TPHA Test Kit

IVD *For In-Vitro and professional use only*
Store at 2- 8°C

INTENDED USE

TPHA test kit is intended to be used for the detection of antibodies of Treponema pallidum (IgG and IgM antibodies) in human serum or plasma based on the principle of passive hemagglutination.

INTRODUCTION

Syphilis is a venereal disease caused by the spirochete micro-organism Treponema pallidum. TPHA test kit has been shown to be a convenient and specific test for detection of treponemal infection. The test is simple to perform and only requires minimum laboratory equipment.

PRINCIPLE OF THE TEST

Indirect hemagglutination (IHA) method are used for detection of antibodies to T.pallidum. Preserved avian erythrocytes are coated with antigenic components of pathogenic T.pallidum (Nichol's strain). Test Cells will agglutinate by the presence of specific antibodies to T.pallidum, and show characteristic patterns in microtitration plates.
Non-specific reactions are detected using the Control Cells, which are avian erythrocytes not coated with T.pallidum antigens. Non-specific reactions may also be absorbed out using these Control Cells.

REAGENT PREPARATION & STABILITY

- The reagents are stable when stored always refrigerated at 2-8º C in an upright position.
- Presence of particles and turbidity will result in reagents deterioration. All the reagents must be allowed to reach room temperature before use.
- **DO NOT FREEZE.** Frozen reagents could change the functionality of the test.
- Do not use haemolyzed, contaminated or lipemic serum or plasma for testing as this will adversely affect the results.
- Under these conditions, kit performance characteristics will be maintained for at least 15 or 18 months from date of manufacture. See expiry date on kit label.
- Discard reagents if contaminate or incorrect activity with the controls are demonstrated.
- The reagents in each kit have been standardized to produce the proper reaction and avoid any interchanges of reagents with those from other batches.

SPECIMEN COLLECTION & HANDLING

- The test is designed for use with serum only.
- Avoid using plasma samples.
- Make sure the samples are free from haemolysis and contamination.
- Store the serum samples at 2-8°C if a preservative is added prior to storage.
- Make sure to store sera at -20°C for long term storage. Avoid any contamination of the reagents or serum dilutions with saliva. This will cause confusing patterns similar to positive results with specimens which should be negative.

MATERIALS

MATERIALS PROVIDED

- Test cells; preserved avian erythrocytes sensitized with T.pallidum antigen
- Control cells; preserved avian erythrocyte
- Diluent
- Positive control serum; (prediluted 1:20), Use neat. This will give an equivalent titer of 1/640:/2560 in the quantitative test
- Negative control serum; (prediluted 1:20), Use neat.

MATERIALS NEEDED BUT NOT PROVIDED

- Accurate pipette for delivering 10:25:75 and 190 microliters.
- U-well microtitration plate

PROCEDURES

A. QUALITATIVE METHOD

Each sample requires 3 wells of a microtitration plate.

1. Add 190µl of diluent to Well 1.
2. Add 10µl serum to Well 1. (Sample dilution 1:20).
3. Mix contents of Well 1 using a micropipette and transfer 25µl to Wells 2 & 3.
4. Ensure that the Test and Control Cells are thoroughly resuspended. Add 75µl of control cells to Well 2. Add 75µl of Test Cells to Well 3.
5. Shake the plate gently to mix the contents thoroughly.
6. Incubate 45-60 minutes at room temperature.
7. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
8. Read results. Results are stable for 24hrs if the plate is cover and the above precautions are observed.

NOTE
Kit controls can be run in parallel and are diluted and ready for use.

B. SEMI-QUANTITATIVE METHOD

Each sample requires 8 Wells of a microtitration plate, Labelled A through to H.

1. Add 25µl of diluent to Wells B to H inclusive.
2. Transfer 25µl of 1:20 serum dilution from screening test to Wells A and B.
3. Take 25µl of diluted serum from Well B and serially dilute from Wells B to H inclusive in 25µl aliquots, discarding 25µl of diluted serum from Well H.
4. Ensure that the Test Cells are thoroughly resuspended. Add 75µl of Test cells to wells A to H inclusive. This will give a dilution of serum of 1/80 in well A through 1/10240 Well H.
5. Shake the plate gently to mix the contents thoroughly.
6. Incubate for 45-60 minutes at room temperature.
7. Caution! Keep the plate away from heat, direct sunlight, and any source of vibration.
8. Read results. Results are stable for 24 hours. If the plate is covered and the above precautions are observed.

