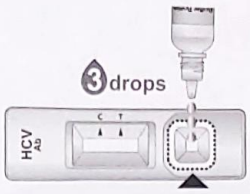
1 Specimen Collection
Using a micropipette or specimen transfer device collect 10µl (till marking) of serum or plasma.



2 Specimen Addition
Add the collected serum or plasma to the specimen well of the cassette.



3 Buffer Addition
Add 3 drops of buffer into specimen well of the cassette.



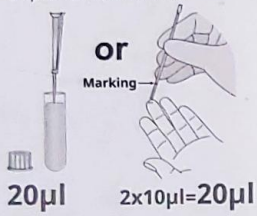
4 Reading Time
Read the test results after 5 minutes.
The test can be read up to 20 minutes.

Read
After 5 mins
Can be read
Up to 20 mins

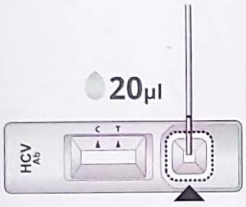
CAUTION Do not read test result after 20 minutes. It may give false results.

2. For Whole Blood specimen

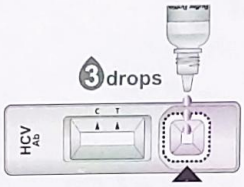
1 Specimen Collection
Collect 20µl of whole blood by using a micropipette or collect two times 10µl of whole blood till the marking of specimen transfer device.



2 Specimen Addition
Add the collected whole blood to the specimen well of the cassette.



3 Buffer Addition
Add 3 drops of buffer into specimen well of the cassette.

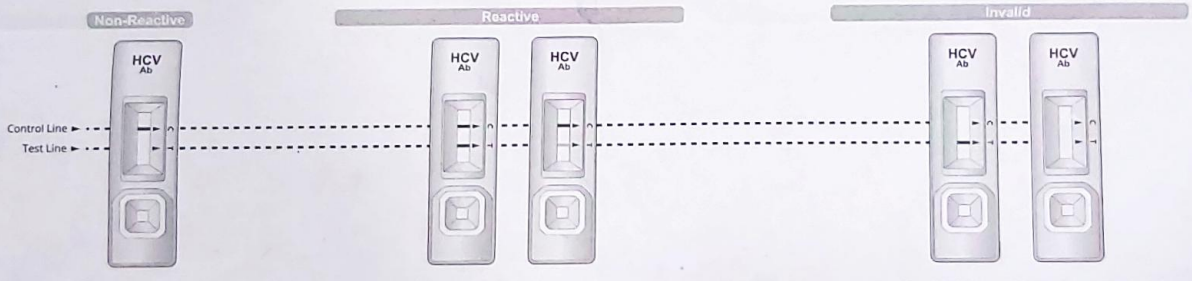


4 Reading Time
Read the test results after 5 minutes.
The test can be read up to 20 minutes.

Read
After 5 mins
Can be read
Up to 20 mins

CAUTION Do not read test result after 20 minutes. It may give false results.

[Interpretation of Test Result]



HCV Non-Reactive

HCV Reactive

Invalid, Re-test with a new cassette.

1. A colored band will appear in the top section of the result window to show that the test is working properly. This band is the control line (C).
2. A colored band will appear in the lower section of the result window. This band is the test line (T).
3. **Even if the control line/test line is faint, or the test line is not uniform, the test should be considered to be performed properly and the test result should be interpreted as a reactive result.**

* Reactive results should be considered in conjunction with the clinical history and other data available to the physician.

EXPLANATION AND SUMMARY

[Introduction]

Hepatitis C virus (HCV) is one of several hepatitis viruses that can cause inflammation of the liver. It is a bloodborne virus and is most commonly transmitted through unsafe injection practices, inadequate sterilization of medical equipment and the transfusion of unscreened blood and blood products. HCV can cause both acute and chronic hepatitis infection. Acute HCV infection is a short-term viral infection, and is usually asymptomatic. About 15-45% of infected persons spontaneously improve or resolve the infection within just several months without treatment. However, the remaining 55-85% of infected persons will develop chronic HCV infection. The chronic HCV infection is a serious disease that it can result in long-term problems in the liver, including liver damage and liver cancer, even death. According to the World Health Organization, about 130–150 million people globally have chronic HCV infection, with more than 350,000 people dying from Hepatitis C-related liver diseases each year. Antiviral medicines can cure approximately 90% of persons with HCV, thereby reducing the risk of death, but access to diagnosis and is low. To establish best practices for early diagnosis for HCV infection can prevent health problems that may result from infection and prevent transmission of the virus. STANDARD Q HCV Ab Test provides significantly fast, easy and accurate system to detect the specific antibodies HCV in human serum, plasma or whole blood. It is essential for the reliable clinical diagnosis of HCV infection and enables supportive treatment decisions.

