TestLine
Clinical
Diagnostics

Instruction for use

EIA Treponema pallidum TOTAL

REF Tp0096

Σ 96

Kit for professional use

IVD CE

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CONTENT

Enzyme immunoassay for the detection of total antibodies to *Treponema pallidum* in human serum or plasma

1 Introduction

Syphilis (lues) is a sexually transmitted disease caused by the spirochete bacterium *Treponema pallidum* subsp. *pallidum*. The primary route of transmission is through sexual contact. However, in approximately 5-10% of cases the infection is transmitted in different way (congenitally from mother to child, rarely by contact with infected blood or skin lesions).

Syphilis proceeds in the following characteristic stages:

Primary syphilis – 2 or 4 weeks after exposure a hard painless ulcer appears at the point of infection. The ulcer disappears spontaneously after 4 to 6 weeks.
Secondary syphilis – the infection spreads in the bloodstream. Skin lesions – exanthema (rash) and highly infectious condylomata lata – are characteristic for this stage of the disease. Lymph nodes are swollen. Later the disease moves to the latent asymptomatic stage. The latent stage can last for years.
Tertiary syphilis – severe skin lesions occur. Various organs (heart and cardiovascular system, eyes, central nervous system and spinal cord) may be damaged.
The disease may also be transmitted from mother who suffers from primary or secondary syphilis to foetus during pregnancy or at birth. This may result in abortion or child infection (congenital syphilis).

EIA Treponema pallidum TOTAL kit is intended for qualitative detection of anti-treponemal antibodies of all classes. The kit is suitable only for determination of antibody presence in a tested sample but not for antibody level monitoring. EIA Treponema pallidum IgM and IgG kits (TestLine, Clinical Diagnostics) are more appropriate for quantitative determination of antibody level.

2 Test Principle

The kit is intended for the detection of anti-treponemal antibodies in a sample by means of a one-step sandwich type of the EIA method (i.e. a solid phase coated with specific antigens – antibody from the analysed sample – conjugate, i.e. specific antigens of *Treponema pallidum* conjugated with horseradish peroxidase). Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific antibodies in the sample.

Antigen used

Selected parts of the specific antigens of *Treponema pallidum*, particularly p17, p47, p41 and p15

3 Materials Provided

MICROPLATE	**Microtitre Plate** coated with antigen, 12 x 8 wells in bag with desiccant	1 pc
CONTROL -	**Negative Control** Solution containing no specific human antibodies, ready to use	1 × 2 ml
CONTROL +	**Positive Control** Solution containing specific human antibodies in cut-off concentration, ready to use	1 × 2 ml
CONJUGATE	**Conjugate** Solution containing specific peroxidase labelled *Treponema pallidum* antigens, ready to use	1 × 8 ml
SUBSTRATE 2	**TMB-Complete 2** Chromogenic substrate solution containing TMB/H_2O_2, ready to use	1 × 15 ml
WASH 20x	**Wash Solution** 20× concentrated buffer	1 × 75 ml
STOP	**Stop Solution** Acid solution, ready to use	1 × 15 ml
	Instructions for use	1 pc

4 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

5 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum and citrate plasma. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

6 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls (positive, negative and CUT-OFF) are ready to use, do not dilute further!

