IMMUTREP® TPHA Ref OD221/OD071/OD081
Treponema pallidum haemagglutination test for the Serodiagnosis of Syphilis.
Store at 2°C to 8°C. DO NOT FREEZE.
For in-vitro diagnostic use only.

INTRODUCTION AND INTENDED USE
Syphilis is a complex disease which is normally sexually transmitted. The causative organism, Treponema pallidum, cannot be grown on conventional laboratory culture media or in the tissue culture. Infection is normally diagnosed by detecting antibodies specific for T. pallidum in the patient’s serum or CSF. Antibody becomes detectable at about 3-4 weeks following exposure, and may remain at detectable levels for long periods after treatment. Two groups of antibodies are formed: one reacting with the non-treponemal antigens used in the VDRL/Carbon Antigen and RPR tests, and the other reacting with the specific antigens of T. pallidum. Antibody to non-treponemal antigens is found normally in active disease and the levels subside after successful treatment. Specific antibody persists long after the infection has been successfully treated. It is necessary to test for both groups of antibodies since the non-treponemal antibody may arise for reasons other than Syphilitic infection.

IMMUTREP TPHA is a specific, sensitive passive haemagglutination test for the detection of antibodies to Treponema pallidum in serum or CSF.
For professional use only.

PRINCIPLE OF THE TEST
IMMUTREP TPHA comprises T. pallidum sensitised formalised tanned fowl erythrocytes; unsensitised formalised tanned fowl erythrocytes, diluent and control sera. When diluted positive samples are mixed with sensitised erythrocytes, antibody to the sensitising antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of a microtitration plate well. In the absence of antibody, they form a compact button in the well.

This test has been calibrated to WHO Reference Serum for Serodiagnostic tests for Treponemal Infections - Ref 3-1980 +/- one double dilution to ensure the correct sensitivity.

PRECAUTIONS
IMMUTREP TPHA reagents contain material of human origin and have been tested and confirmed negative for HCV, HIV I and HIV II antibodies, and HBsAg by approved procedures at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential biohazards in use and for disposal. Do not ingest.

IMMUTREP TPHA reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

IMMUTREP TPHA reagents contain 0.095% sodium azide as a preservative which may be toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

STORAGE
Reagents must be stored upright at temperatures between 2°C to 8°C.

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the bottle and the kit label. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION
Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required. Obtain a sample of CSF from the patient.

Do not use haemolysed, contaminated or lipaemic samples for testing as this will adversely affect the results.

Samples may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at –20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION
All reagents should be brought to room temperature (20°C to 25°C) and fully resuspended prior to use. Do not induce foaming.

The Cell Droppers are provided for use with the Test and Control Cell suspensions. These droppers dispense 75µl drops and these integral droppers should be placed on their corresponding suspensions as follows:
Red Dropper – Test Cells
White Dropper – Control Cells

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<th>Ref OD081</th>
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<tr>
<td>Test Cells</td>
<td>8.5ml 2x8.5ml 2x33ml</td>
<td></td>
</tr>
<tr>
<td>T. Pallidum antigen coated preserved fowl erythrocytes (approx 0.36% w/v) in buffer. Working Strength.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Cells</td>
<td>8.5ml 2x8.5ml 2x33ml</td>
<td></td>
</tr>
<tr>
<td>Preserved fowl erythrocytes (approx 0.36% w/v) in buffer. Working Strength.</td>
<td></td>
<td></td>
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<tr>
<td>Diluent</td>
<td>20ml 2x20ml 3x57ml</td>
<td></td>
</tr>
<tr>
<td>Selected rabbit serum (approximately 0.4%) in buffer. Working Strength.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>1ml 1ml 9ml</td>
<td></td>
</tr>
<tr>
<td>Serum prediluted (1/20) in buffer containing antibodies to T. pallidum. Working Strength.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>1ml 1ml 9ml</td>
<td></td>
</tr>
<tr>
<td>Serum prediluted (1/20) in buffer free of antibodies to T. pallidum. Working Strength.</td>
<td></td>
<td></td>
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</tbody>
</table>

MATERIALS REQUIRED BUT NOT PROVIDED
DyneX M24A U-well microtitration plates are recommended. Microtitration droppers to deliver 25µl or Micropettettes to deliver 10, 25, 75, 100µl and 190µl volumes.
Note: 75µl droppers do not fit and are not supplied for bottles in the 850 Test Kit.
**LIMITATIONS OF USE**

The use of samples other than serum or CSF have not been validated in this test.

**RESULTS AND INTERPRETATION**

Kit controls or known level value samples should be tested with each test run. The kit negative control should give a negative result after 45 minutes. The kit positive control should give a positive result after 45 minutes. If levels of controls or users known samples do not give expected results, test results must be considered invalid.

**Screening Procedure**

Agglutinated cells form an even layer over the bottom of the well. Non-agglutinated cells form a compact button in the centre of the well. Weakly agglutinated cells form a characteristic ring pattern. Agglutination of the Test Cells but not the Control Cells indicates the presence of specific antibody to *T. pallidum*. Absence of agglutination indicates that antibody is below the limit of detection of the system. Do not use the Control Cell pattern as an indication of a negative result since they give a more compact button of cells.

Agglutination of the Control Cells as well as the Test Cells indicates the presence of anti-cell antibody. In this event the test is not valid and should be repeated.

Should the test not be valid the test should be repeated after first performing an absorption of the test serum. To achieve this, dilute the test serum 1/4 with Control Cells and allow to stand at room temperature for 45-60 minutes. After centrifuging the sample (1000rpm/5 mins) dilute the supernatant 1/5 in Diluent. Test this dilution directly, without any further dilution, using Test and Control Cell suspensions. A confirmatory FTA ABS test is also recommended.

**Quantitative Procedure**

As screening procedure. The titre is the highest dilution showing agglutination. The Reactive Control serum should produce a titre within one doubling dilution of 1/2560. The starting dilution for the quantitative procedure is 1/80. Titres of 1/164000 have been detected with IMMUTREP TPHA with no prozone (Hook) effect.

**TROUBLESHOOTING**

Hemagglutination tests are sensitive to the effects of heat, direct sunlight and vibration. Keep away from such sources during test incubation periods.

Do not allow saliva to contaminate the samples or reagents as this will cause erroneous results.

Use a separate disposable tip for each sample to prevent cross contamination.

Replace caps on all reagents immediately after use.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Kit components are matched and should not be interchanged.

**EVALUATION DATA**

Samples were tested at a European reference centre. These samples originated from Antenatal Clinics, Genito – Urinary Medical Clinical and Public Health Laboratories.

<table>
<thead>
<tr>
<th>Positive Samples</th>
<th>Negative Samples</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis Positive</td>
<td>406</td>
<td>6</td>
</tr>
<tr>
<td>Syphilis Negative</td>
<td>3</td>
<td>669</td>
</tr>
<tr>
<td></td>
<td>409</td>
<td>675</td>
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</tbody>
</table>

This study demonstrates:

- A sensitivity of 98.5%.
- A specificity of 99.8%.
- Reproducibility of IMMUTREP TPHA is 100% (+/- one doubling dilution).

**REFERENCES**


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