Certificate

TÜVRheinland®

Quality Management System
EN ISO 13485:2016

Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

Scope: Design and development, manufacture, installation, service and distribution of immuno-biochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics

The Certification Body of TÜV Rheinland LGA Products GmbH certifies that the organization has established and applies a quality management system for medical devices.
Proof has been furnished that the requirements specified in the abovementioned standard are fulfilled. The quality management system is subject to yearly surveillance.

Report No.: 1130650-10
Effective date: 2023-05-19
Expiry date: 2026-05-18
Issue date: 2023-05-11

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TÜV Rheinland LGA Products GmbH

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Location: Moldova
MOLDOVA EUROPEANĂ

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DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

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23560 Lübeck
Germany

The scope of certification also covers the following:

No.	Facility	Scope
/01	c/o EUROIMMUN Medizinische Labordiagnostika AG Seekamp 31 23560 Lübeck Germany	Design and development and manufacture of immuno-biochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for instruments for in vitro diagnostics
/02	c/o EUROIMMUN Medizinische Labordiagnostika AG Werkstr. 1 23942 Dassow Germany	Design and development, manufacture and distribution of immuno-biochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics
/03	c/o EUROIMMUN Medizinische Labordiagnostika AG An der Trave 1 23923 Selmsdorf Germany	Design and development, manufacture, service and distribution of immuno-biochemical test systems, immunofluorescence test systems and instruments / software for in vitro diagnostics

Report No.: 1130650-10
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TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland

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Seekamp 31
23560 Lübeck
Germany

The scope of certification also covers the following:

/04	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Sonnenberg 9 23627 Groß Grönau Germany	Manufacture of immunofluorescence test systems for in vitro diagnostics
/05	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Born 24 23627 Groß Grönau Germany	Design and development of software for in vitro diagnostics
/06	c/o EUROIMMUN Medizinische Labordiagnostika AG Im Kreppel 1 02747 Herrnhut Germany	Manufacture of immuno-biochemical test systems and immunofluorescence test systems for in vitro diagnostics
/07	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Pließnitztal 1 02748 Bernstadt Germany	Manufacture of immuno-biochemical test systems and instruments for in vitro diagnostics

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TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland

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Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

The scope of certification also covers the following:

/08	c/o EUROIMMUN Medizinische Labordiagnostika AG Schloßstr. 11 91257 Pegnitz Germany	Manufacture of immunofluorescence test systems, installation and service of instruments / software for in vitro diagnostic
/09	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Flugplatz 4 23560 Lübeck Germany	Design and development, installation, service and distribution of immuno-biochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics
/10	c/o EUROIMMUN Medizinische Labordiagnostika AG Gewerbestr. 19 23942 Dassow Germany	Manufacture of sheet metal and other components for instruments for in vitro diagnostics

Report No.: 1130650-10
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TÜV Rheinland LGA Products GmbH Zertifizierungsstelle

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Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

The scope of certification also covers the following:

/11	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Berzdorfer See 7 02829 Markersdorf Germany	Warehousing of immuno-biochemical test systems and instruments for in vitro diagnostics

Report No.: 1130650-10
Effective date: 2023-05-19
Expiry date: 2026-05-18
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TÜV Rheinland LGA Products GmbH Zertifizierungsstelle

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CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, 23560 Lübeck, Germany

declares under its sole responsibility as manufacturer that the ELISA product

Anti-Treponema pallidum ELISA (IgM) *EI 2111-9601 M*
(product name, order number)

meets the following demands of:

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

This Declaration of Conformity is valid based on the respective currently valid version of technical documentation.

Lübeck, May 17, 2022
(Place and date of issue)

ppa
Dr. Ewald Müller-Kunert
- Head of Quality Management -

Susanne Aleksandrowicz
- Member of the Executive Board -

CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, 23560 Lübeck, Germany

declares under its sole responsibility as manufacturer that the ELISA product

Anti-Treponema pallidum ELISA (IgG) *EI 2111-9601 G*
(product name, order number)

meets the following demands of:

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

This Declaration of Conformity is valid based on the respective currently valid version of technical documentation.

