TPHA - 100, 200 & 500 Tests

IVD CE +2°C +8°C REF

RAPID BIOTEC™

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

Intended for the qualitative detection of Treponema pallidum IgG and IgM antibodies to syphilis in human serum or EDTA plasma and to determine the titre level of the samples. The intended use population is patients with a suspected syphilis infection or at elevated risk of syphilis infection who attend STI clinics or other healthcare settings. This assay is not intended for automated use. This assay is not intended for blood screening or as a confirmatory assay on donor samples.

PRINCIPLE OF THE TEST

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 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 erythrocytes. This causes the erythrocytes to agglutinate, then settle to form a characteristic pattern in the test well. Non-specific reactions are eliminated by the use of absorbents.

Additional required materials:

Micro-pipettes capable of delivering; 10, 25, 75 & 190µl

REAGENT PREPARATION

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.

STORAGE AND SHELF LIFE AFTER OPENING

Test cells and Control Cells must be stored upright position at 2-8°C. Do not freeze
After opening, Test cells, Control cells, Sample diluent and controls are stable for up to 3 months when stored upright at 2-8°C
Do not use after expiration date.

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	Avian erythrocytes	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	Human antiserum Titre 1/1280	1 mL	1 mL	1 mL
Negative Control	Normal Rabbit Serum	1 mL	1 mL	1 mL

WARNINGS AND PRECAUTIONS

- Rapid Biotec's TPHA is for in vitro diagnostic use only. For professional use only
- Test cells, Control cells, Sample Diluent and Controls contain sodium azide (< 0.1% w/v) as a preservative, which can accumulate in lead or copper pipes to form potentially explosive azides. To prevent azide build-up, flush with large volumes of water after disposing of solutions containing azide into the drains.
- Caution: Controls contain material of human or animal origin. All human origin material in the TPHA has been tested and found negative or nonreactive for HBsAG, HIV 1 Ag [or HIV PCR(NAT)], HIV 1/2 antibody, HCV antibody, and HCV PCR (NAT) as required at the time of collection using FDA licensed test kits. No known test methods can offer total assurance that products derived from human origin will not transmit HIV, hepatitis, or other potentially infectious agents. Therefore, the Controls and all specimens should be handled as potentially infectious.
- Reagents contain material of animal origin. Any bovine albumin used in the manufacture of this product is sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.
- Do not freeze Test cells, Control cells, Sample Diluent and Controls.
- Test cells and Control cells must be thoroughly re-suspended prior to use. Failure to do so could result in an inadequate dilution and erroneous results.
- Test cell and Control cell erythrocytes should be covered by suspension medium during storage, where this has not been the case then erythrocytes should be re-suspended. Failure to do so could result in clumping in the test well.
- Test cells, Control cells and Sample Diluent from the same lot may be pooled using good laboratory practices.
- Reagents showing visible signs of microbial growth or gross turbidity may indicate degradation and should be discarded according to local rules.
- The effects of microbial contamination in specimens cannot be predicted.
- Do not use Test cells, Control cells, Sample Diluent, or Controls after the expiration date.
- Do not interchange caps between the Positive and Negative Control vials. Controls are differentiated by colour coded caps and the vial label. If caps are inadvertently switched, the Control tubes should be discarded.
- Samples exhibiting gross lipemia, haemolysis or icterus may be compromised and may require alternative testing.
- Deviations from the TPHA Instructions for Use can lead to erroneous results.
- Dispose of leftover reagents in a safe manner, in accordance with local regulations.

SAMPLE COLLECTION, HANDLING AND STORAGE

TPHA may be used for testing with either human serum or EDTA plasma specimens for up to 7 days after collection. Specimens should be free of particulate matter to prevent interference with the assay result. If erythrocytes or other visible components are present in the specimen, remove by centrifugation to prevent interference with the test results. Store EDTA plasma and serum specimens at 2-8°C up to 7 days. EDTA plasma and serum specimens can be frozen at less than -20°C for up to one month, thawed and mixed thoroughly prior to testing. Specimens may be frozen and thawed up to 5 times.

