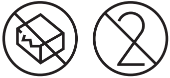
abia Treponema Ab



REF DK.009.01.3
REF DK.009.05.3

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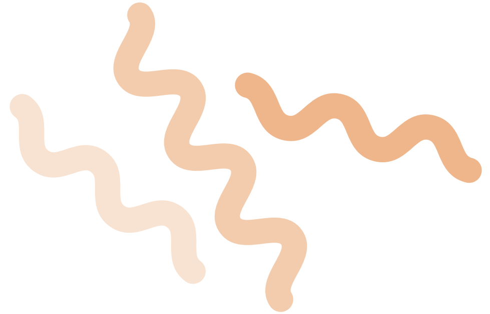

abia

Intended use

abia Treponema Ab is an enzyme immunoassay for the qualitative detection of antibodies to Treponema pallidum in human serum or plasma.

The assay is intended for screening for potentially infectious samples to prevent their use as donor materials, for aid in the diagnosis of patients suspected of having syphilis and for the screening of pregnant woman. For professional use only.

Clinical value

Syphilis is a bacterial sexually transmitted infection (STI) caused by Treponema pallidum that results in substantial morbidity and mortality. Syphilis is transmitted through sexual contact with infectious lesions of the mucous membranes or abraded skin, via blood transfusion, or transplacentally from a pregnant woman to her fetus (congenital syphilis).

The detection of antibodies against T. pallidum is major importance for the diagnosis of syphilis because T. pallidum cannot be isolated in culture and specimens for direct detection are often not available from patients with latent or late stages of the disease.

There are two types of serological tests for syphilis: non-treponemal (RPR, VDRL) and treponemal (TPHA, FTA-ABS, EIA). A presumptive diagnosis of syphilis requires a positive result from at least one of these types of tests. A confirmed diagnosis requires positive results from both types of serologic tests.

Principle of the test

abia Treponema Ab is a two-step noncompetitive assay based on microwells coated with recombinant antigens of Treponema pallidum (rAg). The conjugate is a mixture of HRP-labeled monoclonal anti-human-IgG and anti-human-IgM antibodies (mAb).

Serum or plasma samples are added to the wells and if anti-T. pallidum antibodies are present in a sample, they form stable complexes with antigens immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of HRP labeled anti-human-IgG and anti-human-IgM conjugate.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of anti-T. pallidum antibodies in the specimen and can be read at 450 nm or 450/620-680 nm.

Kit contents

	S	XL	
T. pallidum Ag coated plate	1	5	polystyrene plate 12 × breakable 8-well strips coated with T. pallidum rAg
Conjugate (concentrated 11-fold)	1 × 1.2 ml	2 × 3.0 ml	mixture of HRP-labeled anti-human-IgG/IgM mAb; transparent or slightly opalescent colorless liquid
Conjugate diluent	1 × 12 ml	3 × 20 ml	casein hydrolysate, sodium chloride, TRIS; transparent or slightly opalescent yellow liquid
Sample diluent	1 × 14 ml	2 × 25 ml	phosphate saline buffer, bovine serum albumin, TRIS; opalescent violet liquid
Positive control (inactivated)	1 × 2.5 ml	1 × 2.5 ml	ready to use; human plasma positive for antibodies to T. pallidum; red liquid
Negative control (inactivated)	1 × 2.5 ml	2 × 2.5 ml	ready to use; negative human plasma; green liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	solution containing TMB; colorless liquid
Substrate buffer	1 × 25 ml	1 × 70 ml	citric acid and potassium hydroxide solution, pH 4.2, containing H_2O_2; colorless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	1 × 90 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	2	10	
Plastic dish	2	–	
Plastic zip-lock bag	1	5	

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within 1 month. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- microplate incubator or shaker thermostatically set at 37.0 ± 1.0 °C
- automatic microplate washer
- microplate reader equipped with 450 nm or with 450/620–680 nm filters

Safety notes

- human origin material used in the preparation of the negative control and positive control has been tested by CE-marked tests and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke, or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use undiluted heparin/EDTA/citrate plasma or serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum or plasma as soon as possible to avoid any haemolysis
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- pooled specimens must not be used since the accuracy of test with such specimens has not been validated
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 48 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (18–24 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 400 µl per well is used. When using a microplate washer for which this is not possible, ensure that the well is completely filled with a slight positive meniscus without overflow
- allow a soaking time of at least 40 seconds before aspiration
- perform this procedure 4 times in total
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0
Working solution of conjugate: mix the reagents thoroughly until diluted, avoid foaming **Note:** before use keep the working solution of conjugate at least within 10 min at 18–24 °C **Stability:** 12 hours at 18–24 °C in a dark place												
Conjugate (concentrated 11-fold), ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Conjugate diluent, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
Substrate mixture: mix the reagents thoroughly until dilution **Note:** substrate mixture should be colorless! **Stability:** 10 hours at 18–24 °C in a dark place												
TMB (concentrated 11-fold), ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Substrate buffer, ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

Test procedure

abia Treponema Ab for the qualitative detection of antibodies to T. pallidum in human serum or plasma

Attention! Incubation is possible as two alternative procedures. During test performance follow the same incubation mode. The combination of incubation modes is not supposed.

