Syphilis Ab ELISA KIT
(SERUM / PLASMA)

REF E0610

2°C 8°C IVD

INTENDED USE

The *Aria* Syphilis Ab ELISA Kit is a solid phase enzyme linked immunoabsorbent assay for the qualitative detection of antibodies (IgG, IgM, IgA) against *Treponema pallidum* 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 *Aria* Syphilis Ab ELISA Kit 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 1990[1], 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 reported [2]. In 1995, WHO reported 12 million new cases of syphilis[3]. 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 weeks[4]. These antibodies could last for several years or even decades in the serum of a patient with untreated latent syphilis [5].

Antigens such as Rapid Plasma Cardiolipin 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 leptospires in the serological test. In addition, the composition of extracts may vary from lot to lot.

In contrast, the *Aria* Syphilis Ab ELISA Kit utilizes *Tp* specific recombinant antigens[6-9], which redeems the test highly specific, sensitive, and reproducible.

TEST PRINCIPLE

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

The *Aria* Syphilis Ab ELISA Kit is composed of two key components:

1) Solid microwells pre-coated with recombinant *Tp* antigens;

2) Liquid conjugates composed of recombinant *Tp* antigens conjugated with horse reddish 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 complex 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.

REAGENTS AND MATERIALS PROVIDED

Item	Description	Quantity	Catalog
1.	Microwells coated with Tp antigens	8 wells x12 strips	E0610W
2.	Syphilis Ab negative control	1 mL	E0610N
3.	Syphilis Ab positive control	1 mL	E0610P
4.	HRP–Tp conjugates	6 mL	E0610H
5.	Wash buffer (30 x concentrate)	30 mL	WE3000
6.	TMB substrate A	6 mL	TME2000A
7.	TMB substrate B	6 mL	TME2000B
8.	Stop solution	1 mL	SE1000
9.	ELISA Working Sheet	2 sheets	E0001ES
10.	Product insert	1 set	PI-E0610

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette capable of delivering 50 µL and 100 µL volumes with a precision better than 1.5%.
2. 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.
3. Absorbent paper for blotting the microplate wells.
4. Distilled or de-ionized water
5. Timer.
6. Parafilm or other adhesive film sealant for sealing plate.

STORAGE AND STABILITY

- Test components are stable up to their expiration data when stored at 2°C-8°C. Do not freeze.
- Return all reagents requiring refrigeration immediately after use.
- Reseal the microwells immediately after removing the desired number of wells.
- Do not mix or use components from the kits with different lot numbers. Do not use reagents after their expiration date.

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. 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.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. In the beginning of each incubation and after adding Stopping Solution, gently rock 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.
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Therefore do not allow any metal element to come into contact with the conjugate or substrate solution.
13. 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.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. 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.
16. Avoid strong light during substrate development.

SPECIMEN COLLECTION AND PREPARATION

- Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture techniques.
- This kit is designed for use with serum 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

1. 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	580 mL	20 mL	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

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

3. Mix each reagent before adding to the test wells.

4. 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

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.

2. Add specimens according to the designation on the ELISA Working Sheet

   2.1 Blank well: Leave the blank well alone. Don't add any reagents.

   2.2 Control wells: Add 50 µL of Syphilis Ab Positive, Negative Control into the designated control wells, respectively.

   2.3 Test wells: Add 50 µL of test specimen into each test well, respectively.

3. Add 50 µL of HRP-*Tp* Conjugate solution into each well, but not the blank well.

4. Gently rock the wells for twenty second, then cover the wells.

5. Incubate the wells at 37°C for 60 minutes.

6. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely by inverting and tapping the plate on absorbent paper. Repeat above procedure 4 more times.

7. Drain the wells by firmly tapping the plate on a clean paper towel to remove excess washing solution.

8. Add 50 µL (1 drop) of TMB substrate A and 50 µL (1 drop) of TMB substrate B into each well.

9. Incubate at 37°C in the dark for 10 minutes.

10. Stop the reaction by adding 50 µl (or 1 drop) of stop buffer to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**

11. Set the microplate reader wavelength at 450 nm and measure the absorbance 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.

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.15 + N
N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if the mean OD 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 ≥ 0.80.
The mean OD value of the Syphilis Ab negative controls should be ≤ 0.10.

Check the procedure and repeat assay if above conditions are not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1. The negative result indicates that there is no detectable anti-*Tp* antibodies in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the *Aria* Syphilis Ab ELISA Kit. They should be retested in duplicate before final interpretation.
   If after retesting 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 *Aria* 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 retesting 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 *Aria* Syphilis Ab ELISA Kit, subject to the limitation of the procedure, described below.

LIMITATION OF THE TEST

1. 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.
2. The *Aria* Syphilis Ab ELISA Kit 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.
3. A negative result for an individual subject indicates absence of detectable anti-*Tp* antibodies. However, a negative test result does not preclude possibility of exposure to or infection with *Tp*.
4. A negative result can occur if the quantity of 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.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
2°C 8°C	Store between 2-8°C		Do not reuse	EC REP	Authorized Representative
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
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EC REP **MDSS GmbH**
Schiffgraben 41, 30175 Hannover Germany

PI-E0610-ARIA Rev D
Effective date: 2015-03-03
English version