TPHA TEST KIT

For the detection of antibodies to T.pallidum in human serum using micro haemagglutination.

For In-Vitro diagnostic and professional use only

PRODUCT CODE: C12LX1011-100
C12LX1011-200

INTENDED USE
For the detection of antibodies to T. pallidum in human serum based on haemagglutination.

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 florescence 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 specificity similar to that of the TPI test and 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.

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.
- U-Well microtitration plates
- Package Insert.

MATERIALS NEEDED BUT NOT PROVIDED
- Accurate pipettes for delivering 10:25:75 and 190 microlitres

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.

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

REAGENTS 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.

PROCEDURES
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. 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 re-suspended. 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 re-suspended. 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 24hrs if the plate is covered and the above precautions are observed.

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

*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.

**Note:**
If the result is repeatedly non-specific the sample should be tested by another method eg. Reagin or FTA-ABS.

### INTERPRETATION OF RESULTS.

**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 regain test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.

### PERFORMANCE

**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**
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.

### REFERENCES:

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