abia Treponema Ab



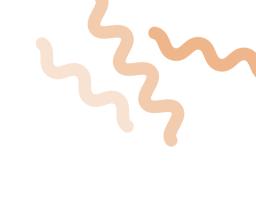


REF DK.009.01.3

IVD

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Note: Changes highlighted ★



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 potentially infectious samples to prevent their use as donor materials, for aiding the diagnosis of patients suspected of having syphilis, and for screening pregnant women. For professional use only.

Clinical value

Syphilis is a bacterial sexually transmitted infection (STI) caused by the bacterium Treponema pallidum (T. pallidum), resulting in substantial morbidity and mortality. It is transmitted through sexual contact with infectious lesions on mucous membranes or broken skin, via blood transfusion or from a pregnant woman to her foetus during pregnancy (congenital syphilis).

The detection of antibodies against Treponema pallidum is important for the diagnosis of syphilis because of the inability to culture this bacterium and the frequent unavailability of specimens for direct detection from patients in the latent or late stages of the disease.

There are two types of serological test for syphilis: non-treponemal (RPR and VDRL) and treponemal (TPHA, FTA-ABS and 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 test.

Principle of the test

abia Treponema Ab is a two-step, non-competitive assay based on microwells that are coated with recombinant Treponema pallidum antigens (rAg). The conjugate is a mixture of horseradish peroxidase (HRP)-labelled monoclonal anti-human-lgG and anti-human lgM antibodies (mAb).

Samples of serum or plasma are added to the wells. If anti-T. pallidum antibodies are present, they form stable complexes with the immobilized antigens.

The antigen-antibody complexes are then identified by adding HRP-labelled anti-human IgG and anti-human IgM conjugate.

Unbound components are then removed by washing. After the addition of a solution containing TMB and hydrogen peroxide, the wells containing the bound conjugate become blue, which turns yellow after the reaction is stopped with sulphuric acid.

The colour 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.

Materials and equipment required but not provided

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

Kit contents*

	S	XL	
MP T. pallidum Ag coated plate	1	5	Polystyrene stripped 96-well plate (breakable wells) coated with T. pallidum recombinant antigenes
CJ Conjugate (concentrated 11-fold)	1 × 1.2 ml	2 × 3.0 ml	Mixture of peroxidase-labelled anti-human-IgG/IgM mouse antibodies in a glycerol-based solution with addition of bovine serum albumin; transparent or slightly opalescent colourless liquid
CD Conjugate diluent	1 × 12 ml	3 × 20 ml	Tris-HCI buffer with addition of urea and caseinat; transparent or slightly opalescent yellow liquid
SD Sample diluent	1 × 14 ml	2 × 25 ml	Phosphate-saline buffer with addition of urea and bovine serum albumin; opalescent violet liquid
PC Positive control	1 × 2.5 ml	1 × 2.5 ml	Inactivated human plasma positive for antibodies to T. pallidum; red liquid
NC Negative control	1 × 2.5 ml	2 × 2.5 ml	Inactivated human plasma negative for antibodies to T. pallidum; contains 0.2 % ProClin 300 as preservative; green liquid
WS Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	Phosphate saline buffer; transparent or slightly opalescent colourless or pale yellow liquid; a sediment may form that dissolves completely when shaken at 35-39 °C
TB TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	Solution containing 3,3',5,5'-Tetramethylbenzidin; transparent colourless liquid
SB Substrate buffer	1 × 25 ml	1 × 70 ml	Citric acid solution containing $H_3O_{\hat{y}}$ transparent colourless liquid
SR Stopping reagent 0.2 M H ₂ SO ₄	1 × 25 ml	1×90 ml	0.2 M sulfuric acid solution (H ₂ SO المرابع); transparent colourless liquid

^{*}Continued on next page

	S	XL	
Protective film	2	10	
Plastic dish	2	-	
Plastic zip-lock bag	1	5	

All components are stable until the kit's expiry date when stored at 2-8 °C in a tightly sealed package. The expiry date is indicated on the package. Once opened, the components should be used within one month. The concentration of preserving agents is \$ 0.1 %, unless otherwise specified.

Safety notes

- Human-origin material used in the preparation of the negative and positive controls has been tested using CE-marked tests and found to be non-reactive for hepatitis B surface antigen (HBsAg), HIV-1 p24 antigen, hepatitis C virus antibodies and human immunodeficiency virus (HIV-1 and HIV-2) antibodies
- As no test method can offer complete assurance that infectious agents are absent, reagents and samples should be handled as if they are capable of transmitting infectious diseases.
 Any equipment that is in direct contact with samples and reagents should be considered contaminated
- Do not eat, drink, smoke, or apply cosmetics in the laboratory
- Do not pipette by mouth
- Avoid contact with the reagents and samples with your skin or mucosa. Wear a lab coat and disposable gloves when handling them; thoroughly wash your hands after work
- Avoid spilling samples or solutions containing samples. Immediately wipe up any spills and decontaminate the affected surfaces
- All materials that have come into contact with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed of in accordance with applicable local laws and regulations

