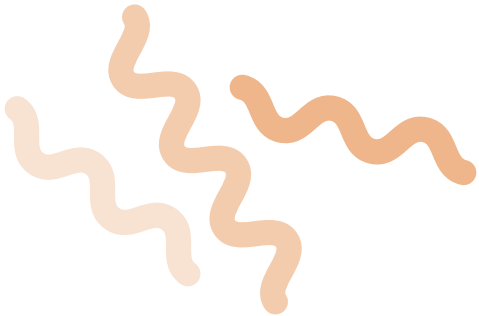
abia Treponema Ab

REF DK.009.01.3
REF DK.009.05.3

IVD

CE

Note: Changes highlighted ★



abia

Intended Use

abia Treponema Ab is a solid-phase enzyme immunoassay for the qualitative detection of antibodies to Treponema pallidum (IgG and IgM) in human serum or plasma.

The assay is intended to be used as a screening test and as an aid in the diagnosis of syphilis in individuals with suspected infection or at risk of infection.

The device is intended for use by trained laboratory professionals in clinical and diagnostic laboratory settings.

Results obtained with this assay must be interpreted in conjunction with clinical findings and other laboratory tests, and in accordance with established diagnostic algorithms.

★ The assay has not been validated for testing of individuals under 18 years of age.

Clinical Value

Syphilis is a sexually transmitted infection (STI) caused by the bacterium Treponema pallidum, which may lead to serious clinical complications if untreated. Transmission occurs primarily through sexual contact, and may also occur from a pregnant woman to her foetus during pregnancy (congenital syphilis).

The detection of antibodies to Treponema pallidum is an established method for the diagnosis of syphilis, particularly in cases where direct detection of the pathogen is not feasible, such as in latent or late stages of infection.

Serological testing for syphilis includes non-treponemal tests (e.g. RPR, VDRL) and treponemal tests (e.g. TPHA, FTA-ABS, EIA). Results should be interpreted in conjunction with clinical findings and other laboratory tests, in accordance with established diagnostic algorithms.

Principle of the Test

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

Samples of serum or plasma are added to the wells. If anti-T. pallidum antibodies are present, they form complexes with the immobilized antigens. The antigen-antibody complexes are detected by the addition of HRP-labelled anti-human IgG and IgM conjugate. After washing to remove unbound components, a substrate solution containing tetramethylbenzidine (TMB) and hydrogen peroxide is added, resulting in a blue colour reaction. The reaction is stopped by the addition of sulphuric acid, leading to a colour change from blue to yellow.

The optical density is measured photometrically at 450 nm or at 450 nm with a reference wavelength of 620–680 nm.

The signal intensity correlates with the presence of anti-T. pallidum antibodies in the sample and is interpreted qualitatively using a predefined cut-off value.

Materials and Equipment Required but not Provided

- Purified water
- Adjustable pipettes or multipipettes (manual or automated)
- Disposable pipette tips
- Microplate incubator or thermoshaker capable of maintaining 37.0 ± 1.0 °C and, if applicable, shaking at 500 rpm
- Microplate washer (manual or automated)
- Microplate reader capable of measuring absorbance at 450 nm or at 450/620–680 nm

Kit Contents*

Symbol	Components	S	XL	Description / Reactive Ingredients	Preservatives ★
	T. pallidum Ag coated plate	1	5	Polystyrene 96-well microplate (breakable wells) coated with recombinant Treponema pallidum antigens (Tp p17, Tp p47 and Tp tmpA).	None
	Conjugate (concentrated 11-fold)	1 × 1.2 ml	2 × 3.0 ml	Mixture of horseradish peroxidase (HRP)-labelled monoclonal anti-human IgG and anti-human IgM antibodies in a glycerol-based solution containing bovine serum albumin; transparent or slightly opalescent colourless or pale yellow liquid.	ProClin 300; gentamycin sulfate
	Conjugate diluent	1 × 12 ml	3 × 20 ml	Tris-HCl buffer containing urea and caseinate; transparent or slightly opalescent yellow liquid.	Thiomersal
	Sample diluent	1 × 14 ml	2 × 25 ml	Phosphate-buffered saline containing urea and bovine serum albumin; opalescent violet liquid; flakes or amorohous sediment may form.	Thiomersal; sodium azide
	Positive control	1 × 2.5 ml	1 × 2.5 ml	Inactivated human plasma positive for antibodies to Treponema pallidum; transparent or slightly opalescent red liquid.	Thiomersal; sodium azide
	Negative control	1 × 2.5 ml	2 × 2.5 ml	Inactivated human plasma negative for antibodies to Treponema pallidum; transparent or slightly opalescent green liquid.	0.2 % ProClin 300; gentamycin sulfate; thiomersal
	Washing solution (concentrated 25-fold)	1 × 50 ml	2 × 120 ml	Phosphate-buffered saline; transparent or slightly opalescent colourless to pale yellow liquid; a sediment may form that dissolves completely upon warming to 35–39 °C.	None
	TMB (concentrated 11-fold)	1 × 2.5 ml	2 × 3.5 ml	Solution containing 3,3‘,5,5‘-Tetramethylbenzidine; transparent colourless liquid.	None
	Substrate buffer	1 × 25 ml	1 × 70 ml	Citric acid solution containing hydrogen peroxide (H_2O_2); transparent colourless liquid.	ProClin 300
	Stopping reagent 0.2M H_2SO_4	1 × 25 ml	1 × 90 ml	0.2 M sulphuric acid (H_2SO_4); transparent colourless liquid.	None

