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TPHA

**TPHA**

Micro haemagglutination

IVD  
Store at 2 - 8°C.

Ref. 221920 TPHA kit – 200 tests

## TPHA

*For detection of antibodies to *T.pallidum* in human sera and plasma using micro haemagglutination*

### CLINICAL SIGNIFICANCE

Syphilis is a chronic infection which progresses through distinct stages of infection: primary, secondary, tertiary and quaternary. These stages produce diverse clinical symptoms, typically producing initial chancres then syphilitic rash followed by long periods of dormancy and may eventually lead to cardiovascular problems and neurosyphilis.

Caused by the spirochaete *Treponema pallidum* infection is usually acquired by sexual contact, and the disease may be transmitted by transfusion of infected blood.

Tests for syphilis fall into four categories: direct microscopic examination; treponemal antibody tests; non-treponemal antibody tests and direct antigen tests. Because of the long periods of dormancy and the non-specific nature of non-treponemal tests, methods which detect specific anti-treponemal antibodies in patient samples have become increasingly popular for screening. TPHA is one such test.

### PRINCIPLE OF THE METHOD

The TPHA kit uses preserved avian erythrocytes coated with antigens of *T. pallidum* (Nichol's strain) to bind with specific antibody present in patient sera or plasma. The cells are suspended in diluent containing components to eliminate non-specific reactions. Positive reactions are characterised by agglutination of the cells, negative reactions by the setting of the cells to a button or small ring.

Although intended to be used primarily as a qualitative test it may also be used semi-quantitatively (titrating of antibody levels by doubling dilution).

Test patterns may be interpreted manually or with an auto-analyzer using an agglutination interpretation program.

### REAGENTS

Test cells:	Avian erythrocytes coated with antigens of <i>T.pallidum</i>	2 x 8,5 ml
Control cells:	Avian erythrocytes, not coated	2 x 8,5 ml
Positive control:	Rabbit sera; <u>Pre-diluted</u> Titre: 1/1280.	1 ml
Negative control:	Rabbit sera; <u>Pre-diluted</u>	1 ml
Diluent:	Saline solution containing absorbents	2 x 20 ml

### PRECAUTIONS

For in - vitro diagnostic use only.

All human samples should be handled and disposed of according to local guidelines.

Reagents contain sodium azide (<0.1%). Reagents containing sodium azide may combine with copper and

lead plumbing to form highly explosive metal azides. Dispose of reagent by flushing with large amounts of water to prevent azide buildup.

### STORAGE AND STABILITY

All reagents are stable at 2 - 8° C until shelf life stated on reagent labels.

Store bottles in an upright position. Do not freeze.

### ADDITIONAL EQUIPMENT, NOT INCLUDED

- U well micro-plates,  
- Accurate pipettes for 10, 25, 75 and 190µl.  
The TPHA reagents may be used in combination with automated liquid handling or pattern interpretation equipment. Consult manufacturers for advice.

### SAMPLES

Fresh serum or plasma, free of blood cells and of microbial contamination. Stability: 7 days at 2-8°C. For longer storage, freeze at -20°C or lower. Frozen specimens should be thawed and well mixed before testing.

### PROCEDURE

Bring all reagents to room temperature prior to use. Ensure that the Test Cells and Control Cells are thoroughly resuspended. The kit positive and negative control must be run with each assay, using the semi-quantitative procedure given below for the positive control.

#### Qualitative Assay

##### 1. Sample Dilution ( To 1/20 )

Add 190 µl of TPHA diluent to a well.

Add 10 µl of sample to the same well.

Ensure thorough mixing.

Note: Positive & negative controls provided are already pre-diluted (i.e. diluted 1/20)

##### 2. Assay

Add 25 µl of diluted sample from step 1. to each of 2 wells. Gently mix Test Cells & Control Cells to ensure thorough resuspension!

Add 75 µl of Test Cells to 1<sup>st</sup> well.

Add 75 µl of Control Cells to 2<sup>nd</sup> well.