Precautions

- Do not use reagents that are unlabelled or have a 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 (acidic, alkaline or aldehyde), dust or metals
- Do not allow the wells to dry once the assay has started
- Do not use the same container or tips for the different liquid components of the kit or samples
- Do not reuse the coated plates
- Do not reuse the protective film that has been removed
- Do not expose the reagents to excessive heat or sunlight during storage or testing
- Do not freeze the reagents

Collection and handling of specimens

- Collect blood specimens according to current practices
- Use undiluted heparin, EDTA or citrate plasma or serum for testing. The performance of the test has not been evaluated on other biological fluids
- Separate the clot or red cells from the serum or plasma as soon as possible to avoid haemolysis
- Do not use contaminated, hyperlipidaemic or hyperhaemolysed specimens
- Samples with hyperproteinaemia and hyperbilirubinaemia were not tested specifically
- Do not use pooled specimens, as the accuracy of the test with such specimens has not been validated
- Before testing, samples containing 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 for up to 48 hours or frozen at -20 °C
- No more than one freeze/thaw cycle is allowed

Procedural notes

- $\,$ Before use, wait 30 minutes for the reagents to stabilise at room temperature (18-24 °C)
- Check the 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 critical; see the "Washing procedure" section for detailed washer settings
- For a description of the test procedure with automated analysers, see the "Automated analysers" section

Washing procedure

Please contact your representative for protocols regarding recommended washers and procedures. The following protocol is generally recommended:

- Flow-through washing with a volume of at least 400 µl per well should be used. If using a
 microplate washer for which this is not possible, ensure the well is completely filled with a
 slight positive meniscus, taking care not to overflow
- Allow a soaking time of at least 40 seconds before aspiration
- Perform this procedure four times in total
- Do not allow the wells to dry out during the assay procedure
- Ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- Avoid tapping out the plate
- A residual volume of less than 10 μl is not critical for the subsequent steps of the test procedure
- When using a microplate washer, clean the wash head frequently to prevent contamination

Automated analysers

Your representative can provide you with validated protocols for automated analysers. For instrumentation without an established, validated protocol, follow the "Test procedure" section and ensure that all the requirements described in the "Precautions" section are met. All protocols for automated analysers must be fully validated prior to use.

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: The Substrate mixture should be colourless! 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 is used for the qualitative detection of antibodies to Treponema pallidum in human serum or plasma.

Attention! Two alternative incubation procedures are possible. Follow the same incubation mode throughout the test. Do not combine incubatione modes.

	Procedure 1 · microplate incubator Procedure 2 · microplate thermoshaker					
1	Take the required number of coated strips. Place any unused strips back into the bag and reseal the foil-lined package in the plastic zip-lock bag. Do not remove the desiccant bag.					
2	2 Add 100 µl of the Positive control to well A1 and 100 µl of the Negative control to wells B1, C1 and D1. Add 90 µl of Sample diluent and 10 µl of the samples to be tested (final sample dilution 1:10) to the remaining wells. Depending on the system and the number of strips used, the position of the controls or the order of distribution can be modified. The violet colour of the solution should change to light blue-green. Then, cover the plate with protective film.					
3	Incubate in a microplate incubator at 37.0 ± 1.0 °C for 30 minutes. Incubate in a microplate thermoshaker at 500 rpm at 37.0 ± 1.0 °C for 15 minutes.					
4	Slowly and carefully remove the protective film to prevent splashes. Aspirate the contents of all wells into a biohazardous waste container containing disinfectant. Add a minimum of 400 µl of Working Washing solution to each well. Leave to soak for at least 40 seconds, then aspirate. Repeat this procedure 4 times.					
5	Add 100 µl of Working solution of Conjugate to each well.					
6	Cover the plate with protective film. Incubate in a microplate incubator at 37.0 ± 1.0 °C for 30 minutes. Incubate in a microplate thermoshaker at 500 rpm at 37.0 ± 10 °C for 20 minutes.					
7	Slowly and carefully remove the protective film to prevent splashes. Aspirate the contents of all wells into a biohazardous waste container containing disinfectant. Add a minimum of 400 µl of Working Washing solution to each well. Leave to soak for at least 40 seconds, then aspirate. Repeat this procedure 4 times.					
8	Add 100 µl of Substrate mixture to all the wells. Leave the plates in a dark place at 18-24 °C for 20 minutes.					
9	Add 150 µl of Stopping reagent to each well.					
10	Read the optical density at 450/620-680 nm using a plate reader. It is also possible to read the absorbanc	e at 450 nm only. Test results remain stable for at least 3 minutes for reading.				

Calculation and interpretation of the results

Assay validation

The results of an assay are valid if the following control criteria are met:

The absorbance (OD) of each Negative control should be less than 0.200. If one Negative control does not meet this criterion, disregard it and recalculate the mean value using the remaining two values. No more than one value may be eliminated in this way. The absorbance (OD) of the Positive control should be greater than 0.600.

Calculation of the cut-off value

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

Interpretation of the results

A non-reactive sample is defined as a sample with an OD value \$ 0.9 cut-off value. Samples with absorbance values less than the cut-off value are considered to be negative by the abia Treponema Ab test.

