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MAST-TPHA

Intended use
HA101. A liquid stable *Treponema pallidum* haemagglutination assay for the serodiagnosis of syphilis.

FOR IN VITRO DIAGNOSTIC USE ONLY

Contents
MAST-TPHA- contains the following components for HA101-200 tests:
1. Sample Diluent, ready to use. 2 x 20ml. Contains phosphate buffer pH 7.2 and 'normal' rabbit serum.
2. Test Cells, ready to use. 2 x 8.5ml each of a suspension of tanned chicken erythrocytes, sensitised with *T. pallidum* (Nichol's strain) antigen in a phosphate buffered base with cell components of *T. pallidum* (Reiter's strain)
3. Control Cells, ready to use. 2 x 8.5ml each of a suspension of unsensitised tanned chicken erythrocytes in a phosphate buffered base with cell components of *T. pallidum* (Reiter's strain).
4. Positive Control, ready to use. 1 x 2ml of diluted human serum with *T. pallidum* specific antibodies. Supplied prediluted 1:20 and has a titre of 1:2560 ± one doubling dilution.
5. Negative Control, ready to use. 1 x 2ml of diluted human serum negative to *T. pallidum*.
6. Instruction leaflet.

Items 1 to 5 above all contain 0.095% sodium azide as preservative.

Stability and storage
Store unopened at 2 to 8°C until the expiry date shown on the pack label. Once opened, MAST-TPHA- should be stored at 2 to 8°C and may be used until the expiry date given on the label. **Do not freeze reagents.**
Samples may be stored at 2 to 8°C for up to 48 hours prior to testing. If longer storage is required, store at minus 20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Warnings and precautions
MAST-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. Do not ingest.
MAST-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. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilise all biohazard waste before disposal.
Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.
Haemagglutination 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.
Do not use damaged or contaminated kit components.
Kit components are matched and should not be interchanged between batches.

Materials required but not provided
Microdiluters or micropipettes capable of delivering 25, 75 and 100µl.
Rigid U well microtitre plates e.g. Dynatech M24A.

Specimen collection and preparation
The test is designed for use with serum and CSF specimens only. Plasma should not be used.
Obtain a sample of venous blood from the patient and allow clot to form and retract. Centrifuge the clotted blood sample and collect the clear serum.
Do not use haemolysed, contaminated or lipaemic sera for testing.
Serum specimens should be fully resuspended before use. Specimens do not require any pre-treatment.
Do not repeatedly freeze-thaw the specimens, as this will cause false results.

Procedure
A. Reagent preparation
1. Allow the MAST-TPHA reagents to equilibrate to room temperature before use.
2. Fully resuspend the Test and Control Cells prior to use. Do not induce foaming.

B. Qualitative (screening) procedure
Each test requires 4 wells of a microtitre plate.
1. Dispense Sample 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.
5. Tap plate gently to mix.
   The final sample dilution in rows 3 and 4 is 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.

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

C. Alternative one well dilution screening procedure
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.
6. Add 75µl of well mixed Control Cells to row 2.
7. Tap plate gently to mix.
8. The final sample dilution in rows 1 and 2 is 1/80.
9. Cover and let stand at room temperature for 45 to 60 minutes (alternatively the plates can be left overnight).
10. Examine for agglutination patterns.

D. 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 sample dilution in rows 1 and row 2 is 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).

Interpretation of results
Screening Procedure
Agglutinated cells form a smooth, 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 or a mat of cells surrounded by a red circle.
Agglutination of the Test Cells but not the Control Cells indicates the presence of specific antibody to *T. pallidum*. Absence of agglutination with the Test Cells indicates that specific antibody to *T. pallidum* is absent or below the limit of detection.
The Control Cell pattern should not be used as an indication of a negative pattern with Test Cells 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.
If the test not valid it 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 to 60 minutes. After centrifuging the sample (1000rpm/5mins) 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
Interpret the results as for the screening procedure. The titre is the highest dilution showing agglutination. The Positive Control serum should give a titre of 1/2560 +/- one doubling dilution. The starting dilution for the quantitative procedure is 1/80. Titres of 1/164,000 have been detected with no prozone (Hook) effect.

Limitations of use
The test has only been validated for use with serum or CSF samples.
Do not reuse this product.
No serological agglutination test can discriminate between antibody due to infection with *T. pallidum* and antibody due to infection with other pathogenic treponemes i.e.
T. pertenue and *T. carateum*. No other interfering factors have been identified however all positive samples should be confirmed e.g. with the FTA-ABS procedure, in conjunction with other clinical findings.
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 give a negative result in cases of early active Syphilis or in late latent Syphilis. To complete the profile of results, it is recommended that a RPR test or VDRL/carbon antigen be performed on the patient's sample, since these tests will detect active Syphilis.

Quality control
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. If levels of controls or users known samples do not give the expected results, test results should be considered invalid.

References
Bibliography available on request.

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