*Continued on next page

Once opened, the components should be used within one month. Unless otherwise specified, preservative concentrations are ≤ 0.1%.

Components	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.

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, all reagents and samples should be handled as potentially infectious. Any equipment that comes into contact with samples or reagents should be considered contaminated.
- Do not eat, drink, smoke, or apply cosmetics in the laboratory.
- Avoid contact of reagents and samples with skin and mucous membranes. Wear appropriate personal protective equipment, including laboratory coat and disposable gloves. Wash hands thoroughly after handling.
- Avoid spilling samples or solutions. In case of spills, immediately clean the affected area and decontaminate using appropriate disinfectants.
- 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.
- Some reagents contain preservatives such as sodium azide, thiomersal and ProClin® 300. Avoid contact with skin and eyes. In case of contact, rinse immediately with plenty of water. ★
- Sodium azide may react with lead or copper plumbing to form metal azides. When disposing of solutions containing sodium azide, flush with large volumes of water. ★
- The stopping reagent contains sulphuric acid and is corrosive. Avoid contact with skin and eyes. ★
- Safety data sheets (SDS) for all components are available upon request and should be consulted for detailed safety information. ★

Precautions

- Do not use reagents that are unlabelled or have damaged labels or packaging.
- Do not use expired reagents.
- Do not modify the assay procedure. Follow the instructions for use exactly and perform all steps without interruption.
- Do not mix reagents from different lots.
- Do not interchange caps between reagent vials.
- Do not perform the assay in the presence of reactive vapours (e.g. acidic, alkaline or aldehyde vapours), dust or metal contaminants.
- Do not allow the wells to dry during the assay procedure.
- Use separate pipette tips or containers for each reagent and sample to avoid cross-contamination.
- Do not reuse coated microplate wells.
- Do not reuse protective films once removed.
- Do not expose reagents to excessive heat or direct sunlight during storage or use.
- Do not freeze reagents unless explicitly stated.
- When using automated systems, ensure that validated protocols are applied. ★

Collection and Handling of Specimens

- Collect blood specimens in accordance with standard laboratory procedures.
- Use undiluted serum or plasma (heparin, EDTA or citrate) for testing. The performance of the assay has not been established for other specimen types.
- Separate serum or plasma from the clot or red blood cells as soon as possible to avoid haemolysis.
- Do not use contaminated, highly lipaemic or haemolysed specimens.
- The performance of the assay has not been established for samples with hyperproteinaemia or hyperbilirubinaemia.
- Do not use pooled specimens, as the performance of the assay with such samples has not been validated.
- Samples containing particulate matter should be clarified by centrifugation prior to testing.
- The presence of fibrin particles or aggregates may lead to incorrect results.
- Do not heat the samples.
- Samples may be stored at 2–8 °C for up to 48 hours or frozen at –20 °C for longer storage.
- Avoid repeated freeze–thaw cycles; no more than one freeze–thaw cycle is recommended.

Procedural Notes

- Allow all reagents to equilibrate to room temperature (18–24 °C) for at least 30 minutes before use.
- Inspect all reagents prior to use. Do not use reagents showing signs of contamination, turbidity or other abnormalities.
- Loss of vacuum in the sealed bag of the coated microplate does not affect assay performance.
- Ensure that pipettes and other equipment are calibrated and functioning correctly.
- The washing procedure is critical for assay performance. Refer to the "Washing Procedure" section for detailed instructions.
- When using automated analysers, follow validated protocols as described in the "Automated Analysers" section.

