TOYO DIAGNOSTICS

INSTRUCTIONS FOR USE

ULTRA SENSITIVE ONE STEP TEST

For Syphilis Antibody (Anti -*Treponema pallidum*) Detection in Whole Blood / Serum / Plasma

Product Code: TTP02

Only for professional *in vitro* diagnostic use

BACKGROUND INFORMATION

In spite of the development of efficient treatment methods, Syphilis still persists as a common Sexually Transmitted Disease in many regions of the world. This disease is caused by *Treponema pallidum*, which is a member of Spirochaetaceae family. Infection of Treponema type is transmitted by direct contact with active lesion. The primary and secondary lesions of sexually transmitted syphilis are highly infectious, and contain a high amount of organism until the healing period. Congenital syphilis occurs when the *Treponema pallidum* is transmitted from a pregnant woman with syphilis to her fetus, during the later phases of pregnancy. The immune response to syphilis involves production of antibodies to a broad range of antigens, including non-specific antibodies and specific treponemal antibodies. The first demonstrable response to infection is the production of specific anti-treponemal IgM, which may be detected towards the end of the second week of infection; anti-treponemal IgG appears later, at about four weeks. By the time that symptoms develop, most patients have detectable IgG and IgM. Use of this rapid diagnostic test is highly advantageous, due to the corresponding technical application difficulties and high cost of many other treponemal tests.

INTENDED USE

Anti-Syphilis Test is a rapid and immunochromatographic procedure for the qualitative detection of Treponemal antibodies (IgA, IgM, IgG) generated against *Treponema pallidum* antigens (17 kDa, 15 kDa, 47 kDa) in human whole blood / serum / plasma with high sensitivity and specificity. This test can be used even in detection of congenital syphilis with the same accuracy.

REAGENTS

Recombinant *Treponema pallidum* antigens, monoclonal goat anti-*Treponema pallidum*, recombinant *Treponema pallidum* antigens (17 kDa, 15 kDa, 47 kDa) conjugated with colloidal gold particles.

METHOD

Anti-Syphilis Test uses immunochromatographic, Ag-Ab-Ag sandwich method for qualitative detection of *Treponema pallidum* antibodies (IgG, IgM, IgA) in human whole blood / serum / plasma. Recombinant *Treponema pallidum* antigens were immobilized on the test area "T" and antibodies generated against *Treponema pallidum* were immobilized on the control area "C" of the nitrocellulose membrane. Other *Treponema pallidum* antigens (17 kDa, 15 kDa, 47 kDa) conjugated with colloidal gold particles were dried on a conjugate pad. Sample is introduced from sampling pad. If there are *Treponema pallidum* antibodies in the sample, *Treponema pallidum* antibodies bind to the mobile recombinant *Treponema pallidum* antigens conjugated with colloidal gold particles. Together they move to the test area "T". *Treponema pallidum* antibodies that have already bound to mobile recombinant *Treponema pallidum* antigens conjugated to colloidal gold particles become immobilized in the test area "T" thus creating a visible colored signal due to the accumulation of colloidal gold particles in the test area "T" (a colored test line), indicating positive test result. If there are no *Treponema pallidum* antibodies in the sample then sample moves to the test area "T" together with unbound (free) recombinant *Treponema pallidum* antigens conjugated to colloidal gold particles. Immobilized recombinant *Treponema pallidum* antigens can not bind to mobilized *Treponema pallidum* antigens conjugated to colloidal gold particles, therefore no visible colored signal in the test area "T" (no colored test line) can be obtained, indicating negative test result. Regardless of *Treponema pallidum* antibodies content of the liquid sample, *Treponema pallidum* antigens conjugated with colloidal gold particles, bind to the immobilized *Treponema pallidum* antibodies in the control area "C" while liquid sample is passing through the control area "C". Therefore accumulation of colloidal gold particles produces a visible colored signal in the control area "C" (a colored control line), indicating a valid test result. The colored line should be visible in control area "C" in every case; if no visible colored line in control area "C", the test result should be indicated as invalid.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new dropper for each sample.
6. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7. This test will indicate only the presence or absence of *Treponema pallidum* antibody in the sample, and should not be used as the only basis for the diagnosis of Syphilis.

