REF NB010/011

Newbio Syphilis EIA TA

An EIA for assay for the qualitative detection of IgG, IgM and IgA antibodies to *Treponema pallidum*

1. INTRODUCTION AND INTENDED USE

Syphilis is a chronic infection caused by the spirochaete *Treponema pallidum.* The disease is naturally acquired by sexual contact, congenital infection also occurs. Infected blood and products may also transmit syphilis.
Syphilis occurs in three distinct phases primary, secondary and tertiary with significant periods of dormancy. Diverse clinical symptoms are observed, typically initial sores known as chancres are followed by a rash, frequently atypical symptoms are present. If left untreated infection may eventually cause cardiovascular problems and neurosyphilis.
As T.pallidum cannot be routinely cultured in artificial media and there are long periods of dormancy diagnosis is routinely carried out by testing for antibodies in blood.

Newbio Syphilis TA is for the detection of antibodies to *Treponema pallidum* in human serum and plasma, **for professional use only.**

2. PRINCIPLE OF THE TEST

Newbio Syphilis TA is a one step sandwich assay using recombinant antigens for enhanced sensitivity and specificity. The assay is for use with plasma and serum samples and will detect antibodies at all stages of infection. Antibodies are captured by recombinant antigens on the plate and marked by HRP recombinant conjugates for visualisation with TMB substrate. The reagents and protocol ensure ease of use and assay control.

3. CONTENTS

Name	Description	96 t NB010	480 t NB011
Positive Control	Human antiserum diluted in stabilisation buffer	1 mL	2 mL
Negative control	Rabbit serum diluted in stabilisation buffer	1 mL	3 mL
Plate	12 x 8 well strips coated recombinant antigen	x 1	x 5
Conjugate	HRP conjugated recombinant antigen	8 mL	30 mL
Substrate	TMB/Peroxidase in stabilisation buffer	7 mL	30 mL
Stop solution	0.5M Sulphuric acid	8 mL	30 mL
Wash buffer	20 x Concentrated	125 mL	250 mL

4. WARNINGS AND PRECAUTIONS

For *in-vitro* diagnostic use only.
Material of human origin has been tested negative by FDA Approved methods for HIV 1&2, HCV antibodies and HBsAg.
All human samples should be handled as if capable of transmitting disease and disposed of according to local guidelines.

5. STORAGE

Store at **2–8°C**.
Substrate is light sensitive.

6. LIMITATIONS OF USE

Newbio Syphilis TA may be used for neat serum and plasma.
Do not use after the stated expiry date.
Opened plate may be used within 1 month if re-sealed with zip-lock.
Diluted wash buffer is stable for 1 month.
Do not use substrate which has turned blue.
Controls containing sodium azide are not valid.

7. SAMPLES

Use fresh serum or plasma samples free of 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. Sample treated at 56°C for 30 minutes may be used.

8. ASSAY PROCEDURE

Equipment Required

Micro-pipettes capable of delivering: 50 and 300µL
Plate reader
Incubator
Newbio Syphilis TA may be used in combination with automated assay equipment. Consult manufacturers for advice.

Bring all reagents and samples to room temperature before use.

Dilute wash buffer in deionised water 1/20 prior to use.

Kit controls must be run with each assay.

The kit positive should be run in duplicate.
The kit negative should be run in triplicate.

Sample and reagent verification

The addition of samples, kit controls and conjugate is verified in **one step**.
The addition of conjugate to sample or kit control will cause the well to turn red, this signifies that both sample and conjugate are present. This can be confirmed by reading the wells at 550nm.
The OD will be $\geq$ 0.4

Substrate verification

The addition of the coloured substrate can be verified visually or by reading the wells at 550nm.
The OD will be $\geq$ 0.08

Protocol

1. Add 50µl neat sample or Kit Control to reaction well.

2. Add 50µl of HRP Conjugate to each reaction well, mix for 20 seconds.

   Verify sample and conjugate addition as described above.

   Cover the plate and incubate at **37°C for 30 minutes**.

3. Wash strips x 5 with diluted wash buffer.

   Use a minimum of 300µl per wash.

   Ensure excess wash is removed.

4. Add 50µl of TMB substrate to each reaction well.

   Cover the plate from light and incubate at **RT for 30 minutes**.

5. Add 50µl of Stop to reaction wells.

6. Read wells at 450nm (reference filter 620–690nm)

   Read within 30 minutes of addition of stop.

Assay Validation

Kit Negative:

The assay is valid if the $OD_{450 - 620}$ value of each control reading is **≤ 0.080**

If one of the values is above 0.080 then the remaining values should be used in the cut off calculation.

Kit Positive:

The value of each control reading should be **≥ 1.500**

Cut-off Value

The cut off is 0.100 plus the mean of the negative values:
0.100 + mean (N1 + N2 + N3)

Example: 0.100 + mean (0.020 + 0.022 + 0.021) = 0.121

9. INTERPRETATION OF RESULTS

Negative

Samples with an OD less than the calculated cut off value are considered negative**.**

Positive

Samples with an OD greater than or equal to the calculated cut off value are considered positive and should be retested in duplicate.

Where both retests are below the cut off then the sample should be considered negative.

Where one of the retests is equal to or above the calculated cut off then it should be considered as a positive result and submitted for further investigation.

10. PERFORMANCE CHARACTERISTICS

Specificity

A study on 300 donor serum showed 100% specificity. (95% confidence limits 98.8 – 100 %)

A study on 300 donor EDTA plasma showed 100 % specificity. (95% confidence limits 98.8 – 100 %)

Clinical Sensitivity

A study on 109 positive samples showed 100 % sensitivity (95% confidence limits 96.7 – 100 %)

Analytical sensitivity

Newbio Syphilis TA has a sensitivity of ≤ 0.0015 IU/ml against the 1st IS for human syphilitic plasma IgG NIBSC code: 05/122.

11. BIBLIOGRAPHY

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12. KEY TO SYMBOLS

REF	Catalogue number
IVD	IVD In Vitro Diagnostic Medical Device
	Manufactured by
	Temperature limitation
	Use by
LOT	Batch code
	Consult instructions for use