

OnSite® Syphilis Ab Combo Rapid Test

REF R0031C C E

Instructions for Use

INTENDED USE

The OnSite Syphilis Ab Combo 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, plasma or whole blood. It is intended to be used by healthcare professionals 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 1990¹, 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 reported². In 1995, WHO (World Health Organization) reported 12 million new cases of syphilis³. To this day, the positive rate of syphilis serological tests among HIV-infected individuals continues to rise.

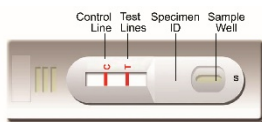
Serological detection of anti-Tp antibodies has long been recognized for the diagnosis of syphilis since the natural course of the infection is characterized by periods without clinical manifestations. Both IgM and IgG antibodies to *Treponema pallidum* were detected in sera from patients with primary and secondary syphilis. The IgM antibody may be detectable in the second week of infection, while IgG antibodies appear later at about 4 weeks⁴. Antibodies to *Treponema pallidum* can last for several years or even decades in the serum of a patient with untreated latent syphilis⁵.

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 nontreponemal 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 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 reproducibility⁶⁻⁹.

The OnSite Syphilis Ab Combo Rapid Test was developed to detect antibodies (IgM, IgG and IgA) to recombinant antigens of Tp in serum, plasma or whole blood. The test can be performed by minimally trained personnel and without cumbersome laboratory equipment.

TEST PRINCIPLE

The OnSite Syphilis Ab Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing recombinant Tp antigens conjugated with colloidal 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 well of the test cassette, the specimen migrates by capillary action across the cassette. 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 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 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

- Individually sealed foil pouches containing:
 - One cassette device
 - One desiccant
- Plastic droppers
- Sample diluent (REF SB-R0031, 5 mL/bottle)
- Instructions for Use

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

- Positive Control
- Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or timer
- Lancing device for whole blood test

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use

- Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
- Do not open the sealed pouch unless ready to conduct the assay.
- Do not use expired devices.
- Bring all reagents to room temperature (15-30°C) before use.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Do not use hemolyzed blood specimens for testing.
- Wear protective clothing and disposable gloves while handling the kit reagents and

- clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
- Handle the negative and positive controls in the same manner as patient specimens.
- The test result should be read 15-20 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
- 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 temperatures over 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.

Whole Blood

- Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

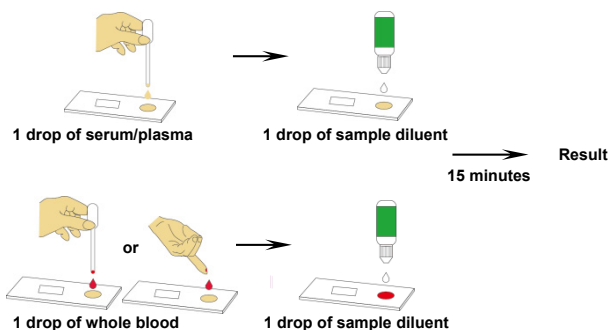
Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

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: 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 the specimen 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 or 1 drop of whole blood (about 40-50 µL) into the sample well making sure that there are no air bubbles.

Immediately add 1 drop (about 35-50 µL) of sample diluent to the sample well with bottle positioned vertically.



- Step 5: Set up timer.
- Step 6: Read results at 15 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. **However, any results interpreted outside 15-20 minutes should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.**

QUALITY CONTROL

- Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.
- 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 new operator uses the kit prior to performing testing of specimens.
- A new lot of test kit is used.
- A new shipment of kits is used.
- The temperature used during storage of the kit falls outside of 2-30°C.
- The temperature of the test area falls outside of 15-30°C.
- To verify a higher than expected frequency of positive or negative results.
- To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

- 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.



- POSITIVE RESULT:** If both the C and T lines develop, 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 positive determination is made.

- 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 specimens were collected from susceptible subjects and tested with the OnSite Syphilis Ab Combo Rapid Test and with a TPPA (Treponema Pallidum Particle Agglutination) test. Comparison for all subjects is shown in the following table:

TPPA	OnSite Syphilis Ab Combo Rapid Test		
	Positive	Negative	Total
Positive	318	0	318
Negative	2	735	737
Total	320	735	1055

Relative Sensitivity: 100% (95% CI: 99.2% - 100%)
 Relative Specificity: 99.7% (95% CI: 99.0% - 99.9%)
 Overall Agreement: 99.8% (95% CI: 99.3% - 100%)

2. Precision

Within run and between run precisions have been determined by testing 15 replicates with three of the samples: a negative, a weak positive and a strong positive sample. The negative, weak positive and strong positive samples were correctly identified in all of the tests performed in each run.

LIMITATIONS OF TEST

- 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, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
- The OnSite Syphilis Ab Combo Rapid Test is limited to the qualitative detection of anti-*Tp* antibody in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
- 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*.
- 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 the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- Infection may progress rapidly. If symptoms persist while the result from the OnSite Syphilis Ab Combo Rapid Test is non-reactive, it is recommended to test with an alternative test method.
- Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

- Centers for Disease Control and Prevention. Chlamydia trachomatis infections: policy guidelines from prevention and control. Morbid. Mortal. Weekly Rep. 1995; 34:53S-74S.
- 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; 350:210-3.
- Gerbase, A. C., J. T. Rowley, D. H. Heymann, *et al.* Global prevalence and incidence estimates of selected curable STDs. Sex. Transm. Infect. 1998; 74:S12-6.
- Luger AFH. Serological Diagnosis of Syphilis: Current methods. In: Young H, McMillan A, eds. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker.1988: 249-74.
- Baker-Zander SA, Hook EW 3rd, Bonin P, *et al.* Antigens of Treponema pallidum recognized by IgG and IgM antibodies during syphilis in humans. J Infect Dis. 1985; 151(2):264-72.
- 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;54:500-6.

- Purcell BK, Chamberlain NR, Goldberg MS, *et al.* Molecular cloning and characterization of the 15-kilodalton major immunogen of Treponema pallidum. Infect Immun. 1989; 57(12):3708-14.
- Bailey MJ, Thomas CM, Cockayne A, *et al.* Cloning and expression of Treponema pallidum antigens in Escherichia coli. J Gen Microbiol. 1989;135 (Pt 9):2365-78.
- Sambri V, Marangoni A, Simone MA, *et al.* Evaluation of recomWell Treponema, a novel recombinant antigen-based enzyme-linked immunosorbent assay for the diagnosis of syphilis. Clin Microbiol Infect. 2001; 7(4):200-5.
- Rufli T. Syphilis and HIV infection. Dermatologica. 1989;179:113-117.

Index of CE Symbols

	Consult instructions for use		For <i>in vitro</i> diagnostic use only		Use by
	Catalog #		Lot Number		Tests per kit
	Store between 2-30°C		Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		



CTK Biotech, Inc.
 13855 Stowe Drive
 Poway, CA 92064, USA
 Tel: 858-457-8698
 Fax: 858-535-1739,
 E-mail: info@ctkbiotech.com



MDSS GmbH
 Schiffgraben 41
 30175 Hannover, Germany

PI-R0031C Rev. E2.0
 Date released: 2020-08-05
 English version

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