OnSite™ Syphilis Ab Rapid Test

REF R0030C CE

INTENDED USE

The *OnSite* Syphilis Ab Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of antibodies (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

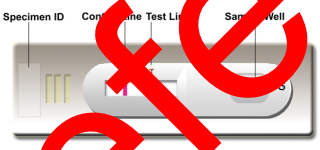
Tp, a spirochete bacterium, is the causative agent of the venereal disease syphilis. Although syphilis rates are declining in the United States after an epidemic outbreak between 1986 and 1990[1], the incidence of syphilis in Europe has increased since 1992, especially in the countries of the Russian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

Serological detection of anti-*Tp* antibodies has long been recognized as an aid in the diagnosis of syphilis since the natural course of the infection is 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 an infection while IgG antibodies appear later at approximately 4 weeks[4]. These antibodies can last for several years or even decades in the serum of a patient with untreated latent syphilis[5].

Antigens such as Rapid Plasma Reagin (RPR) and *Tp* bacterial extracts have been used in syphilis serological tests for decades. However, RPR antigen is a non-*Treponema* antigen derived from bovine heart. Antibodies to RPR antigen do not develop until 1-4 weeks after the appearance of the chancre, thus this antigen lacks 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 borrelia 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 advantage of having high specificity and reproducibility.[6-9]

The *OnSite* Syphilis Ab Rapid Test was developed to detect antibodies (IgM, IgG and IgA) to recombinant antigens of *Tp* in serum or plasma. The test can be performed within 10 minutes by minimally skilled personnel and without the use of cumbersome laboratory equipment.

TEST PRINCIPLE



The *OnSite* Syphilis Ab Rapid Test is a lateral flow chromatographic immunoassay. The test strip in cassette device consists of: 1) a burgundy colored conjugate pad containing recombinant *Tp* antigens conjugated with colloidal gold (*Tp* conjugates) and a control antibody conjugated with colloidal gold, and 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 well of the test cassette, the specimen migrates by capillary action across the test 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 test 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. If the C line does not develop, 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. 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 may lead to 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 specimens 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 handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test result should be read 10-15 minutes after specimen is added to the sample well or sample pad of the device. Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, e.g. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unopened test devices unopened at 2-30°C. The positive and negative controls should be kept at 2-8°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 temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

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

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: 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. 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.

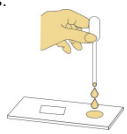
ASSAY PROCEDURE

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

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

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

Step 4: Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 2 drops (about 60- 90 µL) of specimen into the sample well making sure there are no air bubbles.



2 drops of specimen

→ 10 minutes → Result

Note: ***Add 1 drop of Saline or Phosphate-Saline buffer (common buffers used in clinics not provided in the kit) to the sample well if flow migration is not observed in the result window within 30 seconds, which could occur with highly viscous specimens.***

Step 5: Set up timer.

Step 6: Result should be read at 10 minutes. Positive results may be visible in as soon as 1 minute. Negative results must be confirmed at the end of 15 minutes only. ***Any results interpreted outside of the 10-15 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.***

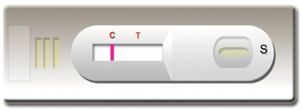
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen. If the C line does not develop, review the entire procedure and repeat the test with a new device.

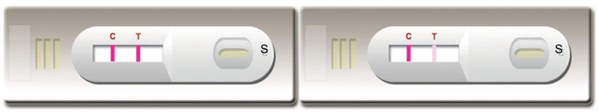
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature 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 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.

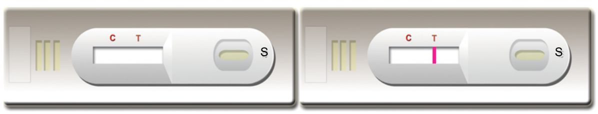


2. **POSITIVE RESULT**: If both the C and T lines developd, the test indicates the presence of anti-*Tp* antibodies in the specimen. The result is positive or reactive.



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.

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 1055 clinical specimens were collected from susceptible subjects and tested by the *OnSite* Syphilis Ab Rapid Test and by a TPPA test. Comparison for all subjects is shown in the following table.

	***OnSite* Syphilis Ab Rapid Test**		
TPPA	**Positive**	**Negative**	**Total**
Positive	318	0	318
Negative	2	735	[illegible]
Total	320	735	[illegible]

Relative Sensitivity: 100%, Relative Specificity: 99.7%, Ove[illegible] Agree[illegible]nt: 99.8[illegible]

2. **Precision**

Within run and between run precisions have been determin[illegible] testing [illegible] replicates with three of the samples: a negative, a weak positive and a [illegible]rong positive. The negative, weak positive and strong positive samples we[illegible]rrectly i[illegible]tified in all of the tests performed in each run.

