TREPONEMA PALLIDUM HAEMAGGLUTINATION ASSAY – TPHA ASSAY

CAT NO	DESCRIPTION	PACK SIZE
SYPTPH1	TPHA FULL KIT (Positive and Negative Control)	100 T
SYPTPH2	TPHA FULL KIT (Positive and Negative Control)	200 T

Intended Use:

The TPHA test is a sensitive and specific indirect haemagglutination test for the detection of antibodies to *Treponema pallidum* in human serum and plasma. For in-vitro diagnostic use by trained professionals only.

Assay Principle:

Preserved avian erythrocytes are coated with antigenic components of pathogenic *T. pallidum*. These test cells agglutinate in the presence of specific antibodies to *T. pallidum* and show characteristic cross-linkage patterns in the wells of microtitre plates. Any non-specific reactions occurring are detected using the control cells which are avian erythrocytes not coated with *T. pallidum* antigens. Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes, included in the test cell suspension.

Appearance, Preparation and Stability:

Reagents must be stored upright at all times.

Discard any reagent that is contaminated or do not demonstrate correct activity with controls.

Unopened products are stable up to expiry when stored tightly capped at 2 - 8°C. Opened vials are also stable up to expiry when stored without contamination and tightly capped at 2 - 8°C. Do not freeze.

Bring all reagents and controls to room temperature before use. Kit controls must be run with each assay. Ensure that the test and the control cells are thoroughly re-suspended.

Reagent Composition:

Name	Description	100 tests	200 tests
TEST CELLS	**Preserved avian erythrocytes sensitised with T. pallidum antigen.**	**8 ml**	**16 ml**
CONTROL CELLS	**Preserved avian erythrocytes.**	**8 ml**	**16 ml**
DILUENT	**Buffered solution**	**20 ml**	**40 ml**
POSITIVE CONTROL	**Human serum containing antibodies against Treponema pallidum. Prediluted 1/20. Will provide a titre of 1/640 to 1/2560 with the quantitative assay.**	**1x1ml**	**1x1ml**
NEGATIVE CONTROL	**Human serum free from antibodies against TP.**	**1x1ml**	**1x1ml**

Warnings and precautions:

- Kit controls contains material of animal origin.
- All human samples should be handled and disposed of according to the local guidelines.
- Reagents contain Sodium azide (0.1%) which can accumulate in lead and copper pipes to form potentially explosive salts.

Specimen Collection:

Use fresh serum or plasma samples free of cells and microbial contamination. After the clear serum is separated it can be stored at 2 - 8°C for 7 days and at -20°C for longer periods.

Materials required but not provided:

Automatic pipettes (10ul, 25ul, 75ul and 190ul), U well microplates.

Procedure:

Qualitative Assay:

i) Each sample requires 3 microwells.
ii) Add 190 µl of diluent to well 1.
iii) Add 10 µl of sample to well 1 and mix well. (Note that the Kit controls are provided pre-diluted)
iv) Transfer 25 µl from well 1 to wells 2 and 3.
v) Ensure that the test and control cells have been thoroughly re-suspended before use.
vi) Add 75 µl of control cells to well 2.
vii) Add 75 µl of test cells to well 3.
viii) Tap the plate gently to mix the contents thoroughly.
ix) Cover the plate and incubate for 45-60 minutes or overnight in an area which is away from heat, direct sunlight and vibration at 15 - 30°C. Read the agglutination patterns. Patterns are stable if undisturbed.

Semi-Quantitative assay:

i) Each sample requires 9 wells of a microtitre plate.
ii) Add 190 µl of diluent to well 1.
iii) Add 25 µl of diluent to wells 4 through to 9.
iv) Make a 1/20 dilution by adding 10 µl of serum to well 1. Mix well.
v) Transfer 25 µl of the 1/20 dilution to wells 2,3 and 4.
vi) Mix the 1/40 dilution created in well 4 and transfer 25 µl to well 5.
vii) Repeat this step until the serial dilution has been completed discarding 25 µl from the last dilution well.
viii) Ensure that the test cells and control cells have been thoroughly re-suspended.
ix) Add 75 µl of Test cells to wells 3,4,5,6,7,8 and 9.
x) Add 75 µl of Control cells to well 2.
xi) Tap the plate gently to mix the contents.
xii) Cover the plate and incubate for 45-60 minutes or overnight in an area which is away from heat, direct sunlight and vibration at 15 - 30°C.

Non-Specific Absorption step:

Very occasionally the presence of non-specific antibodies can result in agglutination of both the test cells and control cells. In this instance the sample should be re-tested once the following absorption step has been completed:

1. Add 10 µl of sample to 190 µl of re-suspended control cells, mix thoroughly and leave for 30 minutes.
2. Centrifuge to deposit the cells at a minimum of 1500g for 3 minutes.
3. Add 25 µl of supernatant from step 2 to each of 2 wells.
4. Ensure Test and Control cells are re-suspended.
5. Add 75 µl of Test cells to the first well.
6. Add 75 µl of Control cells to the second well.
7. Mix wells thoroughly and incubate at 15 - 30°C on a vibration free surface for 45-60 minutes.
8. Read and interpret patterns as above.

Performance Characteristics:

Specificity: A study on 300 donor serum showed 100% specificity. (95% confidence limits 98.8 – 100%)

A study on 300 donor EDTA plasma showed 100% specificity (95% confidence limits 98.8 – 100%)

Sensitivity: A study of 100 syphilis positive samples showed 100% sensitivity (95% confidence limits 96.6- 100%).

Analytical Sensitivity: The TPHA reagents are prepared to a sensitivity between 0.025 and 0.1 IU/ml against the 1st International Standard for human syphilitic plasma IgG and IgM NIBSC code 05/132.

READING AND INTERPRETATION:

Kit controls must give a positive result for the Positive Control and a negative result for Negative Control. When the Positive Control (in the kit) is titrated the expected end point is 640 – 2560.

POSITIVE **EQUIVOCAL NEGATIVE**

- A sample where the Test cell is non-reactive should be considered as negative for *T. pallidum*.
- A sample where the Test cell is reactive indicates presence of antibodies to *T. pallidum* resulting from a syphilis infection. The sample should be repeated in duplicate. Where 2 or more results are positive the sample should be considered as positive for *T. pallidum*.
- A repeated equivocal sample should be considered positive.
- Where a sample is reactive in both Test and Control cells, if the agglutination is greater in the Test cells then the sample is considered positive and should be repeated as above.
- Where a sample has greater or equal agglutination in the Control cells then the sample should be absorbed using the Non specific Adsorption step.
- The result from this test should not be used as the sole criteria for the diagnosis of Syphilis infection. Results should be confirmed using an alternative test method and confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

References:

1. Rathlev T – Haemagglutination tests utilising antigens from pathogenic and apathogenic *Treponema pallidum* WHO/VDT/RES 1965;77:65.
2. Tomizawa T., Kasamatsu S – Haemagglutination tests for the diagnosis of Syphilis: A preliminary report – Japan. J.Med Sci Biol 19, 305-308, 1966.
3. Wasley G.D & Wong H.H.Y: Syphilis Serology Principles and Practice: Oxford Medical Publications 104-105.

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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