Instructions for Use

Treponema pallidum µ-capture IgM ELISA

Enzyme immunoassay for the detection of *Treponema pallidum* specific IgM antibodies in human serum expressed in capture index.

REF **RE58811**

Σ **12x8**

2-8°C

EU: IVD CE U.S.: *For research use only. Not for use in diagnostic procedures.*

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1. INTENDED USE

The *Treponema pallidum* IgM ELISA kit is an enzyme immunoassay for the detection of *Treponema pallidum* specific IgM antibodies in human serum and is used as an aid in the diagnosis of Syphilis infections. The assay must be performed strictly in accordance with the instructions set out in this instruction for use. No responsibility can be held for any loss or damage (except as required by statute) how so ever caused by or arising out of non-compliance with the instructions provided.

2. SUMMARY AND EXPLANATION

The chronic infection Syphilis is caused by the spirochete *Treponema pallidum*. During early primary Syphilis IgM class antibodies appear in the blood. In the secondary phase *T. pallidum* antibodies of both IgM and IgG classes reach peak titers. Thereafter the developed *T. pallidum* IgG antibodies remain present in the blood irrespective the course of the disease. *T. pallidum* specific IgM serum levels are related to disease activity. In the late phases of Syphilis serum IgM remain demonstrable. Successful treatment induces a decrease of IgM levels. Successful treatment of Syphilis in the primary phase shows a more rapid IgM decrease than in a late phase of the disease. A significant decrease in *T. pallidum* IgM titer can be expected within 3-4 months following successful treatment and will disappear usually within 2 years, merely depending on the assay in use. *T. pallidum* specific IgM is commonly detected by the fluorescent treponemal antibody test with absorption in combination with removal of IgG (19S IgM FTA-Abs). It is specific, sensitive, but very laborious. It cannot be automated and only a limited number of samples can be processed at one time. The *T. pallidum* IgM ELISA is based on the IgM class capture principle making use of the *T. pallidum* antigens covalently labelled to peroxidase. It has the advantages of a modern ELISA: the analyte can be quantitative measured, it fits to open automates for ELISA processing and large numbers of serum specimens can be run at one time. In combination with the IBL *T. pallidum* IgG ELISA, the IBL *T. pallidum* IgM ELISA can be used in the differential diagnosis between past (*T. pallidum* IgM negative and IgG positive) and active (or recent) syphilis infection (*T. pallidum* IgM positive). A decline in IgM levels can be used to monitor success of therapy.

3. TEST PRINCIPLE

The *Treponema pallidum* IgM ELISA is an antibody class capture immunosorbent assay for the detection of *Treponema pallidum* specific IgM in human serum. Rabbit antibody specific for the µ-chain of human antibodies is coated to the solid phase of the microtiter strip wells. These antibodies will bind specifically human IgM present in each serum: capture of the IgM. Purified *T. pallidum* antigens labelled covalently to peroxidase (the conjugate) will complex to captured *T. pallidum*-specific IgM. The conjugate will act as the indicator for the immunological reaction between the *T. pallidum* antigen in the conjugate and the *T. pallidum* specific human IgM captured, coated on the wells of the microtiterplate. TMB that acts as a chromogen will induce colour proportionally to the amount of *T. pallidum* specific IgM captured.

4. WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the IBL-Homepage.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the microtiter strips in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma

Either human serum or plasma may be used. Samples must not be hemolysis, nor contain particulate material. To obtain sera for the detection of antibodies, patient blood should be drawn and allowed to clot at room temperature. Centrifuge within one day, transfer the serum into a vial.

Storage:	2-8°C	-20°C - -70°C	Keep away from heat or direct sun light.
Stability:	7 d	> 7 d	Avoid repeated freeze-thaw cycles.

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiterplate** Break apart strips. Coated with polyclonal anti-IgM.
1 x 0.25mL	ENZCONJ CONC	**PO labeled *T. pallidum* conjugate (100 x concentrated)** *T. pallidum* antigen covalently labeled with peroxidase, PBS buffer, BSA and preservatives.
1 x 1.8mL	CONTROL +	**Positive control (ready to use)** Red colored: human serum, highly reactive for IgM against *T. pallidum* prediluted in PBS buffer, BSA, preservatives.
1 x 1.8mL	CONTROL -	**Negative control (ready to use)** Yellow colored: human serum, non reactive for IgM antibodies to *T. pallidum* prediluted in PBS buffer, BSA, preservatives.
2 x 1.5mL	CUT OFF ±	**Cut-off control (ready to use)** Green colored: human serum, with a low reactivity for IgM to *T. pallidum* prediluted in PBS buffer, preservatives.
1 x 0.25mL	CAg CONC	**Control Antigen (100 x concentrated)** Control antigen, obtained from sonicated Hep-2 cells in PBS, BSA and preservatives.
1 x 15mL	TMB SUBS	**TMB Substrate Solution (ready to use)** Contains: TMB, Buffer, stabilizers.
1 x 60mL	WASHBUF CONC	**Wash Buffer (20 x concentrated)** Contains: PBS buffer, Tween® 20 and preservatives.
1 x 120mL	DILBUF	**Diluent Buffer (ready to use)** Blue colored. Contains: PBS Buffer, detergents, BSA, stabilizers.
1 x 20mL	TMB STOP	**TMB Stop Solution (ready to use)** Contains: 0.5 M H_2SO_4.
2 x	BAG	**Plastic Bag** Resealable. For dry storage of non-used strips and incubation.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

