NewBio TPHA

REF NB007 NB008 NB009

IVD 2797

1. Intended Use

Intended for the *in vitro* qualitative detection of *Treponema pallidum* IgG and IgM antibodies to syphilis in human serum, EDTA plasma or CSF 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.

2. Principle of assay

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.

NewBio 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.

3. Components

Name	Description	100 tests NB007	200 tests NB008	1000 tests NB009
Test Cells	Avian erythrocytes coated with inactivated antigens of *T. pallidum*	8.5 mL	2 x 8.5 mL	2 x 40 mL
Control Cells	Avian erythrocytes	8.5 mL	2 x 8.5 mL	1 x 40 mL
Sample Diluent	Saline solution containing absorbents	20 mL	2 x 20mL	4 x 50mL
Positive Control	Rabbit antiserum Titre 1/1280	1.5 mL	1.5 mL	1.5 mL
Negative Control	Normal Rabbit Serum	1.5 mL	1.5 mL	1.5 mL
Instructions for use.				

4. Additional required materials

Micro-pipettes capable of delivering: 10, 25, 75 and 190µL
96-well U well micro-plates

5. 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.

6. Storage and shelf life after first opening

1. Test cells and Control cells must be stored in an upright position at 2–8°C. Do not freeze.
2. After opening, Test cells, Control cells, Sample Diluent and Controls are stable for up to 3 months when stored upright at 2–8°C.
3. Do not use after the expiration date.

7. Warnings and precautions

1. NewBio TPHA is for *in vitro* diagnostic use only. For professional laboratory use only.
2. Inspect the kit box and contents before use. Do not use if damaged.
3. Read these instructions for use carefully. Deviations can lead to erroneous results.
4. Do not use kit reagents or controls after the expiration date.
5. Do not freeze kit reagents and controls.
6. Kit reagents 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 when disposing into the drains.
7. Kit reagents and controls 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. Test cells contain antigens of *T.pallidum* that have been inactivated. All components of biological origin should be handled, stored, and treated as potentially infectious.
8. Care should be taken when handling material of human origin. All laboratory personnel must be trained in the correct handling of human samples according to good laboratory practices. All patient samples should be handled, stored and treated as potentially infectious
9. 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.
10. 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.
11. Test cells, Control cells and Sample Diluent from the same lot may be pooled using good laboratory practices.
12. 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.
13. Reagents showing visible signs of microbial growth or gross turbidity may indicate degradation and should be discarded according to local rules.
14. Samples exhibiting gross lipemia, haemolysis or icterus may be compromised and may require alternative testing.
15. The effects of microbial contamination in samples cannot be predicted.
16. Handle, store, and dispose of patient samples and test components in accordance with appropriate national laboratory safety guidelines or regulations.

8. Sample collection, handling, and storage

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

NewBio TPHA may be used for testing with CSF for up to 5 days after collection. Store CSF samples at 2-8°C for up to 5 days. For longer periods store at less than -20°C.
Allow all patient samples to equilibrate to room temperature before use.

9. Assay procedure

9.1 Serum and plasma testing

Each sample requires 3 wells plus 2 additional wells per test run for Positive and Negative Controls.
Note: For NewBio TPHA 1000 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 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.

9.2 Serum and plasma titration assay procedure (optional)

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

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

B. Titration
1. Leave the second and third wells empty, add 25µL of diluent to well 4 to well 10 in the sequence.
2. Transfer 25µL from step 1 to the second and third wells.
3. 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

C. Test
1. Re-suspend the Test Cells and Control Cells thoroughly
2. Add 75µL of Control Cells to well 2
3. Add 75µL of Test Cells to wells 3 to 10.
(Final sample dilution for Test Cells is 1 in 80 – 1 in 10,240)
4. Mix wells thoroughly.
5. Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes.
6. Read the agglutination patterns. Patterns are stable if undisturbed.
7. The titre of the sample is the reciprocal of the final positive sample dilution.

9.3 CSF testing

1 Dilute CSF sample 1 in 5 with TPHA sample diluent.
2 Place 25µL of diluted sample in to 2 'U' wells.
3 Add 75 µL of Test Cells to well 1
4 Add 75 µL of Control Cells to well 2
5 Incubate on a vibration free surface for 1 hour at room temperature.
6 Interpret result according to pack insert.

