

TPHA TEST KIT

Cat No.	Product Description
TPHA/010	TPHA 100 Tests
TPHA/020	TPHA 200 Tests
TPHA/100	TPHA 1000 Tests

Kits for the qualitative and semi-quantitative detection of antibody to *Treponema pallidum* in human serum or plasma by passive haemagglutination.

CLINICAL BACKGROUND

Syphilis is a chronic infection that progresses through distinct stages of infection: primary, secondary, tertiary, and quaternary. These stages product 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 infection 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 organism has proved virtually impossible to culture in artificial media, and diagnosis of the infection usually depends on the demonstration of antibodies in the blood, which appear soon after initial infection.

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 that detect specific anti-treponemal antibodies in blood specimens have become increasingly popular for screening. **TPHA** is one such test.

INTENDED USE

These kits are intended for use by appropriately trained and qualified personnel for the detection of antibodies to *Treponema pallidum* in human serum and plasma.

PRINCIPLE OF THE TEST

The Lab21 TPHA kits use preserved avian erythrocytes coated with antigens of *T. pallidum* (Nichols strain), which will bind with specific antibody present in patient’s serum or plasma. The cells are suspended in a medium containing components to eliminate non-specific reactions. Positive reactions are shown by agglutination of the cells, negative reactions by the settling of the cells to a button or small ring. Although the kit is intended for use primarily as a qualitative test, antibody levels may be titrated by doubling dilution. Agglutination patterns may be interpreted by eye or by a plate-reader capable of reading agglutination patterns.

KIT CONTENTS				
Name	Reagent	100 test kit TPHA/010	200 test kit TPHA/020	1000 test Kit TPHA/100
R1: Test Cells	Preserved chicken erythrocytes coated with antigens of <i>T. Pallidum</i>	Sufficient for 100 tests	Sufficient for 200 tests	Sufficient for 1000 tests
R2: Control Cells	Preserved chicken erythrocytes, not coated	Sufficient for 100 tests	Sufficient for 200 tests	Sufficient for 1000 tests
R3: Diluent	Saline solution containing absorbents	20ml	2 x 20ml	200ml
R4: Positive Control	Human serum Titre: 1280	1ml	1ml	2ml
R5: Negative Control	Human serum Titre: <80	1ml	1ml	2ml
Instructions for use				

WARNINGS AND PRECUATIONS

For in-vitro diagnostic use only.

All reagents contain sodium azide (<0.1% w/v). Waste fluids arising from use of the kit must be flushed with large quantities of water to avoid accumulation of potentially explosive compounds in laboratory plumbing.

The control materials supplied are derived from human serum. They have been tested at donor level and found negative for Hepatitis B and C, and for HIV 1 and 2. **However, they should be treated as if capable of transmitting disease.**

Specimens of human serum and plasma should be treated as microbiologically hazardous, and handled in accordance with the applicable regulations.

Do not use the kit after its expiry date.

Do not combine or interchange reagents from kits with different lot numbers.

STORAGE

Sotre at 2-8°C when not in use. Store bottles upright. **Do not freeze.**

Shelf life is valid until date stated on kit label.

EQUIPMENT REQUIRED

Properly calibrated and maintained pipettes capable of delivering volumes of 10,25,75 and 190µl.

96 – well microplates (U-well format).

TPHA kits may be automated for both liquid handling and result interpretation. A variety of systems have been used for automation system for advice on automation.

SPECIMENS

Serum or plasma specimens should be free of blood cells and of obvious microbial contamination. They may be stored at 2-8°C for up to 7 days before testing. Specimens needing longer storage should be frozen at -20°C or lower. Frozen specimens should be thawed and well mixed before testing.

ASSAY PROTOCOL (MANUAL)

Bring all reagents and specimens to room temperature prior to use.

Note: The kit positive and negative controls must be run with each of tests.

QUALITATIVE ASSAY

Three wells are needed for each specimen.

NB: The TPHA 1000 Kit (Cat. No. TPHA/1000) is intended for screening large numbers of specimens and contains only a small volume of Control Cells. It is intended that specimens are screened using only Test Cells in the first instance, and the Control Cells be used when repeating tests on specimens giving a positive result when first tested.

- Specimen Dilution (to 1 in 20)**
Add 190µl of the diluent to one well.
Add 10µl of specimen to the same well.
Mix thoroughly.
Note: Positive and negative controls are provided ready to use and do not require dilution.
- Test**
Add 25µl of diluted specimen from step 1 to test well.
Add 25µl of diluted specimen from step 1 to control well.
Resuspend the Test and Control Cell suspensions by shaking the vial. Examine for complete resuspension.
Add 75µl of Test Cells to test well, and 75µl Control Cells to the control well, (Final specimen dilution after addition of cells is 1 in 80)
Mix thoroughly.
Incubate at 15-30°C on a vibration-free surface for a minimum of 45 minutes. (60 minutes may be necessary for optimum results with some plate-readers).
Read the selling patterns. Agglutination patterns are stable for at least three hours if undisturbed.