READING & INTERPRETATION OF RESULTS

RESULTS	TEST CELLS	CONTROL CELLS
Strong Positive	Full cell pattern covering the bottom of the well.	No agglutination tight button
Weak Positive	Cell pattern covers approx. 1/3 of well bottom	No agglutination tight button
Indeterminate	Cell pattern shows a distinctly open center	No agglutination tight button
Negative	Cells settled to a compact bottom, typically with a small clear center.	No agglutination tight button
Non-specific *	Positive reaction	Positive reaction

Non-specific absorption *

1. Add 10µl to a small tube then add 190µl of Control Cells. Mix well and stand for 30 minutes.
2. Centrifuge for 15 minutes at 1000 rpm and test the supernatant by the qualitative method.

STRONG POSITIVE: Folding at the edge of the cell mat. Control well should be observed when the Test well is positive. Control cells should settle to a compact button. Avoid using control cells as a comparison for Non-Reactive serum patterns since the Control Cells will give a more compact pattern than the Test Cells.
WEAK POSITIVE: May show partially not full cell pattern cover the well bottom.
INVALID: Agglutination in the Control well cause by the presence of non-specific agglutinins in the sample.
A serum that gives this result may be absorbed using the Control Cells. Refer * **Non-specific absorption.**
INDETERMINATE: A doubtful reaction with Test Cells. The result indicates a low level of antibody in early primary syphilis or yaws. Retest the sample in the qualitative test then a further sample should be tested later to determine if there is a rising titer. It is also advisable to perform a regain test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.
Negative results are showed by cells settled as a dot at the bottom of the well.

STABILITY OF THE REACTIONS

Results are stable for 24hrs if the precautions step mentioned in the procedures are taken.

PROCEDURES LIMITATION

1. If the result is repeatedly non-specific the sample should be tested by another method eg. Reagin or FTA-ABS.
2. Although TPHA test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders. FTA-ABS test should be used for confirmation of the results.

QUALITY CONTROL

Positive and Negative Control are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

With clinical samples when compared to FTA-ABS and/or clinical diagnosis was 99.7% (298/299)

SPECIFICITY

With clinical samples was 99.3% (301/303).

CROSS REACTIVITY

Reactive results may indicate an active or successfully treated infection. The following have all been shown not to interfere with the test results (10 clinical samples of each)

- Rheumatoid Factor
- Post Hepatitis B vaccination
- Genital Herpes
- Leptospirosis
- EBV Infection
- SLE
- Lyme's Disease

PRECAUTIONS & WARNNINGS

- The reagents are intended for in vitro diagnostic use only
- All reagents contain 0.1% (w/v) sodium azide as a preservative.
- **The reagents which contain sodium azide which are toxic and can be absorbed through the skin. When drained, flush with a large volume of water to prevent azide build up.**
- Make sure to wear protective clothing when handling the reagents, such as disposable gloves and a laboratory coat.
- When the testing is completed, wash hands and clean the test table top with water and soap.
- For each use, make sure to rinse the slide test thoroughly with water and wiped with lint-free tissue.
- Use provided controls to check reactivity of the reagent. The reagents and controls contain 0.1% sodium azide as a preservative. Avoid ingestion and contact with skin or mucus membrane. Normal laboratory precautions should be maintained while handling test reagents.
- Discard the contents immediately if kit damaged or the glass vials are crack or leaking.
- Discard properly the test materials and samples in a biohazard container.
- Once the test is completed, wash hands and the test tabletop with water and soap.
- Perform the test in a very good visibility area at room temperature in a well let area.
- The procedure should be followed exactly as per the instructions in this package insert. Failure to do so may give false results or safety hazard.
- Close the vial tightly after each test.
- Don't drink or eat beside the reagents as it is considered toxic.
- Clean the spillage of reagent with disinfectant. (Handle disinfectant with care as it could be irritable).

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RSPA020N
Rev 1.0 (02.11.2021)

REF	Catalogue Number		Temperature limit		Manufacturer fax number		Fragile, handle with care
IVD	*In Vitro* diagnostic medical device		Caution		Manufacturer telephone number		Use-by date
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)		Keep away from sunlight		Date of Manufacture
LOT	Batch code		Manufacturer		Do not use if package is damaged		Keep dry