9.4 CSF Titration

1. Add 25µl CSF to 100 µL TPHA Diluent (1 in 5 dilution)
2. Prepare a row of 9 wells, leaving the first 2 empty, and adding 25 µL TPHA Diluent to each remaining 7 wells.
3. Add 25µL diluted CSF to each of the first 3 wells of the prepared row
4. Mix the contents of well 3 and transfer 25 µL to well 4, mix and repeat for the remainder of the series, and discard 25µL from the last well.
5. Add 75µL TPHA Control cells to the 1st (control) well.
6. Add 75µL TPHA Test cells to the remaining wells. The dilution series in wells 2-9 is now 1 in 20 to 1 in 2560.
7. Mix, incubate and interpret as above

10. 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 samples or reference material.
The Positive Control should produce a reactive result and the Negative Control should produce a non-reactive 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.

11. 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 negative.

All samples with a positive interpretation should be retested with an appropriate confirmatory test to confirm diagnosis.

Early seroconverters may not be detected leading to false negative results.

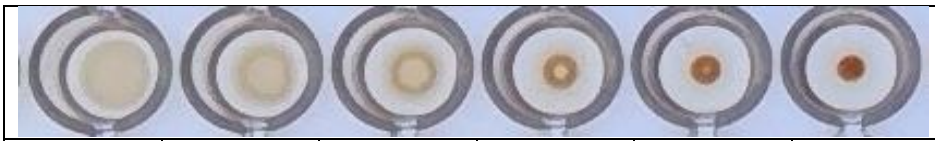
Patient results should be interpreted with careful consideration of the patient's other clinical information.

Where a sample is initial reactive in both Test and Control Cells, if the agglutination is greater in the Test Cells, then the sample should be repeated as above.

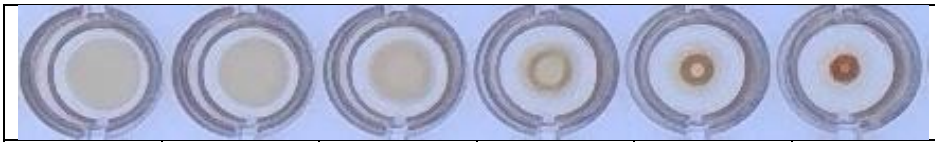
When running the sample titration procedure, a titre of ≥ 1/80 for serum and plasma or ≥ 1/20 for CSF samples is considered reactive and the sample should be repeated in duplicate.

Reactive results may indicate active, past, or successfully treated syphilis infections.

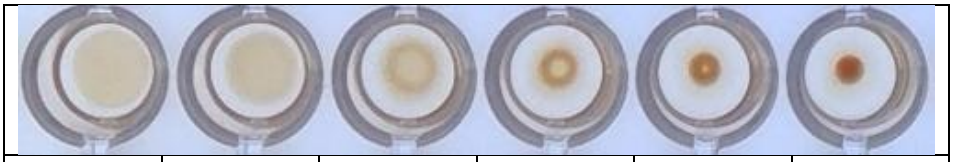
Examples of result interpretation are shown in the figure below.



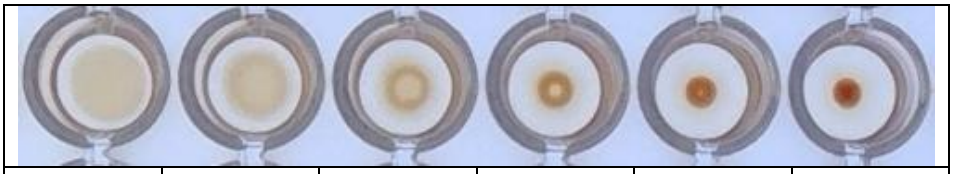
Positive	Positive	Positive	Equivocal	Negative	Negative



Positive	Positive	Positive	Positive	Equivocal	Negative



Positive	Positive	Positive	Positive	Negative	Negative



Positive	Positive	Positive	Equivocal	Negative	Negative

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.

12. Performance characteristics

Limit of detection

NewBio 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 in serum and plasma was assessed using a characterised, mixed titre panel comprising 25 syphilis positive and 5 syphilis negative samples. Testing was completed using 3 different manufactured lots of NewBio TPHA on 5 testing days over a 7-day period, in duplicate, with two separate runs on each testing day at a single site.

Reproducibility Study 1 – 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%
All testing	300	300	100.00%	98.78 – 100%

Assay reproducibility was assessed using a characterised panel of 20 plasma samples to determine variability across the concentration range of the assay. Testing was completed in triplicate on 3 different manufactured lots over 3 days, with 3 different operators at 3 testing sites. Testing sites included Newmarket Biomedical and two additional clinical laboratories in Australia.