Allow all specimens to equilibrate to room temperature before use.

ASSAY PROCEDURE

Each sample requires 3 wells plus 2 additional wells for Positive and Negative Controls.

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

Add 25µL of Positive Control and Negative Control to designated test wells.
Transfer 25µL of diluted sample from step 1 to a test well.
Transfer 25µL of diluted sample from step 1 to a control well.
Re-suspend the Test and Control Cells thoroughly.
Add 75µL of Test Cells to Positive Control and Negative Control wells.
For diluted samples add 75µL of Test Cells to test wells, and 75µL Control Cells to control wells.
(Final sample or Control 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.

Sample titration assay procedure (optional)

9 wells are needed for each sample from 1 in 80 to 1 in 10240 dilution.
2 additional wells for Positive and Negative Controls (if run at 1 in 80 only)
1 additional well is needed if Controls Cells are run

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. Titration

Leave the second and third wells empty, add 25µL of diluent to well 4 to well 10 in the sequence.
Transfer 25µL from step 1 to the second and third wells.
Transfer 25µL from step 1 to the fourth well and mix, then serially dilute along the well sequence, discard the excess 25µL from the final well.
Note: Care must be taken to avoid carryover of sample between serial dilution steps
Kit Positive Control can be titrated if required

3. Test

Re-suspend the Test Cells and Control Cells thoroughly
Add 75µL of Control Cells to well 2
Add 75µL of Test Cells to wells 3 to 10.
(Final sample dilution for Test Cells 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.

CONTROL PROCEDURE

The Positive and Negative Controls must be run with each assay. If required, the Kit Positive can be titrated, and the expected end point is 1/640 – 1/2560. Additional QC testing may be performed by the operator by the inclusion of other characterised specimens or reference material.

The Positive Control should produce a positive result and the Negative Control should produce a negative result with the test. If the appropriate results are not obtained with the controls, the assay is considered invalid and all samples within that assay should be retested.

TPHA Controls are pre-diluted. They should be added directly to the reaction well without being diluted in TPHA Sample Diluent. Test Cells are added directly to the Controls.

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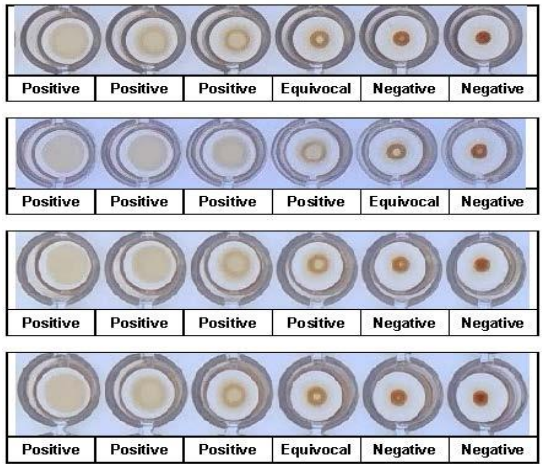
INTERPRETATION OF RESULTS

A sample where the Test Cell well is non-reactive should be considered as **negative for *T.pallidum* antibodies**.
Reactivity less than equivocal is considered negative.
A sample where the Test Cell well is reactive or equivocal indicates antibodies to *T.pallidum* resulting from a syphilis infection. The sample should be repeated in duplicate. Where either repeat duplicate result is reactive or equivocal the sample should be considered as **positive *for T.pallidum* antibodies**. Where both duplicate repeat results are non-reactive then the samples are determined as non-reactive.
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.
When running the sample titration procedure, a titre of ≥ 1/80 is considered reactive and the sample should be repeated in duplicate.
Reactive results may indicate active, past, or successfully treated syphilis infections.
Examples of result interpretation are shown in the figure below.



