REF AR0030S

TRUSTline Syphilis Ab Rapid Test - Dip Strip

IVD

for the qualitative detection of antibodies to *Treponema pallidum* (*Tp*) in human serum or plasma.

INTENDED USE

The TRUSTline Syphilis Ab Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of antibodies including IgG, IgM, and IgA to *Treponema pallidum* (*Tp*) 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 *Tp*.
Any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on 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

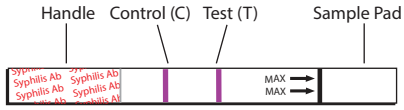
Tp, a spirochete bacterium, is the causative agent of the venereal disease syphilis. Although syphilis rates are declining in the United States after an epidemic outbreak between 1986 and 1990[1], the incidence of syphilis in Europe has increased since 1992, especially in the countries of the Russian Federation, where peaks of 263 cases per 100,000 have been reported[2]. In 1995, WHO reported 12 million new cases of syphilis[3]. Currently, the positive rate of syphilis serological tests in HIV-infected individuals has been rising recently.

Serological detection of anti-*Tp* antibody has been long recognized in the diagnosis of syphilis since the natural course of the infection was characterized by periods without clinical manifestations. Both IgM and IgG antibodies were detected in sera from patients with primary and secondary syphilis. The IgM antibody may be detectable towards the second week of infection, while IgG antibody appears later, at about 4 weeks[4]. These antibodies could last for several years or even decades in the serum of a patient with untreated latent syphilis [5].

Antigens such as Rapid Plasma Cardiolipin antigen (RPR) and *Tp* bacterial extracts have been used in the syphilis serological tests for decades. However, RPR antigen is a non-treponema antigen, derived from bovine heart. Antibody to RPR antigen does not develop until 1-4 weeks after the appearance of the chancre, thus this antigen lacks of sensitivity to primary syphilis. The *Tp* extracts are prepared from inoculated rabbit testis and contain a certain amount of contaminated materials such as flagella, which can lead to cross reactions with borreliae and leptospires in the serological test. In addition, the composition of extracts may vary from lot to lot. Recently, several highly immunogenic *Tp* specific antigens have been identified and used as an alternative to the traditional antigens with the advantages of high specificity and reproducibility [6-9]. The TRUSTline Syphilis Ab Rapid Test permits the measurement of antibodies (IgM, IgG and IgA) to recombinant antigens of *Tp* in serum/plasma rapidly and reliably without instrumentation.

TEST PRINCIPLE

The TRUSTline Syphilis Ab Rapid Test is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing recombinant *Tp* antigens conjugated with colloid gold (*Tp* conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with non-conjugated recombinant *Tp* antigens, and the C line is pre-coated with a control line antibody.



When an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. Anti-*Tp* antibody, if present in the specimen will bind to the *Tp* conjugates. The immunocomplex is then captured on the membrane by the pre-coated *Tp* antigen, forming a burgundy colored T line, indicating a *Tp* antibody positive test result.

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

REAGENTS AND MATERIALS PROVIDED

Kit box package	1. Individually sealed pouches containing:	a. One dip strip b. One desiccant
	2. 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
2. A container for holding test specimen
3. Disposable gloves

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 testing results should be read at 10-15 minutes after removal of the strip from the specimen container. Any results interpreted outside of the 10-15 minutes window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store test strip at 1-30 °C. If test strip stored at 1-8 °C, ensure that the test strip is brought to room temperature before opening.The test strip is stable through the expiration date printed on the sealed pouch. Do not freeze the strip or expose the strip over 30 °C. The test strip is sensitive to humidity and heat. Perform the test immediately after removing the test strip from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 1- 8 °C if not tested immediately for up to 5 days. The specimens should be frozen at -20°Ċ 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 on result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

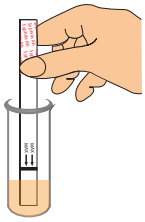
Step 2: Collect at least 150-200 µL or 3-4 drops of serum or plasma in a sample container.

Step 3: **For Kit box package:**
a. Take the desired quantity of sealed pouches from the box.
b. When ready to test, open the pouch at the notch and remove the test strip.

Step 4:. Dip the strip into the specimen for at least 10 seconds.

Don't allow the specimen to reach above the level indicated by the arrows on the strip.

Meanwhile, set up timer.



Step 5: Remove the strip from the specimen, and place it on a flat, dry surface.