LIMITATIONS OF TE[illegible]

1. The Assay Procedure and the In[illegible]pretat[illegible] of Ass[illegible]esult se[illegible]ons must be followed closely when testing for the [illegible]esence of [illegible]ti-*Tp* an[illegible] serum or plasma from individual subjects. Failure [illegible]ollow the pr[illegible]edure may lead to inaccurate results.
2. The *OnSite* Syphilis Ab Ra[illegible] Test i[illegible] qualitative detection of anti-*Tp* antibody in human serum or pla[illegible]e intensity of the test line does not have a linear correlation with the antibody titer i[illegible] specimen.
3. A negative result for an individual s[illegible]ct indicates the absence of detectable anti-*Tp* antibodies. However[illegible] negative test res[illegible]does not preclude the possibility of exposure to or infection with [illegible].
4. A negative res[illegible]an occur if the quantity of the anti-*Tp* antibody present in the specimen is below [illegible] detection limits of the assay or if the antibodies that are detected are not pr[illegible] during [illegible] stage of disease in which a sample is collected.
5. Some [illegible]pecime[illegible] conta[illegible]g unusually high titers of heterophile antibodies or rheu[illegible]toid factor [illegible] affect expected results.
6. If the [illegible]mptoms per[illegible]st while the result from *OnSite* Syphilis Ab Rapid Test is negative or non[illegible]active, it [illegible] recommended to re-sample the patient a few weeks later or test [illegible]h an a[illegible] test device.
7. [illegible]he results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. [illegible]enters for Disease Control and Prevention. Chlamydia trachomatis infections: policy [illegible]uidelines from prevention and control. Morbid. Mortal. Weekly Rep. 1995; 34:53S-74S.
2. Tichonova, L., K. Borisenko, H.Ward, A.meheus, et al. Epidemics of syphilis in the Russian Federation: Trends, origins, and priorities for control. Lancet 1997; 350:210-213.
3. Gerbase, A. C., J. T. Rowley, D. H. Heymann, S. F. Berkley, and P. Piot. Global prevalence and incidence estimates of selected curable STDs. Sex. Transm. Infect 1998; 74:S12-S16.
4. Luger AFH. Serological Diagnosis of Syphilis: Current methods. In: Young H, McMillan A, eds. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Decker, 1988: 249-274.
5. Baker-Zander SA, Hook EW 3rd, Bonin P, Handsfield HH, Lukehart SA. Antigens of Treponema pallidum recognized by IgG and IgM antibodies during syphilis in humans. J Infect Dis. 1985; 151(2):264-72.
6. Norgard MV, Chamberlain NR, Swancutt MA, Goldberg MS. Cloning and expression of the major 47-kilodalton surface immunogen of treponema pallidum in Escherichia Coli. Infect Immun 1986; 54:500-506.
7. Purcell BK, Chamberlain NR, Goldberg MS, Andrews LP, Robinson EJ, Norgard M[illegible] Radolf JD. Molecular cloning and characterization of the15-kilodalton major immunoge[illegible] of Treponema pallidum. Infect Immun. 1989; 57(12):3708-14
8. Bailey MJ, Thomas CM, Cockayne A, Strugnell RA, Penn CW. Cloni[illegible]nd expre[illegible] of Treponema pallidum antigens in Escherichia coli. J Gen Microbiol [illegible]9; 135 (Pt 9):2365-78.
9. Sambri V, Marangoni A, Simone MA, D'Antuono A, Ne[illegible] M, Cev[illegible]i R. Evaluation of recomWell Treponema, a novel recombinan[illegible] antigen-[illegible]ed en[illegible]me-linked immunosorbent assay for the diagnosis of syphili[illegible] in Microbio[illegible]fect 2001; 7(4):200-5.

Index of CE Symbols

[i]	Consult instructions for use	IVD	For *in vitro* diagnostic use only	⌛	Use by
REF	Catalog #	LOT	[illegible] Number	Σ N	Tests per kit
2°C–30°C	Store between 2-30°C	EC REP	Au[illegible] Representative	②	Do not reuse
	Manufacturer		Date of manufacture		

CTK [illegible] Inc.
10110 Mesa Rim [illegible]ad
San Diego, CA 9[illegible]21, USA
Tel: 858-457-869[illegible]
Fax: 858-[illegible]5-1739
E-ma[illegible]tkbiote[illegible]

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover Germany

P[illegible]030C Rev. [illegible]0
D[illegible]eleased: 201[illegible]01
Engl[illegible]ersion

[illegible] Expo[illegible]nly, Not For Re-sale In the USA