[Intended use]

STANDARD Q HCV Ab Test is a rapid chromatographic immunoassay for the qualitative detection of specific antibodies to HCV present in human serum, plasma or whole blood. This test is for *in vitro* professional diagnostic use and intended as an aid to early diagnosis of HCV infection in patient with clinical symptoms with HCV infection. It provides only an initial screening test result. More specific alternative diagnosis methods should be performed in order to obtain the confirmation of HCV infection.

[Test principle]

STANDARD Q HCV Ab Test contains two pre-coated lines, "C" (Control line), "T"(Test line) on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Monoclonal anti-NS3 and monoclonal anti-Core are coated on the control line region and monoclonal anti-human IgG is coated on the test line region. Four recombinant HCV antigens from the Core, NS3, NS4 and NS5 regions conjugated with colloidal gold particles are used as detectors for HCV antibodies. During the test, HCV antibodies in the specimen interacts with recombinant HCV antigens conjugated with colloidal gold particles making antibody-antigen gold particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the monoclonal anti-human IgG. A violet test line would be visible in the result window if HCV antibodies are present in the specimen. The intensity of violet test line will vary depending upon the amount HCV antibodies present in the specimen. If HCV antibodies are not present in the specimen, then no color appears in the test line. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working.

[Materials Provided]

Components	
Cassette	Specimen transfer device
Buffer Bottle	Instruction for use

KIT STORAGE AND STABILITY

Store the RDT Box at room temperature, 2-40°C / 36-104°F out of direct sunlight. Materials provided are stable until the expiry date printed on the RDT box. DO NOT FREEZE.

SPECIMEN COLLECTION AND PREPARATION

[Serum]

1. Collect the whole blood into the commercially available plain tube NOT containing anti-coagulant such as heparin or EDTA by venipuncture and leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
2. If serum in the plain tube is stored in a refrigerator at 2-8°C/36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen in the long-term keeping more than 1 week can cause non-specific reaction. For prolonged storage, it should be at below -20°C / -4°F.
3. It should be brought to room temperature prior to use.

[Plasma]

1. Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin or EDTA by venipuncture and centrifuge blood to get plasma specimen.
2. If plasma in an anti-coagulant tube is stored in a refrigerator at 2-8°C/36-46°F, the specimen can be used for testing within 1 week after collection. Using the specimen in the long-term keeping more than 1 week can cause non-specific reaction. For prolonged storage, it should be at below -20°C/-4°F
3. It should be brought to room temperature prior to use.

[Whole Blood]

- **Capillary whole blood**

1. Capillary whole blood should be collected aseptically by fingertip.
2. Clean the area to be lanced with an alcohol swab.
3. Squeeze the end of the fingertip and pierce with a sterile lancet.
4. Collect the capillary whole blood till the marking of the specimen transfer device for the testing.
5. The capillary whole blood must be tested immediately after collection.

- **Venous whole blood**

1. Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin or EDTA by venipuncture.
2. If venous whole blood in an anti-coagulant tube is stored in a refrigerator at 2-8°C/ 36-46°F, the specimen can be used for testing within 1~2 day after collection.
3. Do not use hemolyzed blood specimen.

CAUTION

- Anticoagulants such as heparin or EDTA do not affect the test result.
- As known relevant interference, haemolytic specimen, rheumatoid factors-contained specimen and lipaemic, icteric specimen can lead to impair the test results.
- Use separate disposable materials for each specimen in order to avoid cross-contamination which can cause erroneous results.

TEST PROCEDURE

[Preparation]

1. *Carefully read instructions for using the STANDARD Q HCV Ab Test.*
2. *Look at the expiry date at the back of the cassette package. Use another lot, if expiry date has passed.*
3. *Allow the RDT kit to come at room temperature before opening the cassette package.*
4. Open the cassette package & check for the cassette & silica gel.
5. Methods for following steps can be changed depending on the specimen or specimen transfer device.

[Test Procedure]

- **For serum or plasma specimen**

1. Using a micropipette or specimen transfer device collect 10µl (till the marking) of serum or plasma.
2. Add the collected serum or plasma to the specimen well of the cassette.
3. Add 3 drops of buffer into the specimen well of the cassette.
4. Read the test results after 5 minutes. Test can be read up to 20 minutes.

- **For whole blood specimen**

1. Collect 20µl of whole blood by using a micropipette or collect two times 10µl of whole blood till the marking of specimen transfer device.
2. Add the collected whole blood to the specimen well of the cassette.
3. Add 3 drops of buffer into the specimen well of the cassette.
4. Read the test results after 5 minutes. Test can be read up to 20 minutes.