Lübeck, May 17, 2022
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ppc
Dr. Ewald Müller-Kunert
- Head of Quality Management -

Susanne Aleksandrowicz
- Member of the Executive Board -

Anti-Treponema pallidum ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2111-9601 M	Treponema pallidum	IgM	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM against Treponema pallidum in serum or plasma for the diagnosis of infections with Treponema pallidum, associated diseases: Lues.

Application: The Anti-Treponema pallidum ELISA (IgM) is suitable as a confirmatory test in the framework of a 2-step strategy for the specific detection of Treponema pallidum infections, but can also be used as specific screening or exclusionary test.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified recombinant antigens of Treponema pallidum. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. Sample buffer buffer containing IgG/RF-absorbent (anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Protective foil	---	2 pieces	FOIL
11. Quality control certificate	---	1 protocol	
12. Test instruction	---	1 booklet	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents should be disposed of according to official regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

The thermostat adjusted ELISA incubator must be set at +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The calibrator an controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors of class IgM from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM-negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with green coloured sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing. Incubate the mixture for at least **10 minutes** at room temperature (+18°C to +25°C). Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation follow the recommendations of the instrument manufacturer.
Incubate **60 minutes** at +37°C ± 1°C.

Washing:
Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	P 6	P 14	P 22								
B	pos.	P 7	P 15	P 23								
C	neg.	P 8	P 16	P 24								
D	P 1	P 9	P 17									
E	P 2	P 10	P 18									
F	P 3	P 11	P 19									
G	P 4	P 12	P 20									
H	P 5	P 13	P 21									

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no quantificated international reference serum exists for antibodies against Treponema pallidum, results are provided in the form of ratios which are a relative measure for the concentration of antibodies in the serum or plasma. The calibration is based on internal reference sera which were used in the evaluation of the test system.The international standard for Human Syphilis Serum; 1. International standard preparation SSI code number HS reacts with ratio 4.0.

For every group of tests performed, the extinction values of the calibrator and the ratio of the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigens: The microplate wells were coated with a mixture of four antigens of Treponema pallidum: p15, p17, p47 and TmpA. The corresponding cDNAs were expressed in E. coli as recombinant antigens.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Treponema pallidum ELISA (IgM) is ratio 0.06.

Cross reactivity: The quality of the antigen used ensures a high specificity and sensitiviy of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Treponema pallidum ELISA (IgM). This ELISA showed no cross reactivity.

Antibodies against	n	Anti-Treponema pallidum ELISA (IgM) positive
Borrelia burgdorferi	10	0%
CMV	8	0%
EBV-CA	10	0%
HSV-1/2	2	0%
Measles virus	10	0%
Mumps virus	9	0%
Parvovirus B19	7	0%
Rubella virus	10	0%
Toxoplasma gondii	10	0%
VZV	4	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (Ratio)**	**CV (%)**
1	1.1	4.4
2	3.3	4.2
3	6.1	3.9

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (Ratio)**	**CV (%)**
1	1.2	5.8
2	3.5	5.4
3	6.2	4.6

Sensitivity and specificity: 72 clinically pre-characterised patient samples (INSTAND and RfB) were investigated with the EUROIMMUN Anti-Treponema pallidum ELISA (IgM). The sensitivity amounted to 100%, with a specificity of 100%. Borderline results were not included in the calculation.

n = 72		**INSTAND/RfB**		
		positive	borderline	negative
EUROIMMUN Anti-Treponema pallidum ELISA (IgM)	positive	17	0	0
	borderline	0	1	0
	negative	0	1	53

The specificity of the Anti-Treponema pallidum ELISA (IgM) was evaluated in a study performed on 37 patient sera which were seropositive for rheumatoid factors. None of the 37 samples tested positive using the Anti-Treponema pallidum ELISA (IgM).

Reference range: The levels of the anti-Treponema pallidum antibodies (IgM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 0.2% of the blood donors were anti-Treponema pallidum positive (IgM).

