

Syphilis ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Determination of *Treponema pallidum* Antibody
level in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-Syph-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Treponema pallidum, is a spirochete bacterium and causative agent of syphilis which is mainly transmitted through sexual contact, congenital transmission and also through non-intact skin. Several specific and nonspecific serological tests has been introduced for Syphilis disease including VDRL and RPR which are employed as nonspecific and FTA, FTA-ABS, TPHA, TPI and ELISA as specific serological tests. Among specific serological tests for syphilis, the ELISA test has higher sensitivity and specificity due to utilizing specific recombinant antigens from outer membrane proteins of *T.pallidum*.

Test Principle

The test principle is based on antigen sandwich enzyme immunoassay test. In this technique, microplate wells are coated with certain amount of 15 kDa and 17 kDa *T. pallidum* specific recombinant antigens. Serum samples with the labeled antigen (15 and 17 kDa-HRP conjugate) are added into the microplate wells and allowed to react with Ag immobilized on the solid phase. If specific antibodies (IgG, IgM and IgA) against *T. pallidum* were presented in sera they will bind to the solid phase antigens (by one of their own Fab) and to the labeled antigens (by their other Fab component). After washing the microplate wells and removing unbound antibodies and labeled antigens, a solution of chromogen-substrate is added and incubated for 15 minutes, resulting in the development of a blue color.

The color intensity is directly proportional to anti *T. pallidum* antibody concentration in specimens. The color development is stopped by the addition of stop solution and measured spectrophotometrically at 450 nm.

Materials provided with the kit

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with *Treponema pallidum* p15, p17 recombinant antigens.
2. Enzyme Conjugate (3 ml / vial): *Treponema pallidum* p15, p17 recombinant antigens labeled with HRP, ready to use .
3. Negative control (1 ml / vial): Contains inactivated human serum without antibody against *Treponema pallidum* and contains 0.05% Kathon CG as preservative. Ready to use.
4. Positive control (1 ml / vial): Contains inactivated human serum containing anti *Treponema pallidum* in buffer containing protein as stabilizer and 0.05% Kathon CG as preservative, ready to use.
5. Wash solution (1 vial, 50 ml): Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative (20X).
6. Chromogen-substrate (1 vial, 12 ml): Contains Tetramethyl benzidine and hydrogen peroxide, ready to use.
7. Stop solution (1vial, 12 ml) : 1 molar hydrochloric acid solution, pH<1.
8. Cardboard sealer.

Materials required but not provided

1. ELISA reader with 450 nm filter.
2. Precision micropipettes.
3. Distilled water.
4. Disposable pipette tips.
5. Absorbent paper or paper towel.
6. Graph paper.
7. 37°C incubator.



General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Assay Procedure

1. Secure the desired number of microplate wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 100 µl of control sera (the control sera are ready to use) and

specimen samples in appropriate wells according to following order:

- Use the first well as blank (BL)
 - Use the next two wells for negative control (NC).
 - Use one well for the positive control. (PC)
 - Use the remaining wells for specimens.
3. Add 25 µl of antigens-HRP conjugate to the microplate wells (Except blank) and shake them gently for 15 seconds to mix the reactants.
 4. Cover the microplate wells with cardboard sealer tightly and incubate wells for 60 minutes at 37°C.
 5. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of working wash solution).
 6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
 7. Dispense 100 µl of chromogen/substrate to each well.
 8. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
 9. Add 100 µl of stop solution to the wells to stop reaction.
 10. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).

Validity of the Assay

The assay is to be considered valid if:

1. The OD (450 nm) of the blanking well is lower than 0.1. Higher values indicate chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
2. After subtracting the blank, the mean OD value (450 nm) for the negative control is lower than 0.20. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The OD value (450 nm) of positive control is higher than the 0.6. Lower



values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available). Subtract OD value of blank from all controls and tests OD.
2. To calculate cut off value for the test, use following formulae:

$$\text{Cut-off} = \text{N.C. mean OD (450 nm)} + 0.2$$

3. Those samples with OD values of greater than cut-off value must be considered as positive for specific anti-*Treponema pallidum* antibody.
4. Those specimens with OD values of lower than cut-off value should be considered as negative for specific anti-*Treponema pallidum* antibody.

Performance Characteristics

Sensitivity & Specificity

Total of 314 donor sera were evaluated. Of these, 5 were confirmed positive and 309 were negative by FTA-ABS. The ELISA test results were compared to the method.

		Pishtaz teb Syphilis ELISA kit			
		+	Equivocal	-	Total
FTA-ABS	+	5	-	-	5
	-	2	-	307	309
Total Samples					314

Sensitivity = $5 / 5 = 100\%$
 Specificity = $307 / 309 = 99\%$
 Accuracy = $312 / 314 = 99\%$

Correlation Test

286 patient's sera were tested by this Syphilis ELISA kit and a reference ELISA kit. 5 sera were positive and 277 were negative by both methods (97 % agreement).

		Pishtaz teb Syphilis ELISA kit		
		+	-	Total
Reference ELISA kit	+	5	-	5
	-	3	278	281
	Total	8	278	286

Related Sensitivity = $5 / 5 = 100\%$
 Related Specificity = $278 / 281 = 99\%$
 Related Accuracy = $283 / 286 = 99\%$

2. Reproducibility

It has been calculated on the Negative and Positive controls tested in replicates in different days. CV's between 4-12% have been obtained depend on their OD (450 nm) values.

Table 1: Intra-assay

	No. of Tests Performed	Means OD	SD OD	CV%
Negative control	24	0.055	0.003	5.4
Positive control	24	1.5	0.04	2.7




Table 2: Inter-assay

	No. of Tests Performed	Means OD	SD OD	CV%
Negative control	10	0.06	0.006	10
Positive control	10	1.49	0.06	4.5

*Each test has been run in duplicate






***Treponema pallidum* ELISA
Test Procedure**
Step 1

Reagent	Blank	Control Serum	Sample
			
Control Serum	None	100 µl	None
Sample	None	None	100 µl
Antigens-HRP conjugate	None	25 µl	25 µl

Shake gently for 15 seconds and cover the microplate wells with cardboard sealer tightly and incubate them for 60 minutes at 37°C.





Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Chromogen-substrate solution	100 µl 	100 µl 	100 µl 
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Incubate wells for 15 minutes at room temperature and dark.


Step 3

Stop Solution	100 µl 	100 µl 	100 µl 
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).