Washing Procedure

The washing step is critical for assay performance and must be performed as described below.

- Perform washing using a volume of at least 400 µl per well for each wash cycle.
- If using a washer that does not support flow-through washing, ensure that each well is completely filled without overflow.
- Allow a soaking time of at least 40 seconds before aspiration.
- Repeat the washing step four times in total.
- Do not allow the wells to dry out during the assay procedure.
- After the final wash, ensure that no residual liquid remains in the wells. Double aspiration is recommended where possible.
- A residual volume of less than 10 µl does not affect assay performance.
- When using an automated washer, clean the wash head regularly to prevent contamination.

Automated Analysers

Validated protocols for automated analysers may be available from the manufacturer.
If unavailable, perform the assay according to the "Test Procedure" section.
All automated procedures must be appropriately validated by the user prior to routine 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: Dilute the Washing solution (25-fold concentrate) with purified water as indicated in the table below. Mix gently but thoroughly. **Stability**: Stable for up to 4 days at 18–24 °C or up to 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 Conjugate solution Dilute the Conjugate (11-fold concentrate) with Conjugate diluent according to the table below. Mix gently to avoid foaming. Allow the working conjugate solution to equilibrate to room temperature (18–24 °C) prior to use. **Stability**: Stable for up to 12 hours at 18–24 °C when kept in the dark.												
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: Prepare the Substrate mixture by diluting the TMB (11-fold concentrate) with Substrate buffer as indicated in the table below. Mix gently. The substrate solution should be colourless prior to use. **Stability**: Stable for up to 10 hours at 18–24 °C when kept in the dark.												
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 may be used. The same incubation mode must be applied throughout the assay. Do not combine incubation modes.

	Instruction	Microplate incubator	Microplate thermoshaker
1	Take the required number of coated strips. Return unused strips to the foil pouch, reseal carefully and store as recommended. Do not remove the desiccant.		
2	Add 100 µl Positive control to A1 and 100 µl Negative control to B1, C1 and D1. Add 90 µl of Sample diluent and 10 µl of sample (1:10) to the remaining wells. Mix gently and cover with protective film.		
3	Incubate at 37 ± 1 °C.	30 minutes	500 rpm for 15 minutes
4	Carefully remove the protective film. Aspirate the contents of all wells. Wash each well with at least 400 µl of Working Washing solution. Allow soaking for at least 40 seconds and aspirate. Repeat the washing step four times.		
5	Add 100 µl of Working Conjugate solution to each well. Mix gently and cover with protective film.		
6	Incubate at 37 ± 1 °C.	30 minutes	500 rpm for 20 minutes
7	Wash as described in step 4.		
8	Add 100 µl of Substrate mixture to each well. Incubate in the dark at 18–24 °C for 20 minutes.		
9	Add 150 µl of Stopping reagent to each well.		
10	Measure the optical density at 450 nm with or without a reference wavelength of 620–680 nm. Measurement at 450 nm only is acceptable. Read the results within 3 minutes after adding the Stopping reagent.		

Calculation and Interpretation of the Results

Assay Validation

The assay is considered valid if the following criteria are met:

- The optical density (OD) of each Negative control is < 0.200.
- If one Negative control does not meet this criterion, it may be excluded and the mean value recalculated using the remaining two values. No more than one value may be excluded.
- The optical density (OD) of the Positive control is > 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 Results

- Samples with an OD value ≤ 0.9 × cut-off are interpreted as non-reactive.
- Samples with an OD value ≥ 1.1 × cut-off are interpreted as reactive.
- Samples with OD values between 0.9 × and 1.1 × the cut-off value are considered borderline (grey zone).

Borderline samples should be retested. It is recommended to collect a follow-up sample after 1–2 weeks and to test paired samples where appropriate.
Results should be interpreted in conjunction with clinical findings and other laboratory results.

Performance Characteristics

The performance of the abia Treponema Ab assay was evaluated using serum and plasma samples from blood donors and patients representing different clinical conditions, including confirmed syphilis infection at different disease stages.

Performance characteristics were established under controlled study conditions and may vary depending on laboratory practice.