A reactive sample is defined as a sample with an OD value ≥ 1.1 cut-off value. Samples with an OD value greater than or equal to the cut-off value are considered to be positive by the abia Treponema Ab test.

If the OD of the tested sample is between 0.9 x and 1.1 x the cut-off value, the sample is to be considered in the "grey zone". In this case, it is necessary to repeatedly test the patient's serum for antibodies to Treponema pallidum one to two weeks after the initial blood sampling. It is advisable to test sera samples simultaneously with previous samples ("pair samples"), as this allows more accurate assessment of specific antibody dynamics.

Performance characteristics

The performance of the abia Treponema Ab test has been determined by testing blood samples from random donors and patients in various clinical categories, including those with confirmed syphilis infection.

401	00.75
	99.75
32	96.90
93	100.00
159	100.00
24	100.00
93	100.00
20	100.00
6	100.00
15	100.00
	93 159 24 93 20 6

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 using the WHO International Standard "1st IS for human syphilitic plasma IgG" (NIBSC code: 05/122) and defined at 0.026 IU/ml.

Precision

Repeatability within one plate was evaluated by testing three positive samples 24 times each. The coefficient of variation (CV) did not exceed 6 %.

Repeatability between plates was evaluated by testing three positive samples 48 times each. The CV did not exceed $7\,\%$.

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

Limits of the test

- A sample should not be defined as positive for anti-Treponema pallidum antibodies based on a single reactive result. Reactive results should be retested; and repeated reactive results confirmed by supplemental assays
- Non-reactive results can occur if the concentration of the marker present in the sample is below the detection limit of the assay, or if the marker is not present at the stage of the disease when the sample was collected
- No test method can offer complete assurance that the anti-Treponema pallidum antibodies are absent
- False positive results may be observed in cases of HIV infection, viral hepatitis, cancer, chlamydiosis, pregnancy, infectious mononucleosis, leprosy, autoimmune diseases, and drug addiction

* References

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- Poltavchenko AG, Rybakov AN, Nadtochi ON. [Dynamics of humoral immune response to Treponema pallidum proteins p17 and p41 at early stages of syphilis]. Zh Mikrobiol Epidemiol Immunobiol. 2004;(3):52-57.
- Castro R, Prieto ES, Santo I, Azevedo J, Exposto F da L. Evaluation of an Enzyme Immunoassay Technique for Detection of Antibodies against Treponema pallidum. J Clin Microbiol. 2003;41(1):250-253.
- Chepurchenko NV, Gladysheva MV, Obriadina AP. [New possibilities of using recombinant antigens in serodiagnostic assays for syphilis]. Zh Clin. Dermat. and Venerol. 2006;(2):28-31.
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- Rostopira N, Tkáciková L, Rayevska G, Pylypenko V, Mikula I, Spivak M. Elaboration of enzyme immunoassay based on recombinant antigens and intended for diagnostics of syphilis. Folia Microbiol (Praha). 2003; 48(4):549-553.
- WHO Guidelines for the Treatment of Treponema Pallidum (Syphilis). World Health Organization. 2016.

Key to symbols used



IVD	For in vitro diagnostic use	SR	Stopping reagent ★
REF	Catalogue number	11X	concentrated 11-fold
LOT	Batch code	25X	concentrated 25-fold
MP	Microplate, coated plate	23/	
Сј	Conjugate	*	Changes highlighted
CD	Conjugate diluent		
SD	Sample diluent		
PC	Positive control		
NC	Negative control		
WS	Washing solution		
ТВ	TMB		
SB	Substrate buffer		

* Hazard and precautionary statements for certain kit components

Negative control



Contains: Mixture: 5-chloro-2-methyl-2H-isothiazol-3-one/2-methyl-2H-isothiazol-3-one (3:1).

H317 May cause an allergic skin reaction.

H412 Harmful to aquatic life with long lasting effects.

P302+P352 IF ON SKIN: Wash with plenty of water / soap.

P333+P313 If skin irritation or rash occurs: Get medical advice / attention.

P362+P364 Take off contaminated clothing and wash it before reuse.

P501 Dispose of contents / containers in accordance with local / national regulations.

Conjugate (concentrated 11-fold)

Contains: Mixture: 5-chloro-2-methyl-2H-isothiazol-3-one/2-methyl-2H-isothiazol-3-one (3:1), 2-Methyl-2H-isothiazol-3-one. EUH208 May produce an allergic reaction.

Substrate buffer

Contains: Mixture: 5-chloro-2-methyl-2H-isothiazol-3-one/2-methyl-2H-isothiazol-3-one (3:1).

EUH 208 May produce an allergic reaction.

EUH210 Safety data sheet available on request.

EUH210 Safety data sheet available on request.

TMB (concentrated 11-fold)

Contains: 2-Chloracetamid. EUH208 May produce an allergic reaction. EUH210 Safety data sheet available on request.

Stopping reagent

EUH210 Safety data sheet available on request.

Conjugate diluent

EUH210 Safety data sheet available on request.