Limitations of the procedure

The test is not intended to be used for the determination of suitability for transfusion, transplantation or cell administration.

Clinical significance

Treponema pallidum pallidum is a helically wound bacteria of the Spirochaeta family. This family includes five genera: Borrelia, Spirochaeta, Cristispira, Treponema and Leptospira. Treponema pallidum is the causative agent of syphilis or lues, a chronic infectious disease. The subspecies T. pallidum endemicum causes veneric syphilis; T. pallidum pertenue leads to a non-veneric infection occuring in tropical regions called framboesia; T. pallidum carateum is the causative agent of Pinta.

In 1905 Fritz Schaudinn (German zoologist, 1871-1906) and Erich Hoffmann (German dermatologist, 1868-1959) at the Charité in Berlin were the first to detect the causative agent of syphilis under the microscope. Spirochaeta were first found in 1913 by the Japanese microbiologist and physician Noguchi Hideyo (1876-1928) in the brain tissue of a patient with progressive paralysis.

Syphilis is transmitted from human to human during sexual acts via the mucosa. Indirect transmission by blood transfusions and wounds is also possible. During pregnancy and at birth the baby can become infected by the mother (syphilis connata). Syphilis is a known risk factor for abortions and stillbirths.

The disease is divided into different stages, the number of which varies in literature, depending on the world region. In German-speaking regions four stages are differentiated (primary, secondary, tertiary and quaternary stage). In Asia and sometimes in the USA, stages three and four are combined into the

tertiary stage. The secondary stage has a wider meaning and is subdivided into an early latent and a late latent phase.

The early latent stage is described as seroreactive, asymptomatic and infectious (approx. one year after infection), while the late latent phase is characterised as seroreactive, asymptomatic and non-infectious (more than one year after infection). In Central Europe the infection is divided into the following four stages:

Primary stage: The ulcus durum (hard-edged ulcer) is characteristic of the primary lesion of the syphilis (stage I) and normally occurs 3 weeks after infection, developing at the place of entry of the pathogen (e.g. penis). It is a painless ulcer, which contains large quantities of the pathogen and is therefore highly contagious. Typically, the clearly defined fibrous or crusted erosion has a raised hard edge. The possible swelling of the regional lymph nodes is painless and the lymph nodes remain displaceable. From that time on, the disease can be diagnosed e.g. using the TPHA test (Treponema pallidum haemagglutination assay). After 2 to 6 weeks the ulcer heals leaving a scar. The infection generally persists and develops into stage II.**Secondary stage:** Approximately 8 weeks after the infection, the disease manifests itself with flu-like symptoms such as fever, fatigue or headache and joint pain. In addition to a generalised swelling of the lymph nodes, 90% of patients show local or generalised skin disorders, which are accompanied by weak or no itching. At first, light pink patches form, which further evolve into hard, coppery nodules (papules). In the foreground are condylomata lata, broad papules which mainly affect skin folds. The liquid excreted by open and weeping papules is highly contagious. Additionally, various organ disorders may develop, for example, ketaritis, iritis, hepatitis, vasculitis, and myocardial disorders.

All skin disorders (syphilids) heal after approximately 4 months. Secondary syphilis is followed by a clinically silent stage (syphilis latens), which can last for years.

Tertiary stage: Typical manifestations of a Treponema pallidum infection in stage III are large papules and ulcers on the skin and mucous membranes, as well as organ or visceral syphilis, including gummatous and interstitial inflammation, perivasculitis, cardiovascular syphilis, neurosyphilis (asymtomatic and symptomatic form), osteitis, and periosteitis.

Quaternary stage: Ten to thirty years after an untreated infection, 8% to 10% of patients experience severe neurological disorders such as neurosyphilis with progressive paralysis and Tabes dorsalis with severe mental and vegetative disorders.

The **diagnosis** of syphilis is based on clinical findings according to the disease stage, microscopic detection of the infectious agent (dark field), and the serological detection of antibodies against Treponema pallidum.

