

TRUSTwell

Syphilis Ab ELISA Kit

REF

AE0610

IVD

- 96-well ELISA kit for the qualitative detection of IgG, IgM and IgA antibodies to *Treponema Pallidum* in human serum or plasma
- Store at 2°C-8°C upon receipt

INTENDED USE

The **TRUSTwell** Syphilis Ab kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of antibodies (IgG, IgM, IgA) against *Treponema pallidum* (Tp) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with Tp. Any reactive specimen with the **TRUSTwell** Syphilis Ab ELISA Test must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

Tp, a spirochete bacterium, is the causative agent of the venereal disease syphilis. Although syphilis rates are declining in the United States after an epidemic outbreak between 1986 and 1990¹, the incidence of syphilis in Europe has increased since 1992, especially in the countries of the Russian Federation, where peaks of 263 cases per 100,000 have been reported². In 1995, WHO reported 12 million new cases of syphilis³. Currently, the positive rate of syphilis serological tests in HIV infected individuals has been rising recently.

Serological detection of anti-Tp antibody has been long recognized in the diagnosis of syphilis since the natural course of the infection was characterized by periods without clinical manifestations. Both IgM and IgG antibodies were detected in sera from patients with primary and secondary syphilis. The IgM antibody may be detectable towards the second week of infection, while IgG antibody appears later, at about 4 weeks⁴. These antibodies could last for several years or even decades in the serum of a patient with untreated latent syphilis⁵.

Antigens such as Rapid Plasma Cardioliolipin antigen (RPR) and Tp bacterial extracts have been used in the syphilis serological tests for decades. However, RPR antigen is a non-treponema antigen, derived from bovine heart. Antibody to RPR antigen does not develop until 1-4 weeks after the appearance of the chancre, thus this antigen lacks of sensitivity to primary syphilis. The Tp extracts are prepared from inoculated rabbit testis and contain a certain amount of contaminated materials such as flagella, which can lead to cross reactions with borreliae and leptospirae in the serological test. In addition, the composition of extracts may vary from lot to lot.

In contrast, the **TRUSTwell** Syphilis Ab Test utilizes Tp specific recombinant antigens⁶⁻⁹, which redeems the test highly specific, sensitive, and reproducible.

TEST PRINCIPLE

The **TRUSTwell** Syphilis Ab ELISA Test is a solid phase enzyme linked immunosorbent assay based on the principle of the double antigen sandwich technique for the detection of antibodies to Tp in human serum or plasma.

The **TRUSTwell** Syphilis Ab ELISA Test is composed of two key components:

- Solid microwells pre-coated with recombinant Tp antigens;
- Liquid conjugates composed of recombinant Tp antigens conjugated with horseradish peroxidase (HRP-Tp conjugates).

During the assay, the test specimen and HRP-Tp conjugates are incubated simultaneously with the coated microwells. Antibodies (IgG, IgM, or IgA) to Tp if present in the specimen, reacts to the Tp antigens coated on the microwell surface as well as the HRP-Tp conjugates, forming sandwich complex conjugates.

Unbounded conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with Stop Solution and absorbances are read using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with Tp antigen	8 wells x 12 strips	AE0610W
2	Syphilis negative control	1 mL	AE0610N
3	Syphilis positive control	1 mL	AE0610P
4	Enzyme Conjugate	6 mL	AE0610H
5	Wash Buffer (30X concentrate)	20 mL	AWE3000
6	TMB substrate A	6 mL	ATME2000A
7	TMB substrate B	6 mL	ATME2000B
8	Stop solution	12 mL	ASE1000
9	ELISA Working Sheet	2 Nos	AE0001ES
10	Product insert	1 No.	PI-AE0610

Materials and reagents required but not provided in the kit

- Pipette capable of delivering 50 µL and 100 µL volumes with a precision better than 1.5%.
- Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable.
- Absorbent paper for blotting the microplate wells.
- Distilled or De-ionized water.
- Parafilm or other adhesive film sealant for sealing plate.
- Timer.

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8 °C.

WARNING AND PRECAUTIONS

For In Vitro Diagnostic Use

- This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
- Do not use expired devices.
- Bring all reagents to room temperature (18°C-28°C) before use.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Do not use hemolyzed blood specimen for testing.
- Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.

- In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
- Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
- The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
- Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
- The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
- Avoid strong light during color development.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

- Bring all reagents, controls to room temperature (18°C-28°C).
- Dilute concentrated Washing Buffer 30 fold with water as following:

Plate	DI water	30 X wash buffer	Final volume
Full plate	290 mL	10 mL	300 mL
Half plate	145 mL	5 mL	150 mL
A quarter plate	72.5 mL	2.5 mL	75 mL

Warm up the concentrated Washing Buffer at 37 °C to dissolve the precipitant if it appears.