CAUTION

- Do not read test results after 20 minutes. It may give false results.

INTERPRETATION OF TEST RESULTS

1. **Non-reactive:** The presence of only one colored band ("C" Control line) within the result window indicates a non-reactive result.
2. **Reactive:** The presence of two colored bands ("C" Control line and "T" Test line) within the result window, no matter which band appears first, indicates a reactive result. Even if the control line/test line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as a reactive result.
3. **Invalid:** If the control band ("C" Control line) is not visible within the result window, the result is considered invalid. The directions may not have been followed correctly. In such case, it is recommended to retest the specimen with a new cassette.

CAUTION

- Even if the control line/test line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as a reactive result.
- Reactive result should be considered in conjunction with the clinical history and other data available to the physician.

LIMITATION OF TEST

1. The test should be used for the detection of HCV antibodies in human serum, plasma or whole blood specimen.
2. Neither the quantitative value nor the rate of HCV antibodies concentration can be determined by this qualitative test.
3. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
4. A non-reactive test result may occur if the level of extracted antibody in a specimen is below the sensitivity of the test or if a poor-quality specimen is obtained.
5. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
6. The test result must always be evaluated with other data available to the physician.

QUALITY CONTROL

[Internal Quality Control]

STANDARD Q HCV Ab Kit has test line and control line on the surface of each cassette. All the test line and control line in result window are not visible before applying specimen. The control line is used for procedural control. It will appear if the test has been performed correctly and the reagents are functional. If it does not appear, the test results are not valid and the test must be repeated. In addition, good laboratory practice recommends the daily use of control materials to confirm the test procedure and to verify proper test performance.

PERFORMANCE CHARACTERISTICS

As per the evaluation conducted at different sites in India, the performance characteristics of STANDARD Q HCV Ab is found to be:

Sensitivity - 100% | Specificity - 99.74%

WARNINGS AND PRECAUTIONS

1. Do not re-use the kit.
2. Do not use the kit if the cassette package is damaged or the seal is broken.
3. Do not use the buffer bottle of another lot.
4. Do not smoke, drink or eat while handling specimen.
5. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after the tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout testing procedures.
9. Dispose off all specimens and materials used to perform the test as bio-hazard waste. Laboratory chemical and bio-hazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
10. Silica gel in cassette packaging is to absorb moisture and prevent humidity from affecting products.
11. Buffer contain sodium azide as a preservative. If these materials are to be disposed off through sink or other common plumbing system, flush with generous water to prevent accumulation of potentially explosive compound.
12. For *in vitro* diagnostic use only.
13. Do not use the kit contents beyond the expiry date printed outside the box.
14. Immediately perform the test after removing the test device from the cassette package.
15. Discard the cassette immediately after reading result.

BIBLIOGRAPHY

1. Smith BD, Teshale E, Jewett A, Weinbaum CM, Nealgus A, Hagan H, et al. Performance of premarket rapid hepatitis C virus antibody assays in 4 national human immunodeficiency virus behavioral surveillance system sites. Clin Infect Dis. 2011;53:780–786.
2. Lee SR, Yearwood GD, Guillon GB, Kurtz LA, Fischl M, Friel T, et al. Evaluation of a rapid, point-of-care test device for the diagnosis of hepatitis C infection. J Clin Virol. 2010;48:15–17.
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4. Larrat, S., Bourdon, C., Baccard, M. et al. Performance of an antigen–antibody combined assay for hepatitis C virus testing without venipuncture. J Clin Virol. 2012;55:220–225.
5. Shah DO, Chang CD, Jiang LX, et al. Combination HCV core antigen and antibody assay on a fully automated chemiluminescence analyzer. Transfusion 2003;43:1067-74.

Product Disclaimer

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product, the product is used outside of the control of the SD BIOSENSOR HEALTHCARE PVT. LTD. and distributor and the result may accordingly be affected by environmental factors and/or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

Warning

The SD BIOSENSOR HEALTHCARE PVT. LTD. and distributors of this product shall not be liable for any losses, liability, claims, costs or damages whether direct or indirect of consequential arising out of or related to an incorrect diagnosis, whether reactive or non reactive, in the use of this product.

SD/018/IN/01-03

Issue date : 2022.03

Manufactured by
SD BIOSENSOR HEALTHCARE PVT. LTD.