QUANTITATIVE ASSAY

8 wells are needed for each specimen.

Note: The kit positive and negative controls must be run with each lot of tests.

- Specimen Dilution (to 1 in 20)**
Add 190µl of the diluent to a well.
Add 10µl of specimen to the same well.
Ensure thorough mixing.
Note: Positive and negative controls are provided ready to use and do not require dilution.
- Titration**
Leaving the 1st well empty, add 25µl of diluent to each of the remaining 7 wells in a row of 8 wells.
Add 25µl from step 1 to the 1st well.
Add 25µl from step 1 to the 2nd well and mix, then serially dilute along the well sequence, discarding the excess 25µl from the final well.
- Test**
Gently mix the Test Cells to ensure thorough resuspension.
Add 75µl of Test Cells to each well. (Final specimen dilution range after addition of cells is 1 in 80 – 1 in 10,240)
Mix thoroughly
Incubate at 15-30°C on a vibration-free surface for a minimum of 45 minutes. (60 minutes may be necessary for optimum results with some plate-readers).
Read the settling patterns. Agglutination patterns are stable for at least three hours if undisturbed.
The titre of the specimen is the reciprocal of the highest dilution giving agglutination.

INTERPRETATION AND ASSAY VALIDATION

Internal Quality Control

For the results to be valid the negative control must give a negative result (see pictorial reading guide) and the positive control must give a titre of 640-2560 (See illustration for guidance on titration endpoint).



Any specimen giving less agglutination than that shown as ‘+/-’ above is Negative. Any specimen giving greater agglutination than that shown as ‘+/-’ above should be noted as provisionally **positive**, and the test procedure repeated as above, but in duplicate, adding the Control Cells provided to one set of wells, and Test Cells to the other.

If the agglutination with Test Cells is greater than with Control Cells, the specimen is **positive** for anti-treponemal antibody, and should be subjected to further tests for confirmation.

If the agglutination with Control Cells is greater or equal to that with Test Cells, the procedure below for absorption of non-specific reactions should be applied.

Absorption of Non-specific Reactions

(Procedure to be used if agglutination is seen in both Test and Control Cells).

1. Add 10µl of specimen to 190µl of resuspended Control Cells, mix well and incubate 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. Gently mix the Test and Control Cells to ensure thorough resuspension. Add 75µl of Test Cells to the 1st well. Add 75µl of Control Cells to the 2nd well.
5. Ensure thorough mixing and incubate at RT for a minimum of 45 minutes. Read and interpret the settling patterns as above.

PERFORMANCE CHARACTERISTICS

Specificity

Two independent studies on 2900 **donor sera** each showed 100% consensus with existing test methods. The initial reactive rate was 0.1%, and the repeat reactive rate was 0%.

An independent study on 200 **antenatal sera** showed 100% specificity (95% confidence 98.04-100%).

Sensitivity

In-house studies on 110 known positive specimens gave 100% positive results (95% confidence 98.04-100%). This included 2 specimens negative by other commercially available TPHA tests but positive by FTA and specific IgM tests.

Clinical specimens

467 specimens submitted to a clinical laboratory for testing for suspected syphilis were examined.

Clinical Category	Number	Positive with TPHA Screening 1000	Positive with other TPHA
Syphilis positive*	217	216	214
Syphilis negative	250	0	0

*Includes treated, untreated and neurosyphilis cases

Sensitivity: 99.5% (95% confidence 97.54-100%)

Specificity: 100% (95% confidence 98.04-100%)

Specificity with Potentially Cross-Reactive Specimens

Clinical category	Number Tested	Positive with TPHA Screening 1000	Negative with TPHA Screening 1000
Rheumatoid Factor positive	10	0	10
EBV infection	10	0	10
Post Hepatitis B vaccination	10	0	10
SLE	10	0	10
Genital Herpes	10	0	10
Lyme disease	10	0	10
Leptospirosis	10	1	9

Analytical sensitivity

This kit has been shown to be capable of detecting 0.05 IU/mL of anti-treponemal antibody by testing dilutions of the First International Standard serum (NIBSC, London, UK).

Precision and Accuracy

For N = 10 assays of a positive sample: CV = 8.1% Accuracy = -2.5%.

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