Ensure thorough mixing.

Note: Final sample dilution after addition of cells is 1/80.

Incubate at room temperature (15-30°C) on a vibration free surface for a minimum of 45 min. (60 minutes may be necessary for optimum results with some plate-readers) Read & interpret the settling pattern. Agglutination patterns are stable for at least 3 hours if undisturbed.

[www.diagnostics.be](http://www.diagnostics.be)

## Semi-Quantitative Assay

### 1. Sample Dilution (to 1/20)

Add 190 µl of TPHA Diluent to a well.

Add 10 µl of sample to the same well.

Ensure thorough mixing.

**Note:** Positive & negative controls provided are already pre-diluted (i.e. diluted 1/20)

### 2. Sample Titration

Leaving the 1<sup>st</sup> well empty add 25 µl of TPHA Diluent to remaining 7 wells in an 8 well sequence.

Add 25 µl from step 1 to the 1<sup>st</sup> well.

Add 25 µl from step 1 to the 2<sup>nd</sup> well and mix, then serially dilute along the well sequence, discard the excess 25 µl from the final well.

### 3. Assay

Gently mix the Test Cells to ensure thorough resuspension!

Add 75 µl of Test Cells to all wells.

Ensure thorough mixing.

#### Note:

- Final sample titration after addition of cells is 1/80 - 1/10240.

- Each sample should be tested for non specific reactions by performing simultaneous a test with Control Cells (25µl sample diluted 1/20+ 75 µl Control Cells).

Incubate at room temperature (15-30°C) on a vibration free surface for a minimum of 45 min. (60 minutes may be necessary for optimum results with some plate-readers)

Read & interpret the settling pattern. Agglutination pattern are stable for at least 3 hours if undisturbed.

The titre is the reciprocal of the highest dilution giving agglutination.

## READING AND INTERPRETATION

### Guidance on titration endpoint:



Positive

Equivocal

Negative

Reactivity less than equivocal is considered negative.

### Result      Interpretation

**Negative**      A sample where the Test Cell well is non-reactive should be considered as negative for *T.pallidum*.

**Positive**      A sample where the Test Cell well is reactive indicates antibodies to *T.pallidum* resulting from a syphilis infection. The sample should be repeated in duplicate. If 1 or more of these duplicates is reactive then the sample should be considered positive.

**Equivocal**      A repeatable 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 procedure below for Absorption of non-specific reactions should be applied.

## Absorption of Non-specific Reactions

1. Add 10 µl of sample to 190 µl of resuspended Control Cells, mix & incubate for 30 min.
2. Centrifuge to compact the cells at 1500g for 3 min.
3. Add 25 µl of supernatant from step 2 to each of 2 wells.
4. Gently mix the Test & Control Cells to ensure thorough resuspension.

Add 75 µl of Test Cells to the 1<sup>st</sup> well.

Add 75 µl of Control Cells to the 2<sup>nd</sup> well.

Ensure thorough mixing & incubate at room temperature for 45-60 min.

Read & interpret the settling pattern

## INTERNAL QUALITY CONTROL

For the results to be valid the negative control must give a negative result and the positive control must give a titre of 640 – 2560.

## PERFORMANCE CHARACTERISTICS

### Specificity

A study on 300 donor serum showed 100% specificity (95% confidence limits 99,7-100%).

A study on 300 donor EDTA plasma showed 100% specificity (95% confidence limits 99,7-100%).

### Sensitivity

A study on 100 syphilis positive samples showed 100% sensitivity (95% confidence limits 99,7-100%).

### Analytical sensitivity

Cypress Diagnostics has a sensitivity of 0,05 IU/ml against the 1<sup>st</sup> IS for human syphilitic plasma IgG and IgM NIBSC code 05/132.

## LIMITATIONS OF USE

No interfering substances have been identified however TPHA can cross react with other treponemal infections such as *T.pertenuis* and *T.carateum* so positive results should be confirmed by another method.

In early primary syphilis, occasionally, specific antibodies may not be detected.

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