As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings..

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F) Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

Kit components : Test devices, droppers and instructions for use.

Additional materials required but not provided : Sample collection tube, centrifuge and timer, lancet (for only fingerstick whole blood), heparinized dispensing bulbs and capillary tubes (for only fingerstick whole blood).

Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

SAMPLE COLLECTION AND PREPARATION

The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.

For whole blood samples : Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.

For Serum Samples : Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.

For Plasma samples : Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.

Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

TEST PROCEDURE

1. Take the test device out of its pouch. Bring the tests and whole blood / serum / plasma samples to room temperature.
2. Draw whole blood / serum / plasma into dropper and put 3 drops (100 µl) into the sample well of the cassette.
   Avoid the formation of any air bubbles.
3. Depending on the concentration of anti- *Treponema pallidum* in the sample, the test can react even in 5 minutes. Results should be read at 15 minutes as shown below. Do not interpret results beyond 20 minutes, results forming after 20 minutes should be regarded as invalid.

INTERPRETATION OF RESULTS

Negative : Only one colored line is visible in "C" area, indicating that *Treponema pallidum* antibody does not exist.
Positive : Two colored lines are visible in "C" and "T" areas, indicating that *Treponema pallidum* antibody exists.
Low concentration of *Treponema pallidum* antibody may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid : No colored line is visible in "C" and "T" areas or only one colored line is visible in "T" area; the test should be repeated using a new test device.

QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Study Number	Sample Status	Sample Anti- T. Pallidum Status	Comparative Assay Used	False Result
200	Positive samples	Positive	VDRL / TPHA	0
500	Blood donors	Negative	VDRL / TPHA	1
28	Clinical	Negative	VDRL / TPHA	0
50	Pregnant women	Negative	VDRL / TPHA	0
10	Bilirubin	Negative	VDRL / TPHA	0
10	Hemoglobin	Negative	VDRL / TPHA	0
10	Triglycerides	Negative	VDRL / TPHA	0
10	Anti-HBs	Negative	WHO standard	0

Ultra Sensitive Anti-Syphilis Test can detect *Treponema pallidum* antibodies (IgA, IgM, IgG) generated against *Treponema pallidum* antigens (17 kDa, 15 kDa, 47 kDa)

Sensitivity : 99,99 % **Specificity: 99,8 %**

+ Predictive V : 99,5 % **- Predictive V: 99,9 %**

Cross Reactivity : Cross reactivity has been tested with below samples, no cross reactivity was found with the Anti-Syphilis Rapid Test.
- Anti-HBs positive whole blood / serum / plasma samples,
- Whole blood / serum / plasma samples from pregnant women.

Interferences: Following potentially interfering substances were tested with Anti-Syphilis Rapid Test: Hemoglobin, Bilirubin, Triglycerides. No interference was observed.
Hemolytic samples should not be used since they can cause to invalid or false results.

REFERENCES

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TÜRKLAB TIBBİ MALZEMELER SAN. TİC. A .Ş.
A.O.S.B 10040 Sok. No:20 Çiğli-İzmir / TURKEY
TEL: +90 232 376 80 81 FAX: +90 232 376 80 40 info@turklab.com.tr www.turklab.com.tr

EC REP **GESAN PRODUCTION S.r.l.**
Via Einaudi, 19 91021 TRE FONTANE - Campobello di Mazara (TP) ITALY

SYMBOLS USED

CE

Manufacturer

EC REP Authorized European Representative

Attention, see instruction for use

Consult instruction for use

IVD *In vitro* diagnostic medical device

Σn Number of test

For single use only

Storage temperature

LOT Lot number

REF Catalog number

Expiry date