Treponema pallidum pallidum has a length of 5 to 15 µm and a width of 0.2 µm with 10 to 20 turns and can rotate around its longitudinal axis. Due to its fine structure, it is difficult to make it visible under the microscope by staining. However, living bacteria can be investigated using dark field microscopy. Detection in cultures has not yet been achieved.

The TPHA (Treponema pallidum haemagglutination assay) is an assay for the indirect determination of antibodies against Treponema pallidum. Erythrocytes marked with proteins and polysaccharides of Treponema pallidum on their surface are mixed with patient serum. The presence of antibodies against Treponema pallidum in the patient serum causes agglutination of the erythrocytes (haemagglutination), which is visible to the naked eye.

When this screening test is positive, further serological investigation is recommended to confirm the result, either using the Anti-Treponema pallidum FTA-Abs Test or using state-of-the-art procedures such as the Anti-Treponema pallidum ELISA or the Anti-Treponema pallidum Westernblot (e.g. Anti-Treponema pallidum EUROLINE-WB). Antibodies against cardiolipin serve as an activity marker of the infection (VDRL or RPR test, EUROLINE WB).

Antibodies against Treponema pallidum can be detected in serum and in CSF. This is diagnostically relevant, for example, in children with congenital syphilis. For the quantitative in vitro detection of human antibodies of immunoglobulin class IgG against Treponema pallidum in CSF, the same ELISA as used for the determination of antibodies against Treponema pallidum in serum is suitable. When determining an infection of the CNS it is necessary to differentiate between intrathecally produced antibodies and antibodies which have migrated from the blood into CSF.

The intrathecal pathogen-specific antibody production is defined by the relative CSF/serum quotient CSQrel (synonym: antibody specificity index). The quotient is calculated from the ratio of agent-specific antibodies to total IgG in CSF in proportion to the ratio of agent-specific antibodies to total IgG in serum. With this method a Treponema pallidum infection in the CNS can be easily and reliably determined.

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Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN accepts no liability for any other use (e.g. non-compliance with the instructions for use and improper use) or for resulting damages.

Technical Support

In case of technical problems you can obtain assistance via the EUROIMMUN website (https://www.euroimmun.de/en/contact/).

Additional information

Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this product to the competent authorities and to EUROIMMUN.

Anti-Treponema pallidum ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG-CLASS	SUBSTRATE	FORMAT
EI 2111-9601 G	Treponema pallidum	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: Infection with Treponema pallidum, associated diseases: Lues.

Principles of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against Treponema pallidum in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified recombinant antigens of Treponema pallidum. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate), catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLEBUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASHBUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Protocol with reference values	---	1 protocol	
14. Protective foil	---	2 pieces	

LOT Lot — Storage temperature

IVD In vitro determination — Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents should be disposed of according to official regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below. The thermostat adjusted ELISA incubator must be set at 37 °C +/- 1 °C

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: Calibrators and controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

Sample incubation: (1. step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.
Incubate **60 minutes** at 37°C ± 1°C.

Wash:
Manual: Remove the protective foil and empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and empty the wells and subsequently wash 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Attention: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to 25°C).

Wash:
Empty the wells. Wash as described above.

Substrate incubation: (3. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C) (protect from direct sunlight).

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**K2**	**P 6**	**P 14**	**P 22**			**K 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**K 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**K 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according the following formula:

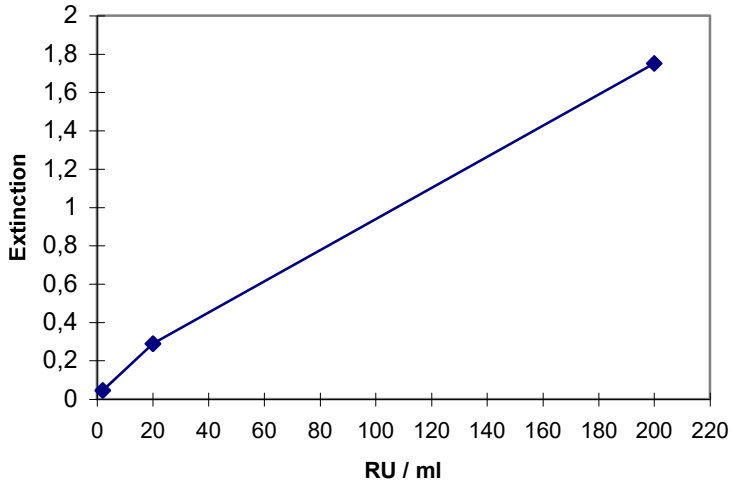
$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result shold be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Test characteristics