	Procedure 1 - microplate incubator	Procedure 2 - microplate thermoshaker
1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant bag.	
2	Add 100 µl of positive control in well A1, add 100 µl of negative control in well B1, C1 and D1. Add 90 µl of sample diluent and 10 µl of samples to be tested (final sample dilution 1:10) in rest of the wells. Depending on system and the number of strips used, it is possible to modify the position of controls or the order of distribution. Violet color of solution should be changed into light blue-green. Then cover the plate with protective film.	
3	Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.	Incubate in microplate thermoshaker for 15 minutes at 500 rpm at 37.0 ± 1.0 °C.
4	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 400 µl of working washing solution into each well. Allow a soaking time of at least 40 seconds and aspirate. Perform this procedure 4 times.	
5	Add 100 µl of working solution of conjugate into each well.	
6	Cover the plate with protective film. Incubate in microplate incubator for 30 minutes at 37.0 ± 1.0 °C.	Incubate in microplate thermoshaker for 20 minutes at 500 rpm at 37.0 ± 1.0 °C.
7	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 400 µl of working washing solution into each well. Allow a soaking time of at least 40 seconds and aspirate. Perform this procedure 4 times.	
8	Add 100 µl of substrate mixture to all the wells. Keep the plates in a dark place for 20 minutes at 18–24 °C.	
9	Add 150 µl of stopping reagent into each well.	
10	Read the optical density at 450/620-680 nm using a plate reader. Reading the absorbance at 450 nm only is possible. Test results remain stable for reading within at least 3 minutes.	

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of each negative control should be less than 0.200.
If one negative control does not respect this norm, disregard and recalculate the mean value using the two remaining values. Only one value may be eliminated by this way.
The absorbance (OD) of positive control should be greater than 0.600.

Calculation cut-off value

Mean OD value of the negative control = (OD value B1 + OD value C1 + OD value D1)/3
Cut-off = mean OD value of negative control + 0.350

Interpretation of the results

Non-reactive sample: sample OD value < 0.9 cut-off
Samples with absorbance values less than the cut-off value are considered to be negative by the abia Treponema Ab test.

Reactive sample: sample OD value ≥ 1.1 cut-off
Samples with absorbance values more than or equal the cut-off value are considered to be positive by the abia Treponema Ab test.

If the OD of the tested sample exceeds the 0.9 x cut-off value but less than 1.1 x cut-off this sample is in the "grey zone". In this case it is necessary to test repeatedly the patient's serum for antibodies to T. pallidum in 1-2 weeks after the first blood sampling. It is advisable to test the sera samples simultaneously with the previous ones ("pair samples"), it will allow to assess specific antibody dynamics more accurately.

Performance characteristics

The performance of the abia Treponema Ab has been determined by testing samples from random blood donors, from patients with confirmed syphilis infection and patients in other clinical categories.
Analytical sensitivity limit has been tested using WHO International Standard 1st IS for human syphilitic plasma IgG (NIBSC Code: 05/122).

Diagnostic sensitivity	Number of tested samples	Sensitivity, %
Anti-Treponema palidum antibodies positive samples	401	99.75
incl. patients with primary stage of syphilis	32	96.90
incl. patients with secondary stage of syphilis	93	100.00
incl. patients with early latent stage of syphilis	159	100.00
incl. patients with latent stage of syphilis	24	100.00
incl. patients with known past syphilitic infection	93	100.00
BBI PSS202, mixed titer performance panel	20	100.00
BBI QSS701, qualification panel	6	100.00
Zeptometrix K-ZMC002, mixed titer performance panel	15	100.00

Diagnostic specificity	Number of tested samples	Specificity, %
Unselected blood donors	5 012	99.78
Hospitalized patients with non-infectious diseases	206	99.03
Pregnant women	40	100.00
Patients with rheumatoid factor	71	94.37
Patients with infectious diseases (HIV)	29	93.10

Analytical sensitivity

The analytical sensitivity was evaluated with WHO International Standard 1st IS for human syphilitic plasma IgG (NIBSC Code: 05/122) and defined at 0.026 IU/ml.

Precision

The repeatability within one plate was evaluated by testing 3 positive samples 24 times each. The CV did not exceed 6%.

The repeatability between plates was evaluated by testing 3 positive samples 48 times each. The CV did not exceed 7%.

The reproducibility between different lots, operators, days was evaluated by testing 3 positive samples 72 times each. The CV did not exceed 11%.

Limitations of test

- a sample should not be defined as positive for anti-Treponema pallidum antibodies based on a single reactive result. Reactive results should be re-tested; and in case of repeated reactive result confirmed by supplemental assays.
- non-reactive results can occur if the concentration of marker present in the sample is below the detection limit of the assay, or if the marker to be detected is not present during the stage of disease when a sample has been collected.
- no known test method can offer complete assurance that the anti-Treponema pallidum antibodies are absent.
- false positive results may be observed at HIV infection, virus hepatitis, cancer, chlamidiosis, pregnancy, infectious mononucleosis, leprosy, autoimmune diseases and drug addiction.

References

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8. WHO guidelines for the treatment of Treponema pallidum (syphilis). 2016

Key to symbols used

Symbol	Meaning
	Manufacturer
	For in vitro diagnostic use
	Catalogue number
	Batch code
YYYY-MM-DD	Expiry date
	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

Code	Statement
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

2020-05-20 IU.009.00.3/3

AB Diagnostic Systems GmbH
Sportfliegerstraße 4
12487 Berlin
Germany

+49 30 208 987 160
+49 30 208 987 199
info@ab-ds.de
www.ab-ds.de