# TPHA Kit

**A passive particle agglutination assay for the qualitative and semi-quantitative detection of IgG and IgM antibodies to *Treponema pallidum***

| KIT NAME | KIT SIZE | CAT. NO |
|---|---|---|
| TPHA Kit | 50T | S01050T |

## INTENDED TO USE

Syphilis is caused by the spirochaete *Treponema pallidum,* and is usually acquired by sexual contact, although the disease may be transmitted by transfusion of infected blood. Intrauterine infection also occurs. The infection is a chronic condition that typically progresses through distinct primary, secondary, tertiary, and quaternary stages of infection. These stages produce diverse clinical symptoms, typically producing initial sores known as chancres, then syphilitic rash followed by long periods of dormancy. Untreated infection may eventually result in cardiovascular problems and neurosyphilis.
The organism cannot be routinely cultured in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

TPHA is for the detection of antibodies to*Treponemapallidum* in human serum and plasma,**for professional use only.**

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## PRICIPLE

TPHA uses preserved avian erythrocytes coated with extracted antigens of T.pallidum (Nichols strain). Specific antibodies present in a sample of plasma or serum bind to these antigens when the sample is incubated with the particles. This causes the particles to agglutinate, then settle to form a characteristic pattern in the test well.

Non-specific reactions are eliminated by the use of absorbents. The assay is intended to be used in combination with the Beckman Coulter PK7200 and PK7300 auto analysers.

## KIT CONTENTS:

| Name | 50 tests |
|---|---|
| Test Cells | 1 Bottle |
| Control Cells | 1Vial |
| Sample Diluent | 1Vial |
| Positive Control | 1 vial |
| Negative Control | 1 vial |

## WARNINGS AND PRECAUTIONS

**For *in-vitro* diagnostic use only.**
Kit controls contain material of human or animal origin.
All human samples should be handled and disposed of according to local guidelines.
Reagents contain sodium azide (< 0.1% w/v) which can accumulate in lead or copper pipes to form potentially explosive salts.

## STORAGE

Store bottles upright at 2–8°C.Do not freeze
Do not use after the expiry date.

## LIMITATIONS OF USE

TPHA may be used for serum and plasma samples. No interfering substances have been identified however TPHA can cross react with other treponemal infections such as *T.pertenue* and *T.carateum* so positive results should be confirmed by anothermethod.
In early primary syphilis, occasionally, specific antibodies may not be detected.

GENUINE BIOSYSTEM

## SAMPLES

Use fresh serum or plasma samples free of cells and microbial contamination.
Samples may be stored at 2-8°C for up to 7 days prior to testing. Samples can be frozen at -20°C or lower, these should be thawed and mixed prior to testing.

## ASSAY PROCEDURE

**Equipment required:**
Micro-pipettes capable of delivering: 10, 25, 75 and 190µL
96-well U well micro-plates.
TPHA may be used in combination with automated liquidhandling or pattern interpretation equipment. Consult manufacturers for advice.

**Bring all reagents and samples to room temperature before use.**
**Kit controls must be run with each assay.**
**Ensure Test Cells are thoroughly re-suspended.**

**Qualitative Assay**
Each sample requires 3 wells.

**1. Sample Dilution (to 1 in 20)**
Add 190µL of sample diluent to the first well.
Add 10µL of sample to the same well.
Mix thoroughly.
***Note: Kit controls are pre-diluted (i.e. diluted 1 in 20)***

**2. Test**
Transfer 25µL of diluted sample from step 1 to test well.
Transfer 25µL of diluted sample from step 1 to control well.
Re-suspend the Test and Control Cells thoroughly.
Add 75µL of Test Cells to test well, and 75µL Control Cells to the control well.
**(Final sample dilution is 1 in 80)**
Mix wells thoroughly.
Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes.
Read the agglutination patterns. Patterns are stable if undisturbed.

**Semi Quantitative Assay**
9 wells are needed for each sample.

**1. Sample Dilution (to 1 in 20)**
Add 190µL of sample diluent to a well.
Add 10µL of sample to the same well.
Mix thoroughly.
***Note: Kit controls are pre-diluted (i.e. diluted 1 in 20)***

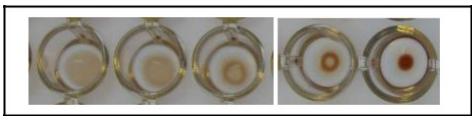
**2. Titration**
Leave the first well empty, add 25µL of diluent all other wells in the sequence.
Transfer 25µL from step 1 to the first well.
Transfer 25µL from step 1 to the second well and mix, then serially dilute along the well sequence, discard the excess 25µL from the final well.

**3. Test**
Re-suspend the Test and Control Cells thoroughly
Add 75µL of Test Cells to each well.
**(Final sample dilution is 1 in 80 – 1 in 10,240)**
Mix wells thoroughly.
Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes.
Read the agglutination patterns.
Patterns are stable if undisturbed.

The titre of the sample is the reciprocal of the final positive sample dilution.

## INTERPRETATION AND ASSAY VALIDATION

The Kit Controls must be give the correct result; Negative is Negative and Positive is Positive. When the Kit Positive is titrated the expected end point is 640 - 2560



**Positive** **Equivocal Negative**

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

Reactivity less than equivocal is considered negative.

A sample where the Test Cell well is reactive indicates antibodies to *T.pallidum* resulting from a syphilis infection. The sample should berepeated in duplicate. Where 2 or more wells are positive the sample should be considered as **positive*for T.pallidum***.

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 sample should be absorbed using the following procedure.

### Absorption of Non-specific Reactions

1. Add 10µL of sample to 190µL 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 25µL of supernatant from step 2 to each of 2 wells.
4. Ensure Test and Control Cells are re-suspended.
5. Add 75µL of Test Cells to the first well.
6. Add 75µL 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 98.78– 100%)

A study on 300 donor EDTA plasma showed 100% specificity. (95% confidence limits 98.78– 100%)

A study on 1819 donor EDTA plasma showed 99.84% specificity (95% confidence limits 99.52 -99.95%)

**Sensitivity**

A study on 100 syphilis positive samples showed 100% sensitivity. (95% confidence limits 96.37– 100%)

**Analytical sensitivity**

TPHA has an expected sensitivity of between 0.1 and 0.025 IU/ml against the 1st IS for human syphilitic plasma IgG and IgM NIBSC code: 05/132

## REFERENCES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