Diagnostic Sensitivity

The overall diagnostic sensitivity was 99.75% (401 samples).
Sensitivity by clinical stage was evaluated as follows:

- Primary stage: 96.9% (n=32)
- Secondary stage: 100.0% (n=93)
- Early latent stage: 100.0% (n=159)
- Latent stage: 100.0% (n=24)
- Past syphilitic infection: 100.0% (n=93)

Diagnostic Specificity ★

The diagnostic specificity was evaluated using samples from different populations:

- Unselected blood donors: 99.8% (n = 5512)
- Hospitalized patients: 99.2% (n = 256)
- Pregnant women: 100.00% (n = 49)
- Samples containing rheumatoid factor: 95.4% (n = 87)
- Samples from HIV-positive individuals: 93.1% (n = 29)
- Samples containing anti-E. coli antibodies: 100 % (n=10)

Overall diagnostic specificity: 99.7% (n=5943)

★ **Analytical Sensitivity**

The analytical sensitivity of the assay was determined using the WHO International Standard 1st IS for human syphilitic plasma IgG and IgM (NIBSC code 05/132) for anti-Treponema pallidum antibodies and was estimated at 0.0034 IU/ml.

Precision

Repeatability (within-run precision) was evaluated by testing three positive samples 24 times each and showed a coefficient of variation (CV) ≤ 6%.

Intermediate precision (between runs) was evaluated by testing three positive samples 48 times each and showed a CV ≤ 7%.

Reproducibility (between lots, operators and days) was evaluated by testing three positive samples 72 times each and showed a CV ≤ 11%.

Limitations of the Test

- A reactive result should not be interpreted as a definitive diagnosis of syphilis. Reactive samples should be retested, and repeatedly reactive results should be confirmed using supplemental assays.
- Non-reactive results do not exclude infection with Treponema pallidum. Such results may occur if the concentration of antibodies is below the detection limit of the assay or if testing is performed during early stages of infection.
★ - Samples with results in the borderline (grey zone) range should be retested. Follow-up testing using a newly collected sample after 1–2 weeks is recommended where clinically indicated.
- False reactive results may occur due to cross-reactivity with other conditions, including viral infections (e.g. HIV, hepatitis), autoimmune diseases, pregnancy, malignancies, or other infections.
- The performance of the assay has not been established for all patient populations or specimen types. Results should always be interpreted in conjunction with clinical findings and other laboratory results.

References

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3. Castro R, Prieto ES, Santo I, Azevedo J, Exposto FL. Evaluation of an enzyme immunoassay technique for detection of antibodies against Treponema pallidum. J Clin Microbiol. 2003;41(1):250–253.
4. Chepurchenko NV, Gladysheva MV, Obriadina AP. New possibilities of using recombinant antigens in serodiagnostic assays for syphilis [in Russian]. Zh Clin Dermatol Venerol. 2006;(2):28–31.
5. Frigo NV, Komarova VD, Obriadina AP, Burkov AN. Comparative results of immunoenzymatic analysis, passive haemagglutination reaction and micro-reaction in serodiagnosis of syphilis [in Russian]. Bull Dermatol Venerol. 2000;(4):34–36.
6. Rostopira N, Tkáciková L, Rayevska G, Pylypenko V, Mikula I, Spivak M. Elaboration of enzyme immunoassay based on recombinant antigens for diagnostics of syphilis. Folia Microbiol (Praha). 2003;48(4):549–553.
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Key to Symbols Used

	Keep away from sunlight
	Keep dry
	Do not use if package is damaged
	Do not re-use
 n	Sufficient for [n] tests
	Consult Instruction for use
	Manufacturer
	Storage temperature limits
 YYYY-MM-DD	Use by date
	CE marking
	For in vitro diagnostic use
	Catalogue number

LOT	Batch code	
UDI	Unique device identification	★
MP	Microplate, coated plate	
CJ	Conjugate	
CD	Conjugate diluent	
SD	Sample diluent	
PC	Positive control	
NC	Negative control	
WS	Washing solution	
TB	TMB	
SB	Substrate buffer	
SR	Stopping reagent	
11X	concentrated 11-fold	
25X	concentrated 25-fold	
★	Changes highlighted	

Hazard and Precautionary Statements for certain Kit Components

Negative control

WARNING

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.
EUH210	Safety data sheet available on request.

Substrate buffer

Contains:	Mixture: 5-chloro-2-methyl-2H-isothiazol-3-one/2-methyl-2H-isothiazol-3-one (3:1).
EUH208	May produce an allergic reaction.
EUH210	Safety data sheet available on request.

TMB (concentrated 11-fold)

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

Stopping reagent

EUH210	Safety data sheet available on request.

2026-04-22 IU.009.00.3/5

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