Anti-Treponema pallidum WESTERNBLOT (IgM)
Instructions for use

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DY 2111-1601 M DY 2111-2401 M	Treponema pallidum	IgM	Antigen coated membrane strips	16 x 01 (16) 24 x 01 (24)

Indication: The WESTERNBLOT test kit provides a qualitative in vitro assay for human antibodies of the immunoglobulin class IgM against Treponema pallidum in serum or plasma to support the diagnosis of infections with Treponema pallidum and associated diseases (lues).

Principles of the test: The test kit contains test strips with electrophoretically separated antigens of Treponema pallidum. The blot strips will be blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples, specific antibodies of the class IgM (and IgA, 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	Format	Format	Symbol
1. Test strips Single strips with electrophoretically separated Treponema pallidum antigens	16 x 1	24 x 1	STRIPS
2. Evaluation matrix with control strip Test strip incubated with a positive control serum	1 pattern	1 pattern	---
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgM (goat), 10x concentrate	1 x 3 ml	2 x 3 ml	CONJUGATE 10x
4. Universal buffer, 10x concentrate	1 x 50 ml	1 x 100 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	1 x 50 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	3 x 8 channels	TRAY
7. Instructions for use	1 booklet	1 booklet	---

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

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

Waste disposal: Undiluted patient sera and incubated blot strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

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Updates with respect to the previous version are marked in grey.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers. Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels (black)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under: ZD 2111-0101 Evaluation protocol visual Anti-Treponema pallidum WESTERNBLOT.

Preparation and stability of the reagents

Note: This test kit may only be used by trained personnel. Test strips and incubation trays are intended for single use. The bag containing the blot strips is printed with a number in addition to the test kit lot number. This number refers to the strip batch and is also printed on the corresponding evaluation template. These two numbers must match to ensure correct evaluation of test results.
All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, if earlier, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the packing with the test strips only when the strips have reached room temperature (+18 °C to +25 °C) to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2°C to +8°C. To ensure correct evaluation of results, the lot number on the bag must match the lot number on the strips as well as on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working-strength enzyme conjugate the required amount should be removed from the bottle using a clean pipette tip and diluted 1:10 with working-strength diluted universal buffer. For 1 test strip dilute 0.15 ml anti-human IgM concentrate with 1.35 ml working-strength diluted universal buffer. The working-strength diluted enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working-strength universal buffer the required amount should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 1.5 ml buffer concentrate should be diluted with 13.5 ml deionised or distilled water. The working-strength diluted buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light .

Warning: The control of human origin, used to incubate the test strip on the evaluation protocol, has 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 sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the serum or plasma 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: The **patient samples** for analysis are diluted **1:51** in ready for use diluted universal buffer using a clean pipette tip. For example, add 30 µl of sample to 1.5 ml ready for use diluted universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

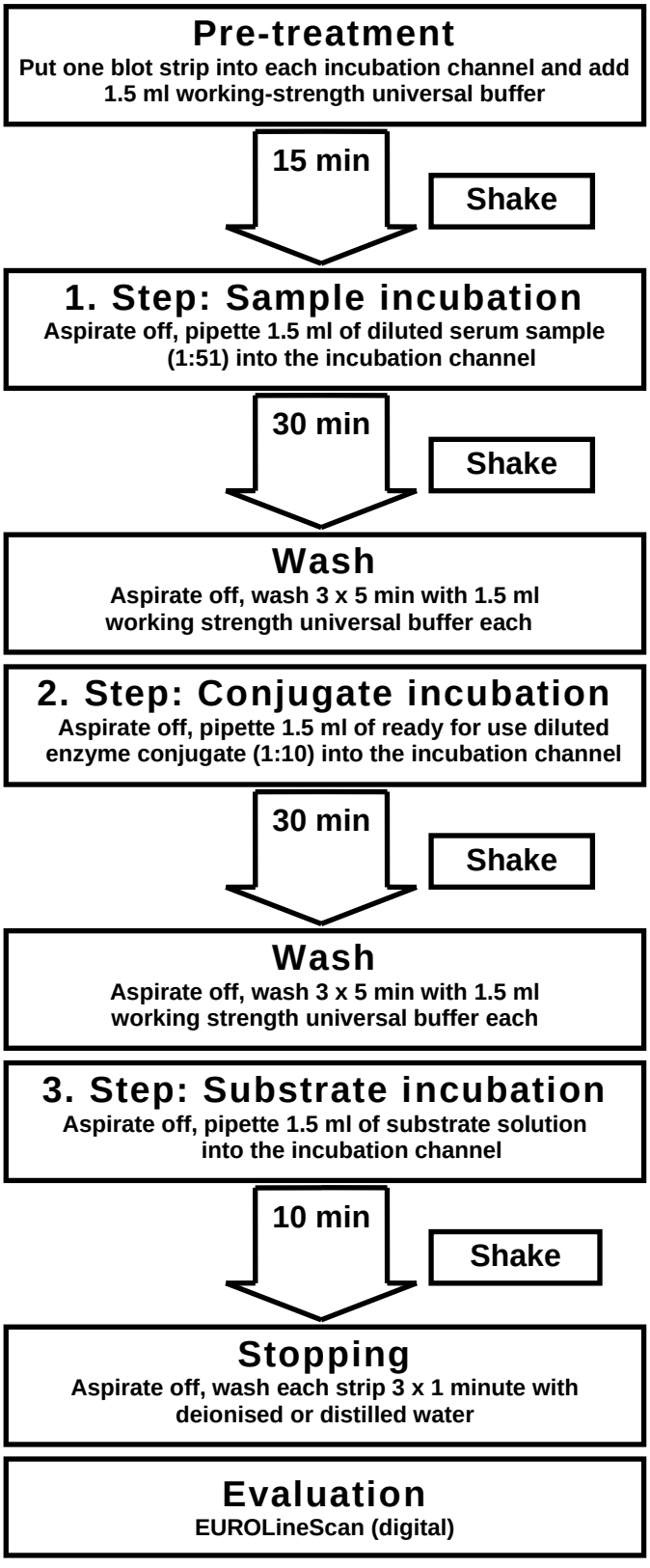
Blocking:	According to the number of serum samples to be tested fill each channel of the incubation tray with 1.5 ml ready for use diluted universal buffer and a blot strip. Remove the required amount of blot strips from the packing using a pair of tweezers. (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip. Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml ready for use diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgM) into each channel. Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