Test cells	Control cells	Repeat	Absorption	Interpretation
+ (strong)	+ (weak)	Y	N	TP positive
+ (equal to CC)	+ (equal to TC)	Y	Y	TP positive
+ (weak)	+ (strong)	Y	Y	TP positive
+	-	Y	N	TP positive
-	-	N	N	TP negative
-	+	Y	N	TP negative

Absorption of Non-specific Reactions (only to be performed where a sample has greater or equal agglutination in the Control cells than the Test Cells)
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.
During absorption of Non-Specific reactions, the supernatant is added directly to the reaction well without dilution in Sample Diluent. Performing this step incorrectly may result in false negative results.

PERFORMANCE CHARATERISTICS

Limit of detection

TPHA has an expected limit of detection of ≤0.1 IU/mL against the WHO 1st IS for human syphilitic plasma IgG NIBSC code:05/122.

Reproducibility

Assay reproducibility was assessed using a characterised, mixed titre panel comprising 25 syphilis positive and 5 syphilis negative samples. The panel was tested using multiple lots of TPHA on 5 testing days over a 7 day period, in duplicate, with two separate runs on each testing day.
Reproducibility Study – rate of agreement

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
Syphilis positive	250	250	100.00%	98.54 – 100%
Syphilis negative	50	50	100.00%	92.89 – 100%
Overall	300	300	100.00%	98.78 – 100%

Cross reactivity and interference

140 syphilis negative samples containing antibodies to infectious diseases (Rubella, Toxoplasma, Borrelia, EBV, HCV, HBV, HAV, HIV, HTLV, Herpes, Chlamydia), ANA antibodies, Rheumatoid Factor antibodies and samples from pregnant (multiparous) subjects were tested in TPHA. All samples gave the expected negative result.
151 syphilis positive samples containing these antibodies and samples from pregnant (multiparous) subjects were tested in TPHA. All samples gave the expected positive result.

Prozone

Prozone effects may be seen at very high antibody levels for haemagglutination assays. In studies for TPHA, no negative results were obtained at high levels of TP antibodies up to 100 IU/mL.

Diagnostic sensitivity

A panel of 205 commercially sourced, well characterised TP positive samples (157 serum and 48 EDTA plasma) were tested using the TPHA in comparison with PK TPHA 500. The true clinical status for the commercially obtained syphilis positive samples was presumed to be that defined by the vendor assay results.
Initial testing for TPHA v PK TPHA 500

Sample	Agreement measure	Agreement N=	Total N=	ROA	95% CI
Serum	PPA	157	157	100.0%	97.68-100.0%
EDTA plasma	PPA	48	48	100.0%	92.60-100.0%
Combined	PPA	205	205	100.0%	98.22-100.0%

Statistical summary against clinical status

Sample	Agreement measure	Agreement N=	Total N=	ROA	95% CI
All samples	Sensitivity	205	205	100.0%	98.22-100.0%

Diagnostic specificity

A panel of 1248 known TP negative EDTA plasma samples were tested using the TPHA in comparison with PK TPHA 500. Initial reactive samples were retested in duplicate with the relevant method.
Initial testing for TPHA v PK TPHA 500

Sample	Agreement measure	Agreement N=	Total N=	ROA (%)	95% CI (%)
EDTA plasma	NPA	1236	1238	99.84	99.42-99.98

Repeat testing for TPHA v PK TPHA 500

Sample	Agreement measure	Agreement N=	Total N=	ROA	95% CI
EDTA plasma	Specificity	1247	1248	99.92	99.55-100.0

Statistical summary by sample type against clinical status — after repeat testing

Sample	Agreement measure	Agreement N=	Total N=	ROA	95% CI
EDTA plasma	NPA	1245	1246	99.92	99.55-100.0

LIMITATIONS

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

REFERENCES

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Consult instructions for use	REF Catalogue number
+2°C +8°C Store between 2-8ºC	Manufacturer
IVD In-vitro diagnostic use	Date of manufacture
Use by	LOT Batch code or lot number

Manufactured By:
Rapid Labs Ltd
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