TPHA - 100, 200 & 500 Tests

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

REF IVD +2°C +8°C CE

For professional *in vitro* diagnostic use only

Cat. No.	Product Description
RL-TPHA100	TPHA 100 Test Kit
RL-TPHA200	TPHA 200 Test Kit
RL-TPHA500	TPHA 500 Test Kit

INTRODUCTION AND INTENDED 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.

Rapid Labs-TPHA is for the detection of antibodies to *Treponema pallidum* in human serum and plasma, **for professional use only.**

PRINCIPLE OF THE TEST

Rapid Labs-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 can be run and interpreted manually or with an auto-analyzer using an agglutination interpretation program.

KIT CONTENTS

Name	Description	100 tests	200 tests	500 tests
Test Cells	Avian erythrocytes coated with antigens of *T. pallidum*	7.6 mL	2 x 7.6 mL or 1 x 15.2mL	2 x 20 mL or 1 x 40 mL
Control Cells	Preserved chicken erythrocytes, not coated	7.6 mL	2 x 7.6 mL or 1 x 15.2mL	2 x 20 mL or 1 x 40 mL
Sample Diluent	Saline solution containing absorbents	20 mL	2 x 20mL or 1 x 40mL	2 x 50mL
Positive Control	Rabbit antiserum Titre 1/1280 **Pre-diluted**	1 mL	1 mL	1 mL
Negative Control	Normal Rabbit Serum **Pre-diluted**	1 mL	1 mL	1 mL

WARNINGS AND PRECAUTIONS

For *in-vitro* diagnostic use only.
Kit controls contain material of 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

Rapid Labs TPHA may be used for serum, plasma and CSF 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 another method. In early primary syphilis, occasionally, specific antibodies may not be detected.

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.
Rapid Labs-TPHA may be used in combination with automated liquid handling 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 and Control Cells are thoroughly re-suspended.

Qualitative Assay

Each sample requires 3 wells.
Note: For Rapid Labs-TPHA 500 only run Control Cells on retest.

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 must be run as samples (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
Assay Control

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 be repeated 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.
Add 75µL of Test Cells to the first well.
Add 75µL of Control Cells to the second well.
5. Mix wells thoroughly and Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes
6.Read and interpret patterns as above.

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 99.6 – 100%)

Analytical sensitivity

Rapid Labs-TPHA has a sensitivity of 0.05 IU/ml against the 1st IS for human syphilitic plasma IgG and IgM NIBSC code: 05/132

REFERENCES

1. Rathlev T. - Haemagglutination tests utilizing antigens from pathogenic and apathogenic Treponema pallidum WHO/VDT/RES 1965 ; 77 : 65.
2. Tomizawa T, Kasamatsu S. - Haemagglutination tests for diagnosis of syphilis. A preliminary report. Japan. J. Med. Sci. Biol. 19, 305-308, 1966.
3. Rathlev T. - Haemagglutination test utilizing pathogenic Treponema pallidum for the serodiagnosis of syphilis. Br J Vener Dis 1967 ; 43 : 181-5
4. Tomizawa T. Kasamatsu S. Yamaya S. - Usefulness of the haemagglutination test using Treponema pallidum antigen (TPHA) for the serodiagnosis of syphilis. Jap J Med Sci Biol 1969 ; 22 : 341-50.
5. Sequeira P,J,L. Eldridge A,E. - Treponemal Haemagglutination test. Br J Vener Dis 1973 ; 49 : 242-8.
6. Larsen S.A., Hambie E.A., et coll., Specificity, sensitivity and reproducibility among the fluorescent treponemal antibody absorption test, the microhemagglutination assay for Treponema pallidum antibodies, and the hemagglutination treponemal test for syphilis. J. Clin. Microbiol., 1981 ; 14 : 441 – 445.
7. Wasley G.D. & Wong H.H.Y. Syphilis Serology Priciples and Practice. Oxford Medical Publications 104 - 105

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IVD	*In vitro* diagnostic medical device		Use by date
LOT	Batch code or lot number	REF	Catalogue number
+2°C +8°C	Temperature limit		Consult Instruction for use (IFU)
	Manufacturer		Date of manufacture