Manufacturing site: Plot No. 38, Sector - 4, IMT Manesar, Gurugram, Haryana - 122052, India

Head Office: Unit No - 202 A-D, 2nd Floor, Tower A, Unitech Signature Towers, South City 1, Gurugram, Haryana - 122001, India

Any inquiries regarding the instruction provided should be addressed to: care@sdbiosensor.co.in or call at - **1800-10-23105**
www.sdbiosensor.co.in

CAT Catalogue number | IVD In vitro Diagnostics | Consult Instructions for Use | Contains Sufficient for <n> Tests | Caution | To indicate the temperature limitations in which the transport package has to be kept and handled. | Do not re-use. | Use by | LOT Batch code | Manufacturer | Date of manufacture | Indicate that you should keep the product dry | Keep away from sunlight | Do not use if packaging is damaged | This way up

OnSite CE

REF R0160C IVD In vitro Diagnostic

INTENDED USE

The Typhoid IgG/IgM Rapid Test is a lateral flow immunoassay for the qualitative detection and differentiation of anti-*Salmonella typhi* (*S. typhi*) and *paratyphi* IgG and IgM in human serum or plasma. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with *S. typhi* and *paratyphi*.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

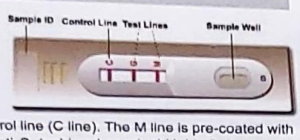
SUMMARY AND EXPLANATION OF THE TEST

Typhoid fever and paratyphi fever are bacterial infections caused by *Salmonella typhi and paratyphi A, B, and C respectively*, which are transmitted through the ingestion of tainted food and water. Worldwide an estimated 17 million cases and 600,000 associated deaths occur annually[1]. Patients who are infected with HIV are at significantly increased risk of clinical infection. 1-5% of patients become chronic carriers harboring *S. typhi* in the gallbladder.

The clinical diagnosis of infections depends on isolation of *S. typhi and paratyphi* from blood, bone marrow or a specific anatomic lesion. In facilities that can not afford to perform this complicated and time-consuming procedure, Filix-Widal test is used to facilitate diagnosis. However, many limitations lead to difficulties in the interpretation of the Widal test[3,4].

In contrast, the Typhoid IgG/IgM Rapid Test is a simple, fast laboratory test that simultaneously detects and differentiates IgG and IgM antibodies to *S. typhi* and *paratyphi* antigen[5] thus aiding in the determination of current or previous exposure to *S. typhi* and *paratyphi*. IgM positive or IgM /IgG both positive suggest current infection, while IgG positive suggests late stage of infection, previous infection, or latent infection.

TEST PRINCIPLE



The Typhoid IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing recombinant H antigen and O antigen conjugated with colloidal gold (HO conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The M line is pre-coated with monoclonal anti-human IgM for the detection of anti-S. *typhi* and *paratyphi* IgM, G line is pre-coated with reagents for the detection of anti-S. *typhi* and *paratyphi* IgG, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the cassette, the test specimen migrates by capillary action across the test cassette. IgM antibodies if present in the patient specimen will bind to the HO conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody, forming a colored M line, indicating an anti-S. *typhi* or *paratyphi* IgM positive test result.

IgG antibodies if present in the patient specimen will bind to the HO conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a colored G line, indicating an anti-S. *typhi* or *paratyphi* IgG positive test result.

Absence of any test lines (M and G) suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies regardless of the color development on any of the test lines. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Sample diluent (REF SB-R0160, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The test results should be read 15 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 15 minutes window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperature above 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature, if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.

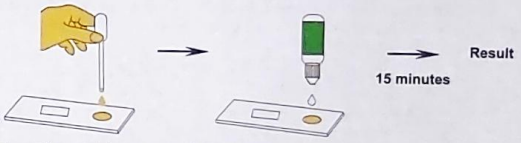
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of serum/plasma into the center of the sample well, making sure that there are no air bubbles.

Then immediately add 1 drop (about 35-50 µL) of sample diluent into the center of the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Results should be read at 15 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***Any results interpreted outside of the 15 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

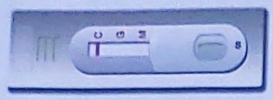
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. Otherwise, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE OR NON-REACTIVE RESULT:** If only the C line is present, the absence of any color in the both test lines (M and G) indicates that no anti-*S. typhi* or *paratyphi* antibody is detected in the specimen. The result is negative or non-reactive.

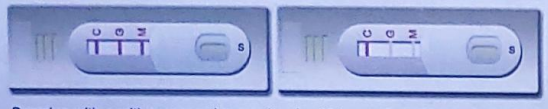


2. **POSITIVE OR REACTIVE RESULT:**

2.1 In addition to the presence of C line, if only M line develops, the test indicates for the presence of anti-*S. typhi* or *paratyphi* IgM in the specimen. The result is IgM positive or reactive.

