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 antibody 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.

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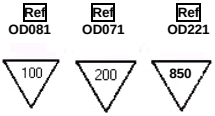

Test	Cells	8.5ml	2x8.5 ml	2x33 ml

T. Pallidum antigen coated preserved fowl erythrocytes (approx 0.36% w/v) in buffer. Working Strength.

Control	Cells	8.5ml	2x8.5ml	2x33 ml

Preserved fowl erythrocytes (approx 0.36% w/v) in buffer. Working Strength.

DIL	20ml	2x20ml	3x57ml

Diluent. Selected rabbit serum (approximately 0.4%) in buffer. Working Strength.

Control	+	1ml	1ml	9ml

Positive Control. Serum prediluted (1/20) in buffer containing antibodies to T. pallidum. Working Strength.

Control	–	1ml	1ml	9ml

Negative Control. Serum prediluted (1/20) in buffer free of antibodies to T. pallidum. Working Strength.

CELL DROPPERS	2	2	0
INSTRUCTION LEAFLET	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED

Dynex M24A U-well microtitration plates are recommended. Microtitration droppers to deliver 25µl or Micropipettes 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.

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

LIMITATIONS OF USE

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

No serological haemagglutination test can discriminate between antibody due to **T.pallidum** infection and antibody due to infection with other pathogenic treponemes, i.e. **T.pertenue** and **T.carateum**.
No other interfering factors have been specifically identified however positive results should be confirmed, eg. by FTA-Abs, and complemented by clinical findings.

There is no reuse protocol for this product.

A low or suspected positive result should be re-assessed. Diagnosis should not be made solely on the findings of one clinical assay. When making an interpretation of the test it is strongly advised to take all clinical data into consideration.

The test may also be negative in early active Syphilis or in late latent Syphilis. To complete the profile of results to aid the physician, it is also recommended that a VDRL/Carbon Antigen or RPR test is performed on the patient's sample since these tests will detect an active case of Syphilis. OMEGA's IMMUTREP VDRL, IMMUTREP CARBON ANTIGEN and IMMUTREP RPR are available for this purpose.

ASSAY PROCEDURE

Allow samples and reagents to reach room temperature and ensure that samples and all reagents are fully resuspended before use. Samples do not require any pretreatment.

QUALITATIVE (SCREENING) PROCEDURE

Each test requires 4 wells of a microtitre plate.
1. Dispense Diluent into the microtitration plate as follows:
- 25µl in rows 1, 3 & 4 and 100µl in row 2.
2. Dispense 25µl of each sample into a well in row 1.
- Mix well and transfer 25µl from row 1 to row 2.
- Mix well and transfer 25µl from row 2 to row 3.
- Mix well and discard 25µl from row 3.
- Transfer 25µl from row 2 to row 4.
- Mix well and discard 25µl from row 4
3. Add 75µl of well mixed Control Cells to row 3.
4. Add 75µl of well mixed Test Cells to row 4.
   Tap plate gently to mix.
   The final dilutions in row 3 and 4 are 1/80.
5. Cover and let stand at room temperature for 45 to 60 minutes (alternatively the plates can be left overnight).
6. Examine for agglutination patterns.

Note: Kit controls are prediluted and should be added directly into individual wells in row 3 and 4 (no diluent required).

ALTERNATIVE ONE WELL DILUTION PROTOCOL FOR SCREENING.

1. Dispense 190µl of diluent into row 1
2. Dispense 10µl of sample to row 1 and mix
3. Discard 150µl from row 1
4. Add 25µl from row 1 to row 2
5. Add 75µl of well mixed Test Cells to row 1
   Add 75µl of well mixed Control Cells to row 2
   Tap plate gently to mix.
   The final dilutions in wells in rows 1 and 2 are 1/80.
6. Cover and let stand at room temperature for 45 to 60 minutes (alternatively the plates can be left overnight).
7. Examine for agglutination patterns.

QUANTITATIVE PROCEDURE

If it is intended to routinely quantitate positive results the screening procedure may be modified by omitting the Control Cells and preparing only one final dilution. Most samples will be negative or genuinely positive, and the Control Cells may be used in the quantitative procedure below.
1. Prepare dilutions in a microtitration plate as follows:
- For each sample, dispense 25µl of diluent into each well in one column of the plate. For titrations of controls dispensing should commence from row 3.
- Transfer 25µl from row 2 of the original screening plate to row 1 of the quantitative plate.
- Mix and discard 25µl.
- Transfer 25µl from row 2 of the original screening plate to row 2 of the quantitative plate.
- Prepare 25µl doubling dilutions from row 2 to row 8 (for controls doubling dilutions should commence from row 3).
2. Add 75µl of well mixed Control Cells to row 1.
3. Add 75µl of well mixed Test Cells to rows 2 to 8.
4. Mix by gentle tapping.
   The final dilutions in row 1 and row 2 are 1/80.
5. Cover and let stand for 45 to 60 minutes at room temperature (or overnight).

   Note: Kit controls are prediluted and 25µl should be added directly into individual wells in rows 1, 2 and 3 with doubling dilutions commencing from row 3 (no diluent is required in row 1 or row 2).

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.

	Positive Samples	Negative Samples	Total
Syphilis Positive	203	3	206
Syphilis Negative	3	669	672
	206	672	878

This study demonstrates:
A sensitivity of 98.5%
A specificity of 99.6%
Reproducibility of **IMMUTREP TPHA** is 100% (+/- one doubling dilution).

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