SYPHILIS TPHA liquid

Hemagglutination Test

Package Size

REF	50101	100 Tests	Complete test kit

IVD

Intended Purpose

SYPHILIS TPHA **liquid** is an indirect hemagglutination test for the qualitative and semi-quantitative determination of specific antibodies against *Treponema pallidum* in human serum. The assay is intended for professional use and should be used by trained personnel only.

Clinical Significance

Syphilis is a chronic infection that progresses through multiple stages of infection. Typical symptoms initially are sores (chancres), then syphilitic rash followed by long periods of dormancy. Untreated infection may result in cardiovascular problems and neurosyphilis. The infection is caused by the *Treponema pallidum* subsp. *pallidum* spirochete and is usually acquired by sexual contact, although the disease may also be transmitted by transfusion of infected blood or vertically *in utero*. The organism has proved virtually impossible to culture in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

Method

Avian erythrocytes coated with *T. pallidum* antigen agglutinate in the presence of syphilis antibodies to form characteristic patterns in microtitration plates. The cells are suspended in an absorbent medium designed to eliminate antibodies to non-pathogenic treponemes. Coated avian erythrocytes lacking the antigen are used as control to reveal non-specific reactivity. Specimens are examined for syphilis antibodies using the qualitative test. The titre of reactive antibodies in positive specimens is determined in the semi-quantitative test.

Contents

STC	2x4 ml	**TPHA Test Cells** (white cap) Stabilised avian erythrocytes coated with *T. pallidum* antigen, ready to use
SCC	2x4 ml	**TPHA Control Cells** (blue cap) Stabilised avian erythrocytes coated with non-specific protein, ready to use
PC	0.5 ml	**TPHA Control Serum Positive** (red cap) Stabilised liquid positive control, reactive with test cells, pre-diluted 1/20, ready to use
NC	0.5 ml	**TPHA Control Serum Negative** (green cap) Stabilised liquid negative control, pre-diluted 1/20, ready to use
DIL	20 ml	**TPHA Diluent** Buffered saline containing absorbers to remove cross-reacting antibodies, ready to use

All reagents and controls contain < 0.1% sodium azide.

Materials Required but not Supplied

Micropipettes or microdilutor (10 µl, 25 µl, 75 µl, 190 µl); rigid-type U-profile 96-well microtitration plates (e.g. Rotilabo 9291.1 U-profile microtest plates, Carl Roth GmbH).

Stability

Stable if unopened up to the expiry date when stored at 2-8 °C. Do not freeze! Reagents are stable for 6 months after opening.

Specimen

Serum. Avoid contamination and haemolysis. Fresh specimens may be stored up to 7 days at 2-8 °C, or at -20 °C for up to one month. Thawed samples must be mixed prior to testing. Do not repeatedly freeze-thaw the specimens as this will cause false results.

Procedure

Allow all reagents and samples to reach room temperature (18-30 °C) before use. Ensure test and control cells are thoroughly re-suspended.

Qualitative Test

The test requires 3 wells in a microtitration plate (1 well for dilution and 2 wells for the test itself) and 50 µl of specimen diluted in DIL to 1 in 20 (25 µl diluted specimen per well).

1. Dilute specimen to 1 in 20 by mixing thoroughly 190 µl DIL and 10 µl specimen in well 1 (dilution well). **Note:** Do not dilute PC and NC in this step if quality control is being carried out.
2. Transfer 25 µl of diluted specimen (or non-diluted PC and NC) to each of wells 2 (control well) and 3 (test well).
3. Resuspend SCC and STC by rocking the vials gently. Add 75 µl of SCC to the control well and 75 µl of STC to the test well. Final specimen dilution after addition of cells is 1 in 80.
4. Tap plate gently to mix. Cover and let stand for 45-60 minutes at ambient temperature. Agglutination patterns are stable for up to one hour if undisturbed.
5. Examine for agglutination patterns.

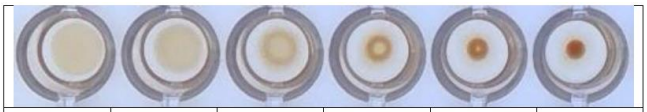
Semi-quantitative Test (Titration)

The test requires 10 wells in a microtitration plate (1 well for dilution and 9 wells for the test itself) and 50 µl of specimen diluted in DIL to 1 in 20.

1. Dilute specimen to 1 in 20 by mixing thoroughly 190 µl DIL and 10 µl specimen in well 1 (dilution well). **Note:** Do not dilute PC and NC in this step if quality control is being carried out.
2. Dispense 25 µl DIL to wells 4-10.
3. Transfer 25 µl of diluted specimen from well 1 (or non-diluted PC and NC from the respective vials) to each of wells 2, 3, and 4 (control well and test wells 1/80 and 1/160, respectively). Mix well 4 thoroughly.
4. Dilute sample from well 4 serially in wells 5-10 in a doubling manner: transfer 25 µl from well 4 to well 5, mix well 5, transfer 25 µl from well 5 to well 6, mix well 6, etc. Discard the excess 25 µl from the last well.
5. Resuspend SCC and STC by rocking the vials gently. Add 75 µl of SCC to well 2 (control well) and 75 µl of STC to each of wells 3-10 (test wells). Final specimen dilution after addition of cells is 1 in 80 in the control well and from 1 in 80 to 1 in 10240, in doubling steps, in the test wells.
6. Tap plate gently to mix. Cover and let stand for 45-60 minutes at ambient temperature. Agglutination patterns are stable for up to one hour if undisturbed.
7. Examine for agglutination patterns and determine the titre (the highest dilution showing agglutination).