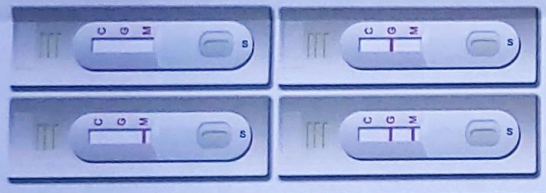
2.2 In addition to the presence of C line, if only G line develops, the test indicates for the presence of anti-*S. typhi* or *paratyphi* IgG in the specimen. The result is IgG positive or reactive.

2.3 In addition to the presence of C line, both M and G lines develop, the test indicates for the presence of anti-*S. typhi* or *paratyphi* IgG and IgM in the specimen. The result is both IgG and IgM positive or reactive.



Samples with positive or reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis decision is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of any color in the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance for IgM Test**

A total of 334 specimens were collected from susceptible subjects and tested by the Typhoid IgG/IgM Rapid Test and by a commercial *S. typhi* IgM EIA. Comparison for all subjects is shown in the following table:

	Typhoid IgG/IgM Rapid Test		
IgM EIA	**Positive**	**Negative**	**Total**
Positive	31	3	34
Negative	2	298	300
Total	33	301	334

Relative Sensitivity: 91%, Relative Specificity: 99.3%, Overall Agreement: 98.5%

2. **Clinical Performance for IgG Test**

A total of 314 specimens were collected from susceptible subjects and tested by the Typhoid IgG/IgM Rapid Test and by a commercial *S. typhi* IgG EIA kit. Comparison for all subjects is shown in the following table.

	Typhoid IgG/IgM Rapid Test		
IgG EIA	**Positive**	**Negative**	**Total**
Positive	13	1	14
Negative	2	298	300
Total	15	299	314

Relative Sensitivity: 92.9%, Relative Specificity: 99.3%, Overall Agreement: 99.0%

3. **Performance Comparison with Blood Culture**

Nine (9) *S. paratyphi* A positive and eleven (11) *S. typhi* positive specimens confirmed with the blood culture were tested with the Typhoid IgG/IgM Rapid Test. The Typhoid IgG/IgM Rapid Test correctly indentified 9 *S. paratyphi* A and 10 *S. typhi* specimens. The agreement was 95%.

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to *S. typhi* or *paratyphi* in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Typhoid IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to *S. typhi* or *paratyphi* in human serum or plasma. The intensity of the test line does not have linear correlation with the antibody titer in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-*S. typhi* or *paratyphi* antibodies. However, a negative test result does not preclude the possibility of exposure to *S. typhi* or *paratyphi*.
4. A negative or non-reactive result can occur if the quantity of anti-*S. typhi* or *paratyphi* antibodies present in the specimen is below the detection limit of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Infection may progress rapidly. If the symptom persists, while the result from Typhoid IgG/IgM Rapid Test is negative or non-reactive result, it is recommended to test with an alternative test method, such as bacterial culture method.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Ivanoff BN, Levine MM, Lambert PH. Vaccination against typhoid fever: present status. Bulletin of the World Health Organization 1994; 72: 957-71.
2. Gotuzzo E, Frisancho O, Sanchez J, Liendo G, Carillo C, Black RE, Morris JG. Association between the acquired immunodeficiency syndrome and infection with Salmonella typhi or Salmonella paratyphi in an endemic typhoid area. Archives of Internal Medicine 1991; 151: 381-2.
3. Clegg A, Passey M, Omena MK, et al. Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. Acta Tropica 1994; 57:255-63.
4. Pang T. False positive Widal test in nontyphoid Salmonella infection. Southeast Asian Journal of Tropical Medicine and Public Health 1989; 20: 163-4.
5. Ismail A, Hai OK, Kader ZA. Demonstration of an antigenic protein specific for Salmonella typhi. Biochem Biophys Res Commun, 1991;181(1):301-5.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

Mfd. by: M/s. CTK Biotech, Inc,
13855 Stowe Dr, Poway, California 92064,
United States having factory premises at M/s.
Beijing Genesee Biotech, Inc., 36, Yanqi Donger
Road, Huairou Yanqi Industrial Development
Zone 101407 Beijing, China

EC REP MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

PI-R0160C-ATH Rev. HP1.0
Date released: 2019-09-05
English version

For Export Only, Not For Re-sale in the USA

9. False positive results are likely if the test is read more than one minute after mixing on the slide test.
10. Any deviation in test procedure could result in variable results.
11. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
12. Use a separate disposable tip for each sample to prevent cross contamination.
13. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1: 80 in the slide and tube test when tested with the TYDAL® antigen suspensions.
2. *The negative control should show no agglutination with any of the TYDAL® antigen suspensions.*
3. *Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.*
4. *Reproducibility of TYDAL® antigen suspensions is 100% (+/- one double dilution).*