Step 6: Read the result in 10-15 minutes. Positive results may be visible in as short as 1 minute. ***Any results interpreted outside of the 15 minutes window should be considered invalid and must be repeated. Discard used strips after interpreting the result following local laws governing the disposal of devices.***

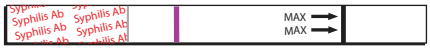
QUALITY CONTROL

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

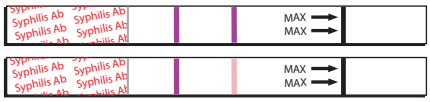
2. **External Control:** Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit falls outside of 1-30 °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 RESULT**: If only the C line develops, the test indicates that no detectable anti-*Tp* antibody is present in the specimen. The result is negative or non-reactive.



2. **POSITIVE RESULT**: If both C and T lines develops, the test indicates for the presence of anti-*Tp* antibody in the specimen. The result is positive or reactive.



Samples with positive or reactive results should be confirmed with alternative testing method(s) such as TPHA test and clinical findings before to make diagnostic decision.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 596 samples from susceptible subjects were tested with TRUSTline Syphilis Ab Rapid Test and with a commercial Syphilis Ab ELISA kit. Comparision for all the subjects is shown in the following table.

	TRUSTline Syphilis Ab Rapid Test		
ELISA	**Positive**	**Negative**	**Total**
Positive	72	0	72
Negative	2	522	524
Total	74	522	596

Relative Sensitivity: 100%, Relative Specificity: 99.6%, Overall Agreement: 99.6%

2. **Cross Reactivity**

The negative specimen was spiked with serum specimens of infectious diseases and then tested according to the standard procedure. The results showed that the TRUSTline syphilis Ab Rapid Test had no cross-reaction with the following tested serum specimens of infectious disease.

Specimen	Sample Size	Syphilis Ab Reactivity
Dengue Positive Serum	10	Negative
HAV Positive Serum	10	Negative
HCV Positive Serum	10	Negative
HIV Positive serum	10	Negative
HBsAg Positive Serum	10	Negative
ANA Positive Serum	5	Negative
RF positive Serum (≤2,500 IU/ml)	5	Negative

3. **Precision**

Within run and between run precisions have been determined by testing 20 replicates with four catelogy of the specimens; negative, weak, medium, and strong positive Specimens.The negative, weak, medium, and strong positive Specimens were correctly identified in all of the tests perfomed in each run.

4. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the TRUSTline Syphilis Ab Rapid Test. This was studied by spiking of these substances into three levels of Syphilis Ab standard control. The results are presented in the following table and demonstrate that at the concentrations tested, the substances studied did not affect the performance of the TRUSTline Syphilis Ab Rapid Test.

Note: -: Negative; +: Weak positive; +++: Strong positive

Potential Interfering Substances Spiked	Syphilis Ab Reactivity		
	Negative	Weak Positive	Strong Positive
Control	-	+	+++
Bilurubin 15 mg/dL	-	+	+++
Creatinine 5 mg/dL	-	+	+++
Glucose 120 mg/dL	-	+	+++
Albumin 5g/L	-	+	+++
Salicylic Acid 4.34 mmol/L	-	+	+++
EDTA 3.4 µmol/L	-	+	+++
Urea 40 mg/dL	-	+	+++

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of anti-Tp antibody in serum or plasma from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The TRUSTline Syphilis Ab Rapid Test is limited to the qualitative detection of anti-Tp antibody in human serum or plasma. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates the absence of detectable anti-Tp antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with Tp.
4. A negative result can occur if the quantity of the anti-Tp antibody present in the specimen is below the detection limits of the assay or if the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. False negative results may arise because of hook effect due to very high titer of antibodies in sample. Repeat the test by using different dilution of same sample.
6. Infection may progress rapidly. If the symptoms persist and the result from the TRUSTline Syphilis Ab Rapid Test is non-reactive, it is recommended to test with an alternative method or to re-sample the patient a few weeks later.
7. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
8. A positive result indicates a past or present infection. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of Symbols

Consult instructions for use		REF	Catalogue number		
IVD	For *in vitro* diagnostic use only	LOT	Batch code		Use-by date
1°C 30°C	Temperature limit 1-30 °C		Do not re-use	Σ N	Tests per kit
	Manufacturer		Date of manufacture		Keep dry
NON STERILE	If device is non-sterile		Warnings / Precautions		
Do not use if package is damaged			Keep away from sunlight		

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