Interpretation of Results

Outcome of the semi-quantitative test with the PC



Positive	Positive	Positive	Positive	Negative	Negative

Strongly agglutinated cells form an even layer over the bottom of the well. Weakly agglutinated cells form a characteristic ring pattern. Non-agglutinated cells form a compact ring or button at the centre of the well.

Qualitative: Absence of agglutination indicates that any reactive antibody, if present, is below the limit of detection of the assay. Agglutination (weak or strong) of STC but not of SCC indicates the presence of a specific antibody to *T. pallidum*. Specimens inducing agglutination should be re-assayed qualitatively or quantitatively and, if positive, should be tested by additional methods for confirmation. Non-specific antibodies can induce agglutination of both STC and SCC and render the result invalid. Such antibodies can be absorbed by pre-incubation with SCC (see Important Note 2).

Semi-quantitative: The titre is the highest dilution showing agglutination.

Quality Control

Correct performance of STC and SCC should be verified with the positive (PC) and negative (NC) control sera with each batch of patient samples. PC makes STC agglutinate to form a characteristic pattern at the bottom of the well. Upon titration, PC gives a titre of 1/1280 ± 1 doubling dilution (see image above). The degree of agglutination decreases as dilution grows. In contrast, PC induces no agglutination with SCC - a compact button of cells forms at the bottom of the well. NC induces no agglutination with either STC or SCC. **Note:** PC and NC are ready to use, and, unlike a specimen, they are not to be diluted before they are combined with SCC or STC.

Important Notes

1. Positive samples should be retested with a confirmatory test. Samples from patients with primary syphilis, mostly containing specific IgM antibodies, may yield negative results. If syphilis infection is still suspected in such cases, the test should be repeated after about 2 weeks with a freshly drawn sample. Clinical evidence should always be considered. Due to long-term persistence of specific antibodies in the circulation in patients with treated syphilis, a positive test result may indicate past or present infection. Although the TPHA test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders. Antibodies against other pathogenic treponemes can also cause false positive results. The final diagnosis therefore should consider further clinical and diagnostic findings.
2. To eliminate from the specimen non-specific antibodies reactive against STC and SCC, mix 10 µl specimen and 190 µl SCC, incubate for 30-60 minutes at room temperature and centrifuge for 3 minutes at 1500 g. The supernatant is equivalent to a specimen diluted to 1 in 20 in DIL. As such, it is to be used in step 2 of the qualitative test or in step 3 of the semi-quantitative test.

3. The kit components are matched and should not be replaced with components of other lots.

Limitations

The test is not intended for screening of blood donors or donated blood to be used in the context of a blood transfusion.

Performance Characteristics

Specificity

A study on sera and EDTA plasma of 300 healthy donors showed 100% specificity (95% confidence 98.8-100%) for both sample types.

Sensitivity

Two clinical studies on a total of 100 samples positive for anti-treponemal antibodies showed 100% sensitivity (95% confidence 96.6-100%).

Precision and Accuracy

Between-run accuracy (QC-1280 control sample, n = 10): %CV = 8.1%, bias = 2.5%.

Analytical Sensitivity

The kit is capable of detecting 0.05 IU/ml of anti-treponemal antibody by testing dilutions of the WHO 1st International Standard for human syphilitic plasma IgG and IgM (NIBSC code 05/132). No high-dose hook (prozone) effect has been detected with specimens with concentrations of treponemal antibodies in a clinically relevant range.

Safety Notes

All patient specimens, calibrators and controls should be handled as potentially infectious. All materials of animal origin avoid many risks associated with the use of human serum (e.g. Hepatitis B and C, HIV). Nevertheless, all material of animal origin should still be treated as potentially infectious material.

P234 Keep only in original packaging.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Rathlev T., Haemagglutination tests utilizing antigens from pathogenic and apathogenic *Treponema pallidum*, WHO Document WHO/VDT/RES**77**, 65 (1965)
2. Tomizawa T., Kasamatsu S., Haemagglutination tests for diagnosis of syphilis. A preliminary report, Japan. J. Med. Sci. Biol. **19**, 305-308 (1966)
3. Rathlev T., Haemagglutination test utilizing pathogenic *Treponema pallidum* for the serodiagnosis of syphilis, Br J Vener Dis **45**, 181-5 (1967)
4. Tomizawa T. *et al*., Usefulness of the haemagglutination test using *Treponema pallidum* antigen (TPHA) for the serodiagnosis of syphilis, Jap J Med Sci Biol **22**, 341-50 (1969)
5. Sequeira P. J. L., Eldridge A. E., Treponemal Haemagglutination test, Br J.Vener Dis **49**, 242-8 (1973)
6. Larsen S.A. *et al*., Specificity sensitivity and reproducibility among the fluorescent treponemal antibody absorption test, the microheamagglutination assay for *Treponema pallidum* antibodies, and the heamagglutination treponemal test for syphilis, J. Clin. Microbiol. **14**, 441-445 (1981)
7. Houng H., Syphilis: New Diagnostic Directions, Intern. J. STD and AIDS **3**, 391-413 (1992)
8. van der Sluis J. J., Laboratory Techniques in the Diagnosis of Syphilis: a Review, Genitourin Med. **68**, 413-9 (1992)

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