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBILIOGRAPHY

(1). Cruickshank R., (1982), Medical Microbiology, 12th Edition, 403. (2).Felix A., (1942), Brit. Med. J., 11, 597-600. (3). Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

Temperature limitation	Manufacturer	Contains sufficient for <n> tests
Use By	Consult Instructions for use	This way up
Date of Manufacture	REF Catalogue Number	CONTROL + Positive control
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	CONTROL - Negative control
Danger H350-H317 P201;P281; P308+P313 P280;P333+P313;P363 Formaldehyde	May be fatal if swallowed or enters airways Harmful if inhaled, or if swallowed or if in contact with skin May cause irritation to skin and/or eyes May cause irritation to the airways and/or drowsiness or dizziness. May cause an allergic skin reaction	EC REP Authorised Representative in the European Community

Manufactured by
TULIP DIAGNOSTICS (P) LTD.

Registered Office
GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

Manufacturing Unit
PLOT NO. UTILITY VIII, PHASE III B, VERNA IND. ESTATE, VERNA, GOA - 403 722, INDIA.

EC REP
CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

0520-9/0421/VER-05

CE

TYDAL®

WIDAL ANTIGEN SET / ANTIGENS FOR SLIDE AND TUBE TESTS

INTENDED USE

TYDAL® is a Widal slide and tube agglutination test that detects the presence of the serum agglutinins (O, H) in the patient's serum, with typhoid and paratyphoid fever.

SUMMARY

Enteric fever occurs when pathogenic microorganisms like S. typhi, S. paratyphi A, S. paratyphi B, S. paratyphi C infect the human body. During the course of disease, the body responds to this antigenic stimulus by producing antibodies whose titre rises slowly in early stages, to a maxima and then slowly falls till it is undetectable. Antibodies to *Salmonella* organisms may be detected in the patient serum from the second week after onset of infection. Information regarding the titres and whether or not they are rising or falling can be obtained by performing serological tests using TYDAL® antigen suspensions. Usually tube titres of 1:80 and above are taken as diagnostically significant, however for endemic areas higher cut-offs may need to be established.

REAGENT

TYDAL® contains ready to use concentrated, smooth antigen suspensions of the bacilli; *S. typhi 'O', S. typhi 'H', S. paratyphi 'AO', S. paratyphi 'BO', S. paratyphi 'AH', S. paratyphi 'BH', S. paratyphi 'CH', S. paratyphi 'CO'* and / or polyspecific positive control reactive with these antigens.
Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE .Keep the reagents away from direct sunlight.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRESENTATION

Σ		4x5 ml	8x5 ml*	2x5 ml	2x5 ml	2x5 ml	2x2x 5 ml	2x2x 5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
REF		105200045	105200085	105210025	105200025	105220025	105210225	105200225	105220005	105230005	105240005	105250005	105280005	105260005	105270005	105290005
Antigens		O, H, AH, BH	O, H, AH, BH, CH, AO BO, CO	O, H	O, H	O, H	O, H	O, H	O	H	AO	BO	CO	AH	BH	CH
Control	+	0.4 ml	2.0 ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml								
Control	-		2.0 ml		0.4ml	0.4ml		0.4ml								
MIXING STICKS LADDER		4	6		4		4	4								
DISPENSER PP TUBES		50	50		50		50	50								
RUBBER TEAT		1	1		1		1	1								
SLIDE		1	1		1		1	1								
PACKAGE INSERT		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

* 8 x 5 ml pack is marketed as TYDAL® PLUS.

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes.
Quantitative method: Timer, Kahn tubes / test tubes, Pipettes (0.1ml, 1ml), Physiological saline, Incubator (37°C), Test tube rack.

PRINCIPLE

When the coloured, smooth, attenuated TYDAL® antigen suspensions are mixed / incubated with patient serum, anti-*Salmonella* antibodies present in the patient serum react with the antigen suspensions to give agglutination. Agglutination is a positive test result, indicating presence of anti-*Salmonella* antibodies in the patient serum. No agglutination is a negative test result indicating absence of anti-*Salmonella* antibodies.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. *The S. typhi 'O', S. paratyphi 'CO' reagents contain 0.5% Phenol, S. typhi 'H', S. paratyphi 'AH', S. paratyphi 'BH', S. paratyphi 'CH' reagents contain 0.3% Formaldehyde and S. paratyphi 'AO', S. paratyphi 'BO' reagents contain 0.7% Ethanol along with 0.1% Sodium azide as preservatives. Avoid contact with skin and mucosa. Do not breathe vapour. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Sodium azide may react with lead and copper in plumbing and form highly explosive metal oxides, on disposal flush with large quantities of water.*
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive and negative controls. Positive control provided with the kit only for TYDAL® 4 x 5 ml set (REF.: 105200045), 2 x 5 ml set (REF.: 105210025 & REF 105200025), 2 x 2 x 5 ml set (REF.: 105210225) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085). Negative Control provided with the kit only for 8 x 5 ml (REF.: 105200085), 2 x 5 ml (REF 105200025) and 2 x 2 x 5 ml (REF 105200225).
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only clean and dry slides / tubes must be used. Clean the slide / tube with distilled water and dry.
6. *It is necessary* to use the calibrated dropper provided in the reagent vial to dispense a reagent drop.
7. TYDAL® antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
8. Accessories provided with the kit only must be used for optimum results. (Applicable only for TYDAL® 2 x 2 x 5 ml set (REF.: 105210225, 105200225), 2 x 5 ml set (REF.: 105200025), 4 x 5 ml set REF.: 105200045) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085).
9. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactivate the serum.
4. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for upto 72 hours.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.
Shake and mix antigens well before dispensing.

