ATLAS TPHA TEST KIT

For the detection of antibodies to T.pallidum in human Serum and Plasma using micro haemagglutination.

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

INTRODUCTION
Syphilis is a venereal disease caused by the spirochaete micro-organism *Treponema pallidum*. As this organism cannot be cultured on artificial media the diagnosis of syphilis depends on the correlation of clinical data with the specific antibody demonstrated by serological tests. Serological screening tests for syphilis using cardiolipin and lecithin as antigens are simple to perform but biological false positive (BFP) reactions occur frequently because the tests use non-treponemal antigens.

The TPI and FTA-ABS tests utilize pathogenic *Treponema pallidum* as the antigen but these tests present some difficulties for routine serodiagnosis. The TPI test requires living pathogenic *T.Pallidum* and the FTA-ABS test requires a flourescence microscope. Both tests require a high level of expertise.

TPHA test kit has been shown to be a convenient and specific test for the diagnosis of treponemal infection, having a specificity similar to that of the TPI test and a sensitivity comparable to that of the FTA-ABS test. It requires minimum laboratory equipment and is very simple to perform.

TPHA reagents are used to detect human serum antibody to *T.pallidum* by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic *T.pallidum* (Nichol's strain). These Test Cells agglutinate in the presence of specific antibodies to *T.pallidum,* and show characteristic patterns in microtitration plates.

Any non-specific reactions occurring 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.

Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes, included in the cell suspension. Test results are obtained in 45-60 minutes and the cell agglutination patterns are both easily read and long lasting

KIT PRESENTATION
TEST KIT 100/200/1000 contain:-

1. Test cells; preserved avian erythrocytes sensitised with T.pallidum antigen.
2. Control cells; preserved avian erythrocyte.
3. Diluent.
4. Positive control serum; (prediluted 1:20). Use neat. This will give an equivalent titer of 1/640:/2560 in the quantitative test.
5. Negative control serum; (prediluted 1:20). Use neat.
6. Package Insert.

Additional Requirements
Accurate pipettes for delivering 10:25:75 and 190 microlitres.
U-Well microtitration plates.

STORAGE
The kit should be stored at 2-8º C in an upright position at all times. 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. Reagents should be discarded if they become contaminated or do not demonstrate correct activity with the controls.
The reagents in each kit have been standardized to produce the proper reaction and reagents should not be interchanged with those from other batches.

SAMPLE PREPARATION
The test is designed for use with serum only. Plasma samples should not be used. The samples should be free from haemolysis and contamination.
Serum samples may be stored at 2-8º C if a preservative is added prior to storage. For long term storage sera should be stored at -20º C.
Strictly avoid contaminating any of the reagents or serum dilutions with saliva. This will cause confusing patterns similar to positive results with specimens which should be negative.

TEST REAGENTS
All the reagents must be allowed to reach room temperature before use. Do not freeze any of the reagents.

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.
3. Using a micropipette, mix contents of Well 1 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. Tap 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 covered and the above precautions are observed.

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

QUANTITATIVE TEST
Each sample requires 8 Wells of a microtitration plate. Labeled 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.
   Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
7. Read results. Results are stable for 24hrs. if the plate is covered and the above precautions are observed.

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 100µl of test serum to a small tube then add 400µl of Control Cells. Mix well and stand for 1 hour.
2. Centrifuge for 15 minutes at 1000 rpm and test the supernatant by the qualitative method.

Note: The sample is now at 1/5, this should be taken into account when preparing the dilutions.
If the result is repeatedly non-specific the sample should be tested by another method eg. Reagin or FTA-ABS.

INTERPRETATION OF RESULTS.
Strong positive reactions may show some folding at the edge of the cell mat.
When the Test well is positive , the Control well should be observed.
The Control cells should settle to a compact button. They should not be used as a comparison for Non-Reactive serum patterns since the Control Cells will give a more compact pattern than the Test Cells.
Agglutination in the Control well indicates the presence of non-specific agglutinins in the sample, the test should be reported as **INVALID**. A serum that gives this result may be absorbed using the Control Cells as detailed under Non-specific absorption.
A doubtful reaction with Test Cells should be reported as **INDETERMINATE.** This result may indicate a low level of antibody in early primary syphilis or yaws. This sample should be first retested in the qualitative test then a further sample should be tested at a later date to determine whether or not there is a rising titer. It is also advisable to perform

a reagin test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.
Syphilis antibodies detected in the Antec TPHA test persist after successful treatment. Therefore a positive test may indicate past or present infection.
In common with other serological tests Antec TPHA cannot distinguish between syphilis and other pathogenic treponemal infections, eg. Yaws.
Clinical evidence should always be considered.
Although the Antec TPHA test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders.
For confirmation the FTA-ABS test should be used, since it allows a differentiation between IgG and the early IgM antibodies. The FTA-ABS test is also very useful in very early syphilis where the haemagglutination test may be negative.
For therapeutic control it is advisable to use a quantitative test such as VDRL or RPR test. These reagents are available from Antec.
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).

SAFETY PRECAUTIONS
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.

REFERENCES BY REQUEST

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