Reproducibility Study 2 – rate of agreement

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
TPHA Lot 1	540	540	100.00%	99.32 – 100%
TPHA Lot 2	540	540	100.00%	99.32 – 100%
TPHA Lot 3	540	540	100.00%	99.32 – 100%
Site 1	540	540	100.00%	99.32 – 100%
Site 2	540	540	100.00%	99.32 – 100%
Site 3	540	540	100.00%	99.32 – 100%
Day 1 testing	540	540	100.00%	99.32 – 100%
Day 2 testing	540	540	100.00%	99.32 – 100%
Day 3 testing	540	540	100.00%	99.32 – 100%
All Syphilis positive	1215	1215	100.00%	99.70 – 100%
All Syphilis negative	405	405	100.00%	99.09 – 100%
All testing	1620	1620	100.00%	99.77 – 100%

Repeatability

Assay reproducibility was assessed using a characterised panel of 20 plasma samples to determine variability across the concentration range of the assay. Testing was completed using 1 lot of NewBio TPHA in 3 testing runs on 1 day by a single operator. Each sample was tested 4 times per assay.

Repeatability Study– rate of agreement

Samples	Agreement N=	Total N=	Rate of Agreement	95% CI
Syphilis positive	180	180	100.00%	97.97 – 100%
Syphilis negative	60	60	100.00%	94.04 – 100%
All testing	240	240	100.00%	98.47 – 100%

Cross reactivity and interference

140 syphilis negative serum and plasma 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 NewBio TPHA. All samples gave the expected negative result.

151 syphilis positive serum and plasma samples containing these antibodies and samples from pregnant (multiparous) subjects were tested in NewBio TPHA. All samples gave the expected positive result.

Drug interference

Five common therapeutic drugs were tested for potential interference. Each drug was spiked into 4 anti-treponemal samples and 4 non-reactive samples at levels recommended in CLSI guidelines. Results were compared to unspiked reference samples. Substances tested were selected based on their relevance to the intended use population.

Each drug was found to non-interfering at the claimed concentration.

Substance	Tested concentration (µM)	Tested Concentration (mg/L)
Benzathine benzylpenicillin	200	182
Doxycycline hydrochloride	65	31.2
Ibuprofen	2425	500
Paracetamol	4340	656
Tenofovir disproxil	3.41	1.78

Prozone

Prozone effects may be seen at very high antibody levels for haemagglutination assays. In studies for NewBio TPHA with serum and plasma, 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 NewBio TPHA in comparison with NewBio PK TPHA 2000. 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 NewBio TPHA v PK TPHA 2000

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 NewBio TPHA in comparison with NewBio PK TPHA 2000. Initial reactive samples were retested in duplicate with the relevant method.

Initial testing for NewBio TPHA v PK TPHA 2000

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

Repeat testing for NewBio TPHA v PK TPHA 2000

Sample	Agreement measure	Agreement N=	Total N=	ROA	95% CI
EDTA plasma	NPA	1245	1246	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	Specificity	1247	1248	99.92	99.55-100.0

CSF sample testing
CSF samples collected for routine screening for neurosyphilis in clinical laboratories in Europe were tested using NewBio TPHA in comparison with Serodia TPPA. Although TPPA does not have a published claim for CSF samples, this method has been widely considered as state of the art as an aid to diagnosis of neurosyphilis.

		Serodia TPPA	
		Positive	Negative
NewBio TPHA	Positive	34	0
	Negative	0	46

Agreement measure	Agreement N=	Total N=	ROA (%)	95% CI (%)
Diagnostic sensitivity	34	34	100	89.72 - 100
Diagnostic specificity	46	46	100	92.29 - 100
OPA	80	80	100	95.49 - 100

13. Limitations
NewBio TPHA may be used for serum, EDTA 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.

14. **Australian Sponsor Information:**

Southern Cross Diagnostics Pty Ltd.
Unit 7, 17 Green Street, Banksmeadow, NSW 2019, Australia
Tel: 1-800-146-900 Web: www.scdiagnostics.com.au

15. Key to symbols

REF	Catalogue Number	CE	CE Mark of Conformity
IVD	In vitro diagnostic medical device	Σ	Contains sufficient for <n> tests
	Manufacturer		Temperature Limit
EU REP	EU Authorised Representative		Use by date
	EU Importer	LOT	Batch code
	Distributor		Consult Instructions for Use

16. Post Market Surveillance

Should this IVD be implicated in any serious incident a report shall be made to the manufacturer and competent authority of the Member State in which the user and/or the patient is established.

www.new-bio.com info@new-bio.com

17. Summary of Safety and Performance

SSP can be obtained from the EUDAMED website https://ec.europa.eu/tools/eudamed.

18. Literature references

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