Slide Screen Method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 50 µl of physiological saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate TYDAL® antigen suspension to the reaction circles containing Positive control & physiological saline.
5. Add one drop of appropriate TYDAL® antigen suspensions to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination **macroscopically at one minute**.

Slide Semi-Quantitative Method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested on 5 different reaction circles on the slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, & 1:320 respectively.
2. Follow step No. 5-7 of slide screen method.

Note: This method is recommended for obtaining quick approximate titres only.

Quantitative Method

Tube-test Procedure

1. Take appropriate number of sets (as required; one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette into tube No. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
6. Transfer 1 ml of the diluted serum sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 7 in each set.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Now the dilutions of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1: 320, 1:640, 1: 1280. Tube No. 8 in all the sets, serves as a saline control.
9. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed TYDAL® antigen suspensions from the reagent vials and mix well.
10. Cover and incubate at 37°C overnight (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

INTERPRETATION OF RESULTS

Slide Screen Method

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum. No agglutination is a negative test result and indicates absence of the corresponding antibody in the patien serum.

Slide Semi-Quantitative Method

Agglutination is a positive test result. The titre of the patient serum corresponds to the visible agglutination in the test circle with the least amount of serum sample.

Quantitative Method

The titre of the patient serum using TYDAL® antigen suspensions is the highest dilution of the serum sample tha gives a visible agglutination.

REMARKS

1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
2. TAB vaccinated patients may show a high titre of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
3. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
4. A rising titre is more significant than a single high titre. It is therefore necessary to evaluate two or more serum samples taken at 4- 6 days intervals after the onset of the disease.
5. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being flagellar antigen brings about larger, loose, flocculant agglutination.
6. While the 'O' antigen is species specific, the 'H' antigen is specific to the serotype.
7. Serological findings are not intended as a substitute for culture. An appropriate attempt should be mad recover and identify the etiologic organisms through various culture and biochemical tests.
8. Generally antibody titres of 1:80 or more are considered clinically and diagnostically significant. Howeve significant titre may vary from population to population and needs to be established for each area.

ASPEN® Syphilis

Syphilis Rapid Test Strip (Serum/Plasma/WB) Package Insert

A rapid test for the diagnosis of Syphilis to detect antibodies (IgG and IgM) to Treponema Pallidum (TP) qualitatively in serum/plasma/whole blood. For professional in vitro diagnostic use only.

INTENDED USE

The Aspen Syphilis Rapid Test strip (Serum/Plasma/Whole blood) is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM) to Treponema Pallidum (TP) in serum, plasma or whole blood to aid in the diagnosis of Syphilis.

SUMMARY

Treponema Pallidum (TP) is the causative agent of the venereal disease Syphilis. TP is a spirochete bacterium with an outer envelope and a cytoplasmic membrane.[1] Relatively little is known about the organism in comparison with other bacterial pathogens. According to the Center for Disease Control (CDC), the number of cases of Syphilis infection has markedly increased since 1985.[2] Some key factors that have contributed to this rise include the crack cocaine epidemic and the high incidence of prostitution among drug users. One study reported a substantial epidemiological correlation between the acquisition and transmission of the HIV virus and Syphilis.

Multiple clinical stages and long periods of latent, asymptomatic infection are characteristic of Syphilis. Primary Syphilis is defined by the presence of a chancre at the site of inoculation. The antibodies response to the TP bacterium can be detected within 4 to 7 days after the chancre appears. The infection remains detectable until the patient receives adequate treatment.[3]

The Syphilis Rapid Test strip (Serum / Plasma / whole blood) utilizes a double antigen combination of a Syphilis antigen coated particle and Syphilis antigen immobilized on membrane to detect TP antibodies (IgG and IgM) qualitatively and selectively in serum / plasma / whole blood.