Calibration: As no quantificated international reference serum exists for antibodies against Treponema pallidum, the calibration is performed in relative units (RU). The international standard for Human Syphilis Serum; 1. international standard preparation SSI code number HS reacts with >200 RU/ml. Using dilution curve result would be 1882 RU/ml.

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigens: The microplate wells were coated with a mixture of four antigens of Treponema pallidum: p15, p17, p47 and TmpA. The corresponding cDNAs were expressed in E. coli as recombinant antigens.

Linearity: The linearity of the Anti-Treponema pallidum ELISA (IgG) was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression was calculated and R^2 amounts to > 0.95 in all samples. The Anti-Treponema pallidum ELISA (IgG) is linear at least in the tested concentration range (19 RU/ml to 166 RU/ml).

Detection limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The lower detection limit of the Anti-Treponema pallidum ELISA (IgG) is 0.3 RU/ml.

Cross reactivity: The quality of the antigen used ensures a high specificity and sensitiviy of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Treponema pallidum ELISA (IgG). This ELISA showed no cross reactivity.

Parameter	HSV-1	EBV-CA	CMV	VZV	Adenovirus	RSVi	Parainfl. Pool	Influenza A
n	12	12	12	12	12	12	12	12
Anti-Treponema pallidum ELISA (IgG)	0	0	0	0	0	0	0	0

Parameter	Influenza B	Mycoplasma pn.	Mumps	Measles	Rubella	Toxoplasma gondii	Chlamydia pneu.	Heliiobacter pyl.
n	12	12	12	12	12	12	12	12
Anti-Treponema-pallidum-ELISA (IgG)	0	0	0	0	0	0	0	0

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	45	3.1
2	139	1.7
3	146	1.7

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	43	5.2
2	145	3.2
3	150	2.8

Specificity and sensitivity: 75 clinically characterized patient samples (interlaboratory test samples from INSTAND, Labquality Finland) were examined with the EUROIMMUN Anti-Treponema pallidum ELISA (IgG). The test showed a specificity and a sensitivity of 100% each. Values for 2 of the samples were borderline and were not included in the calculation.

n = 75		**INSTAND / LABQUALITY**		
		positive	borderline	negative
EUROIMMUN ELISA	positive	46	0	0
	borderline	2	0	0
	negative	0	0	27

139 clinically characterized patient samples were examined with the EUROIMMUN Anti-Treponema pallidum ELISA (IgG). The specificity with respect to the reference method was 100%, with a sensitivity of 98.6%.

n = 139		TPHA	
		positive	negative
EUROIMMUN ELISA	positive	137	0
	negative	2	0

Reference range: The levels of the anti-Treponema pallidum antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 0.4 % of the blood donors were anti-Treponema pallidum positive (IgG) which reflects the known percentage of infections in adults.

Limitations of the procedure

The test is not intended to be used for the determination of suitability for transfusion, transplantation or cell administration.

Clinical significance

Treponema pallidum is a helically wound bacteria of the Spirochaeta family [1]. This family includes five genera: borrelia, spirochaeta, cristispira, treponema and leptospira. Treponema pallidum is the causative agent of syphilis or lues, a chronic infectious disease. The subspecies T. pallidum endemicum causes veneric syphilis; T. pallidum pertenue leads to a non-veneric infection occuring in tropical regions called framboesia; T. pallidum carateum is the causative agent of Pinta [2, 3, 4].