The Conjugate is ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

7 Assay Procedure

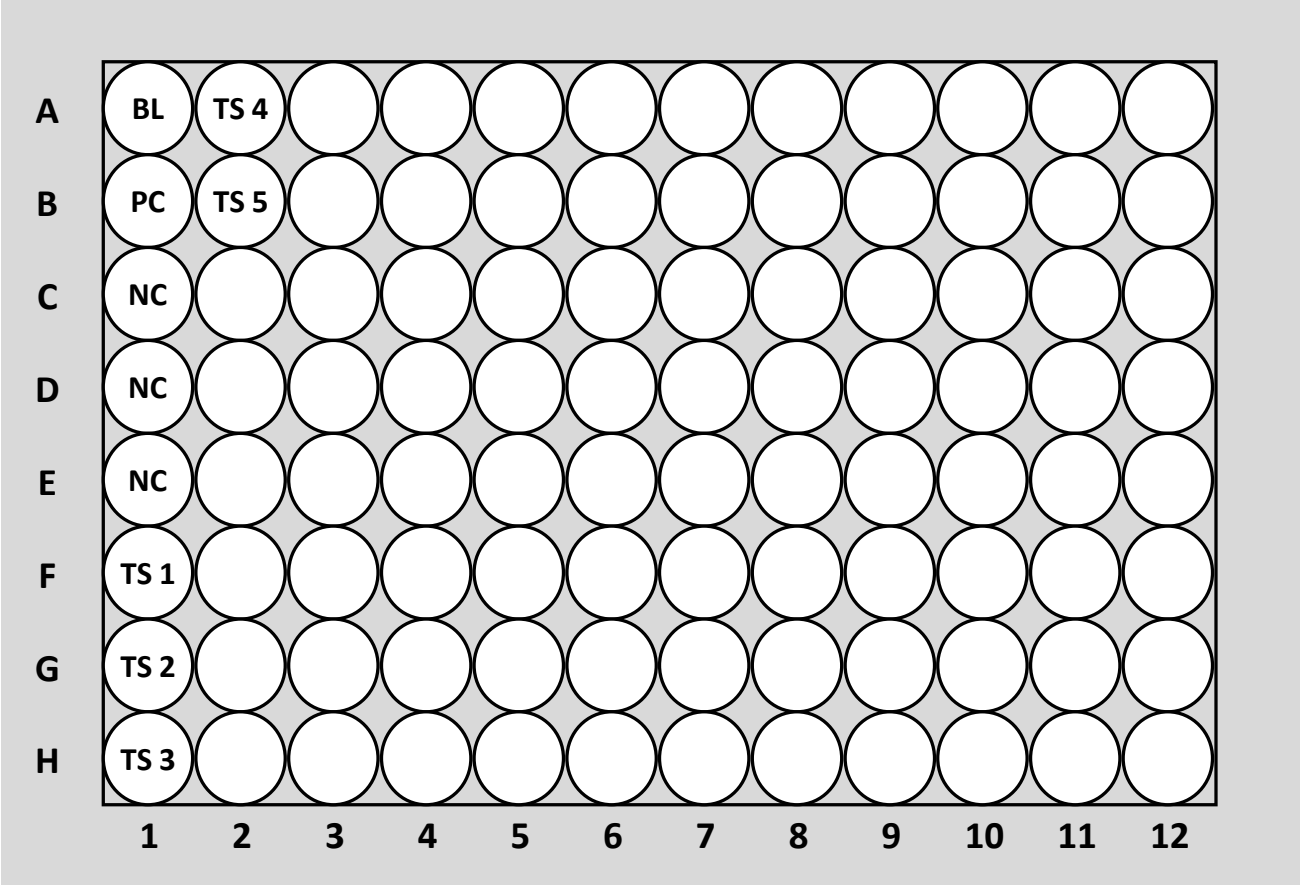
Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls and the undiluted samples according to the working schedule.

- Leave A1 well empty (blank).
- Pipette 50 µl of the Positive Control into 1 well.
- Pipette 50 µl of the Negative Control into 3 wells.
- Pipette 50 µl of the undiluted samples into the other wells.
- Pipette 50 µl of the Conjugate into all wells except A1 well.
- Mix thoroughly for 30 seconds (using a microplate shaker).

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see 13 Procedural Notes.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
6. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
7. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction. Clean the bottoms of the wells prior to measurement.

8 Working Schedule



BL	Blank (empty well)	
PC	50 µl	CONTROL +
NC	50 µl	CONTROL -
TS 1-x	50 µl	Undiluted tested sample

9 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.060.

BLANK < 0.060

The absorbance of the Negative Control is lower than 0.060.

CONTROL - < 0.060

The absorbance of the Positive Control is higher than 0.900.

CONTROL + > 0.900

10 Results Interpretation

Calculation of CUT-OFF

To calculate CUT-OFF, sum up the mean absorbance of the Negative Control and 0.170.

CUT-OFF = Mean absorbance of NC + 0.170

When calculating the mean absorbance of the Negative Control, eliminate a potential invalid value of the Negative Control and calculate the mean of remaining two values.

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the absorbance of CUT-OFF calculated in the same test run:

$$IP = \frac{\text{Absorbance of the sample}}{\text{CUT-OFF}}$$

Interpretation of the test results is described in Table 1.

Table 1 Interpretation of results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

11 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

12 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

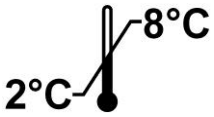
The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

13 IFU Symbols

	Temperature limitation
	Keep dry
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

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Notes

Summary of EIA Treponema pallidum TOTAL Protocol

Step No.	Symbol	Test steps
1		Do not dilute samples
2		Pipette Controls and diluted samples – 50 µl Blank = empty well
3		Pipette Conjugate – 50 µl Blank = empty well, shaker 30 sec
4		Incubate at 37°C for 30 min
5		Aspirate and wash the wells 5×
6		Pipette Substrate (TMB-Complete) – 100 µl Including blank
7		Incubate at 37°C for 30 min
8		Pipette Stop Solution – 100 µl Including blank
9		Read colour intensity at 450 nm