PRINCIPLE

The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) is a qualitative membrane based immunoassay for the detection of TP antibodies (IgG and IgM) in whole blood, serum or plasma. In this test procedure, recombinant Syphilis antigen is immobilized in the test line region of the test. After specimen is added to the specimen well of the test strip, it reacts with Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized Syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains TP antibodies, a colored line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a colored line will not appear in this region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENT

The test contains Syphilis antigen coated particles and Syphilis antigen coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date. Do not use test if pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or test strips are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing, disposable gloves and eye protection when specimens are assayed.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

The test is stable through the expiration date printed on the sealed pouch 2-30°C. DO NOT FREEZE. Do not use after the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Aspen Syphilis rapid test strip can be performed using Serum / Plasma / Whole blood.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimen at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimen to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimen should not be frozen and thawed repeatedly.

MATERIAL PROVIDED

- Test strips
- Buffer
- Droppers
- Package insert
- Strip support

Material required but not provided

Specimen Collection containers, Centrifuge, Timer, Test tubes

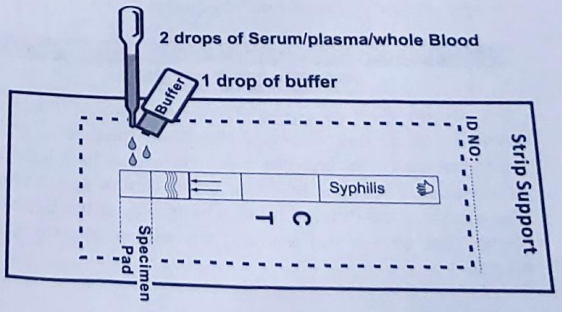
DIRECTIONS FOR USE

Note: Bring the test device, specimen and buffer to the room temperature if stored at 2-8°C.

Remove the strips from the sealed pouch.

- Peel off the tape from the strip support and stick the test strip in middle of the strip support as shown in below picture.

1. Add **2 drops** (50µl) of **Serum/ Plasma / Whole blood** to the specimen pad of the test strip using dropper/ pipette.
2. Add **1 drop** of **buffer** (40µl). Read result at **10 minutes.** (Do not interpret the result after 30 minutes).

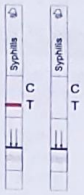


INTERPRETATION OF RESULTS

NEGATIVE : Pink/Purple line at **C** only

POSITIVE : Pink/Purple lines at **C & T**

INVALID : If control line does not appear, the test is invalid. In this case, please repeat the test using another device following the test procedure correctly.



QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms adequate membrane wicking.

LIMITATIONS

1. The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) is for in vitro diagnostic use only. The test should be used for the detection of TP antibodies in serum, plasma or whole blood specimens only. Neither the quantitative value nor the rate of increase in TP antibodies can be determined by this qualitative test.
2. The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) will only indicate the presence of TP antibodies in the specimen and should not be used as the sole criteria for the diagnosis of TP infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of TP infection.

EXPECTED VALUES

The Aspen Syphilis Rapid Test Strip (Serum/Plasma/Whole blood) has been compared with a leading commercial TPPA Syphilis test, demonstrating an overall accuracy greater than or equal to 99.8%.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The Aspen Syphilis Rapid Test strip (Serum /Plasma /Whole blood) has correctly identified specimens of a performance panel and has been compared to a leading commercial TPPA Syphilis test using clinical specimens. The results show that the relative sensitivity of the Syphilis Rapid Test strip is >99.9% and the relative specificity is 99.7%.

Method	TPPA		
Aspen Syphilis Rapid Test Strip(serum/Plasma/WB)	Results	Positive	Negative	Total Result
	Positive	130	1	131
	Negative	0	299	299
Total Result		130	300	430

Relative sensitivity: >99.9% (95%CI*: 97.7%~100.0%);
Relative specificity: 99.7% (95%CI*: 98.2%~100.0%);
Accuracy: 99.8% (95%CI*: 98.2%~100.0%).
*Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 15 replicates of four specimens: a negative, a low positive, a medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same four specimens: a negative, a low positive, a medium positive and a high positive. Three different lots of the Syphilis Rapid Test strip (Serum/Plasma/WB) have been tested over a 3-day period using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Aspen Syphilis Rapid Test Strip (Serum/Plasma/WB) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb,HCV, HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Syphilis negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin 1.1 mg/dL
Bilirubin: 1g/dL	Oxalic Acid: 600mg/dL

None of the substances at the concentration tested interfered in the assay.

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3. Johnson PC. Testing for Syphilis. Dermatologic Clinic (1994); 12 Jan: 9-17.

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