In 1905 Fritz Schaudinn (German zoologist, 1871-1906) and Erich Hoffmann (German dermatologist, 1868-1959) at the Charité in Berlin were the first to detect the causative agent of syphilis under the microscope. Spirochaeta were first found In 1913 by the Japanese microbiologist and physician Noguchi Hideyo (1876-1928) in the brain tissue of a patient with progressive paralysis [1].

Syphilis is transmitted from human to human during sexual acts via the mucosa [1, 5]. Indirect transmission by blood transfusions and wounds is also possible. During pregnancy and at birth the baby can become infected by the mother (syphilis connata) [1, 6, 7, 8, 9]. Syphilis is a known risk factor for abortions and stillbirths [7, 10].

Primary stage: The ulcus durum (hard-edged ulcer) is characteristic of the primary lesion of the syphilis (stage I) and normally occurs 3 weeks after infection, developing at the place of entry of the virus (e.g. penis) [1]. It is a painless ulcer, which contains large quantities of the pathogen and is therefore highly contagious. Typically, the defined, limited fibrous or crusted erosion has a raised hard edge. The possible swelling of the regional lymph nodes is painless and the lymph nodes remain displaceable. From that time on, the disease can be diagnosed e.g. using the TPHA test (Treponema pallidum haemagglutination assay). After 2 to 6 weeks the ulcer heals leaving a scar. The infection generally persists and develops into stage II [1].

Secondary stage: Approximately 8 weeks after the infection, the disease manifests itself with flu-like symptoms such as fever, fatigue or head and joint pains. In addition to a generalised swelling of the lymph nodes, 90% of patients show local or generalised skin disorders, which are accompanied by weak or no itching. At first, light pink patches form, which further evolve into hard, coppery nodules (papules). In the foreground are condylomata lata, broad papules, which mainly affect skin folds [1]. The liquid excreted by open and weeping papules is highly contagious. Additionally, various organ disorders may develop, for example, ketaritis, irititis, hepatitis, vasculitis, and myocardial disorders [1].

All skin disorders (syphilids) heal after approximately 4 months. Secondary syphilis is followed by a clinically silent stage (syphilis latens), which can last for years [1].

Tertiary stage: Typical manifestations of a Treponema pallidum infection in stage III are large papules and ulcers on the skin and mucous membranes, as well as organ or visceral syphilis, including gummatous and interstitial inflammation, perivasculitis, cardiovascular syphilis, neurosyphilis (asymtomatic and symptomatic form), osteitis, and periosteitis.

Quaternary stage: Ten to thirty years after an untreated infection, 8% to 10% of patients experience severe neurological disorders such as neurosyphilis with progressive paralysis and Tabes dorsalis with severe mental and vegetational disorders [1].

The **diagnosis** of syphilis is based on clinical findings according to the disease stage, microscopic detection of the infectious agent (dark field), and the serological detection of antibodies against Treponema pallidum [1].

Treponema pallidum pallidum has a length of 5-15µm and a width of 0.2µm with 10-20 turns and can rotate around its longitudinal axis [1]. Due to its fine structure, it is difficult to be made visible under the microscope by staining. However, living bacteria can be investigated using dark field microscopy. Detection in cultures has not yet been achieved [1].

The TPHA (Treponema pallidum haemagglutination assay) is an assay for the indirect determination of antibodies against Treponema pallidum. Erythrocytes marked with proteins and polysaccharides of Treponema pallidum on their surface are mixed with patient serum. The presence of antibodies against Treponema pallidum in the patient serum causes agglutination of the erythrocytes (haemagglutination), which is visible to the naked eye [1, 11, 12].

If the screening test is positive, additional testing is recommended e.g. using an FTA-ABS assay. For further serological diagnosis, modern serological test methods such as anti-Treponema pallidum ELISA, Westernblot, e.g. EUROLINE WB, and IIFT have become widely accepted and have proven successful [1, 7, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34]. Antibodies can be found in the serum and CSF, particularly in children having congenital syphilis [6, 8, 9, 20, 35]. Antibodies against cardiolipin serve as an activity marker of the infection (VDRL or RPR test, EUROLINE WB) [36, 37, 38, 39, 40, 41, 42].

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