For automated incubation with the EUROBlotMaster select the program **Euro02 Inf WB30**.

For automated incubation with the EUROBlotOne select the program **Euro01/02**.

Anti-Treponema pallidum WESTERNBLOT (IgM)

Incubation protocol



Anti-Treponema pallidum WESTERNBLOT (IgM)
Evaluation and Interpretation

Handling: For evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN). The code for entering the **Test** in EUROLineScan is **T_pal_WB_IgM**.

If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must match the lot number on the blot strips.** Clearly recognisable bands on the blot strips which concur with the labelled bands on the evaluation matrix are noted in the evaluation protocol.

Antigens: The antigen source for the **EUROIMMUN Anti-Treponema pallidum WESTERNBLOT** is provided by particularly suitable Treponema pallidum antigen preparations. These antigens have been separated using discontinuous polyacrylamide gel electrophoresis according to molecular masses and transferred onto nitrocellulose membrane. From each nitrocellulose membrane, control blot strips have been removed and incubated with a reference sera. One of these stained strips is included in the kit, the other remains with EUROIMMUN for documentation purposes.

Specificity of the antigens on the test strips:

Band	Antigen	Specificity
47 kDa	membrane protein, TpN47	**specific**
45 kDa	tmpA	**specific**
17 kDa	membrane protein, TpN17	**specific**
15 kDa	membrane protein, TpN15	**specific**

In the lower part of the test strip there is a conjugate control membrane chip (IgA, IgG and IgM). Below the conjugate control, there is a membrane chip with a control band (Control).

Attention: A correctly performed determination of antibodies of class IgM against the antigens described above is indicated by a positive reaction of the control band and a positive reaction of the IgM band.

If one of these bands only shows a very weak reaction or none at all, the result is not valid.

IgG class antibodies against Treponema pallidum

Interpretation of results: The results of the Anti-Treponema pallidum WESTERNBLOT test can be divided into negative, borderline and positive results. In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration, as negative sera sometimes produce weak signals in individual bands.

Result	Characteristics
Negative	No bands of specific antigens.
Borderline	One distinctive band of the specific antigens: p15 kDa, p17 kDa, p45 kDa or p47 kDa.
Positive	More than one distinctive band of the specific antigens: p15 kDa, p17 kDa, p45 kDa or p47 kDa.

IgM class antibodies against Treponema pallidum

Interpretation of results: The results of the Anti-Treponema pallidum WESTERNBLOT test can be divided into negative, borderline and positive results. In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration, as negative sera sometimes produce weak signals in individual bands.
For the diagnosis of a fresh Treponema pallidum infection, a positive IgM result should be confirmed with a positive IgG result using a fresh blood sample 3 to 6 weeks later.

Result	Characteristics
Negative	No bands of specific antigens.
Borderline	One weak band of the specific antigens: p15 kDa, p17 kDa, p45 kDa or p47 kDa. It is recommended that a fresh sample be taken and the test repeated after a few weeks.
Positive	At least one distinctive band of the specific antigens: p15 kDa, p17 kDa, p45 kDa or p47 kDa.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The Westernblot is a qualitative method. No measurement range is provided.

Inter- and intra-assay variation: The inter-assay and intra-assay variation were determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This Westernblot displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera showed no effect on the analytical results.

Cross reactions: The quality of the antigen substrates and the antigen source guarantee a high specificity of the Westernblot. The measurement of cross reactions is not necessary for Westernblots because with this test system a direct differentiation between specific and unspecific antigens is possible.

Specificity and sensitivity: A panel of 19 serologically defined samples, pre-characterised by an approved reference test (certified by the Paul-Ehrlich Institute) and 50 samples of healthy blood donors, all with negative results in TPHA test, were investigated with the EUROIMMUN Anti-Treponema pallidum Westernblot.

IgG		**Characterised panel** (see above)	
	n = 69	**positive**	**negative**
EUROIMMUN Anti-Treponema pallidum Westernblot	**positive**	**19**	**0**
	negative	**0**	**50**

The Anti-Treponema pallidum Westernblot IgG has a specificity of 100% and a sensitivity of 100%. The positive predictive value is 100%.

IgM		Characterised panel (see above)	
	n = 69	positive	negative
EUROIMMUN Anti-Treponema pallidum Westernblot	positive	17	1
	negative	0	51

The Anti-Treponema pallidum Westernblot IgM has a specificity of 98% and a sensitivity of 100%. The positive predictive value is 94%.

Neither IgM nor IgG antibodies against Treponema pallidum were detected in the samples of healthy blood donors.

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 bacterium 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 called framboesia occuring in tropical regions; 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, asympto-matic 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 haem-agglutination 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 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, keratitis, 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.

Literature

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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.