

IVD
Store at 2 - 8°C.

Ref. 22192 TPHA kit – 200 tests



CE

TPHA

TPHA

Micro haemagglutination

TPHA

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

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 by eye or plate reader.

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.

REAGENTS

Test cells:	Preserved chicken erythrocytes coated with antigens of <i>T.pallidum</i>	17 ml
Control cells:	Preserved chicken erythrocytes, not coated	17 ml
Positive control:	Human sera; Titre: 1280	0,5 ml.
Negative control:	Human sera; Titre: <80	0,5 ml.
Diluent:	Saline solution containing absorbents	40 ml

All reagents contain less than 0.1% sodium azide.

PRECAUTIONS

For in - vitro diagnostic use only.

All human blood components have been tested negative for HBsAg, HCV antibody and HIV 1 & 2 antibodies by FDA approved methods. However as no known test methods

offer total assurance materials should be treated as if capable of transmitting disease.

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

U well plates, calibrated pipettes for 10, 25, 75 and 190µl.

SAMPLES

Fresh serum or plasma, free of blood cells and of obvious 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.

The kit positive and negative control must be run with each lot of tests, 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 should be treated as samples (ie. 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 1st well.

Add 75 µl of Control Cells to 2nd 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 pattern are stable for at least 3 hours if undisturbed.

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 should be treated as samples (ie. diluted 1/20)

2. Sample Titration

Leaving the 1st 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 1st well.

Add 25 µl from step 1 to the 2nd 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

	<u>Test Cells</u>	<u>Control Cells</u>
Positive		
Strong	Full cell pattern covering the bottom of the well.	Negative button.
Weak	Cell pattern covers approx. 1/3 of the well bottom.	Negative button.
Indeterminate	Cell pattern shows a distinctly open center.	Negative button.
Negative	Cells settled to a compact button , typically with a small clear centre.	Negative button.
Non- specific*	Positive reaction	Positive reaction.

*If Control Cells show positive reaction, the procedure below for Absorption of non-specific reactions should be applied.

Guidance on titration endpoint:



CC 1:80 1:160 1:320 1:640 1:1280 1:2560 1:5120

Any specimen giving less agglutination than that shown at “+/-“ above is Negative.

Any specimen giving greater agglutination than that shown as “+/-“ above should be noted as positive.

*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 1st well.

Add 75 µl of Control Cells to the 2nd well.

Ensure thorough mixing & incubate at room temperature for a minimum of 45 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

Two independent studies on 2900 donor sera have shown 100 % consensus with existing test methods. Initial reactive rate was 0.1% Repeat reactive rate was 0%

An independent study on 200 antenatal sera has shown 100 % specificity.

Sensitivity

In house studies on 110 characterized positive samples gave 100 % positive results. This included 2 samples tested negative by other commercial TPHA tests but confirmed FTA positive and I_gM EIA positive.

An independent study on characterized sera including positive samples from various stages of syphilis and disease conditions other than syphilis have shown excellent performance characteristics.

Langdorp, April 2005