- Mix each reagent before adding to the test wells.
- Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

- Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
- Add specimens according to the designation on the ELISA Working Sheet
 - Blank well:** Leave the blank well alone. Don't add any reagents.
 - Control wells:** Add 50 µL of Syphilis Positive, Negative Control into the designated control wells, respectively.
 - Test wells:** Add 50 µL of test specimens into each test well, respectively.

To ensure better precision, use pipette to handle solution.

- Add 50 µL of the HRP-Tp conjugates to each well, except the blank well. Gently rock the wells for twenty second, then cover the wells.
- Incubate the wells at 37 °C for to 60 minutes.
- Carefully remove the incubation mixture by emptying the solution into a waste container. Fill each well with diluted wash buffer (350 µL/well) and shake gently for 20-30 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
- Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the blank well.
- Incubate at 37 °C in dark for 10 minutes.
- Stop the reaction by adding 100 µL of stop buffer to each well. Gently mix for 20-30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
- Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620–690 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1. Secure strips in microwell frame		Number of strips
2. Add controls or specimens		50 µL
3. Add conjugate Gently rock		50 µL 20 seconds
4. Incubate		37°C, 60 minutes
5. Wash: manual or automatic		5 times 350 µL/well
6. Add TMB substrate A and B Gently rock		50 µL + 50 µL 20 seconds
7. Incubate		37°C, 10 minutes
8. Add Stop Solution. Gently rock		100 µL 20 seconds
9. Read result		450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = **0.15 + NC**

NC: Mean OD value of Negative Control

Use NC = 0.05 for calculation of the cut-off value, if the mean OD of NC is less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the Syphilis Ab positive controls should be ≥ 0.50 .
The mean OD value of the Syphilis Ab negative controls should be ≤ 0.10 .
If above specification are not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

D. Interpretation of the results

Specimen OD ratio

- | | |
|----------|-------------|
| Negative | < 1.00 |
| Positive | ≥ 1.00 |
- The negative result indicates that there is no detectable anti-Tp in the specimen.
 - Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
 - Specimens with cut-off ≥ 1.00 are initially considered to be positive by the **TRUSTwell** Syphilis Ab ELISA Kit. They should be retested in duplicate before final interpretation.
If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the **TRUSTwell** Syphilis Ab ELISA Kit.
Non repeatable reactions are often caused by:
 - Inadequate microwell washing.
 - Contamination of negative specimens by serum or plasma with a high antibody titer.
 - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
 - Contamination of the stopping solution
If after re-testing the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the **TRUSTwell** Syphilis Ab ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 1132 patient specimens from susceptible subjects were tested by the **TRUSTwell** Syphilis Ab ELISA Kit. Comparison for all subjects is showed in the following table:

Ref. Syphilis Ab EIA	TRUSTwell Syphilis Ab ELISA Kit		
	Positive	Negative	Total
Positive	95	0	95
Negative	2	1035	1037
Total	97	1035	1132

Relative Sensitivity:100%,Relative Specificity:99.8%,Overall Agreement:99.82%

Precision

Intra-assay precision was determined by assaying 20 replicates of three negatives, three weak positives and three strong positives

Specimens	Number of specimens	Number of replicates	CV
Negatives	3	20	3.5 – 15 %
High positives	3	20	1.8 -5.2 %
Low positives	3	20	4.6 -9.7 %

Cross reactivity

No false positive Syphilis Ab ELISA test results were observed on 10 positives specimens from each of the following disease states or special conditions, respectively:

HIV HCV HBsAg Dengue Malaria Typhoid

Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the **TRUSTwell** Syphilis Ab ELISA Kit. Interference was studied by spiking these substances into 3 Syphilis Ab clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the **TRUSTwell** Syphilis Ab ELISA Kit.

List of potentially interfering substances and concentrations tested:

- Salicylic acid 4.34 mmol/L
- Sodium citrate 1.3 %
- Creatinine 442 µmol/L
- EDTA 3.4 µmol/L
- Glucose 55 mmol/L
- Heparin 3,000 U/L
- Bilirubin 10 mg/dL

LIMITATION OF THE TEST

- The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of anti-Tp antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
- The **TRUSTwell** Syphilis Ab Test is limited to the qualitative detection of anti-Tp antibodies in human serum or plasma. The intensity of the color does not have linear correlation with the antibody titer in the specimen.
- A negative result for an individual subject indicates absence of detectable anti-Tp antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with Tp.
- A negative result can occur if the quantity of anti-Tp antibodies present in the specimen is below the detection limit of the assay, or the antibody that are detected are not present during the stage of disease in which a specimen is collected.
- Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

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