- Pipettes to deliver volumes between 10 µL and 1000 µL (trueness ± 2%, precision 1 %)
- Volumetric laboratory glassware
- Deionised (or distilled) water
- Incubator thermostatically controlled at 37°C ± 1ºC
- Clean disposable tubes for diluting patients sera (capacity appr. 3 mL)
- Clean disposable tubes for diluting conjugate and TMB (capacity 12 mL)
- Automatic plate washer (optional) with dispense volume 300-350 µL, wash cycle = 5 times

- Microtiter plate reader, equipped for measuring absorbances at 450 nm (optionally equipped for dual wavelength measurement at 450 and 620 nm), absorbance range; 0.0 to 3.0 absorbance units
- Vortex tube mixer
- Timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-23 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch.

10. PRE-TEST SETUP INSTRUCTIONS

Bring all reagents to room temperature (18-23 °C) before assaying. Perform all assay steps in the order given and without any appreciable delays between the steps. Check the expiry date before use.

10.1. Preparation of Components

Amounts for reagents are given for one complete microtiter plate.

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
60 mL	WASHBUF CONC	with 1140 mL	bidist. water	1:20	-	18 - 23°C	7 days
						2 – 8°C	1 month

Dilution scheme for preparation of Enzyme conjugate (working solution).			
Number of **strips** in use	**Dilution buffer** **DILBUF** (BLUE)	***Enzyme conjugate*** **ENZCONJ CONC** (100x)	**CAg / Control Antigen** **CAg CONC** (100 x)
1	1.5 mL	15 µL	15 µL
2	3.0 mL	30 µL	30 µL
6	9.0 mL	90 µL	90 µL
12	18.0 mL	180 µL	180 µL

Note: The diluted conjugate can be used for 5 days after preparation, provided it is stored at 2-8°C. Validation criteria should be met in all runs.

10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum / Plasma	generally	**DILBUF**	1:101	e.g. 10 µL + 1000 µL

11. TEST PROCEDURE

1.	Dispense **100 µL** of the **controls** and **diluted samples** into the respective wells of the Microtiter Plate. We recommend to pipette positive (RED) and negative control (YELLOW) in duplicates and cut-off control (GREEN) in quadruplicate.
2.	**Incubate** the wells in the resealable bag or in a 100% moist atmosphere **for 60 min** at **37°C**.
3.	**Prepare** the needed volume *T. pallidum*-PO conjugate (see 10.1.)
4.	When incubation has completed, discard incubation solution. Wash plate **5 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **150 µL** of **Enzyme Conjugate (ready to use)** into each well.
6.	**Incubate** the wells in the resealable bag or in a 100% moist atmosphere **for 60 min** at **37°C**.
7.	When incubation has completed, discard incubation solution. Wash plate **5 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
8.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
9.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
10.	**Incubate 30 min** at **18-23°C** in the dark.
11.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
12.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **10 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated on the **QC Certificate**. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

13. CALCULATION OF RESULTS

1. Calculate the mean absorbance value of the cut-off control, the positive control and the negative control.
2. The abundance of *T. pallidum* specific IgM is determined by calculating the Capture Index (C.I.). Divide the absorbance value of a patient sample by the mean absorbance of the cut-off control:

$$\textbf{\textit{T. pallidum} IgM Capture Index} = \frac{\text{(Mean) absorbance (OD) of control or patient sample}}{\text{(Mean) absorbance (OD) of the Cut-off control}}$$

Note: Use not more than one decimal to express the abundance. Be sure to compare the absorbance value of each patient sample with the cut-off value computed for the plate containing the sample.

14. INTERPRETATION OF RESULTS

To estimate (active or recent) *Treponema pallidum* infection by serology interpretation of the abundance of *Treponema pallidum* antibodies in a serum is as follows:

RESULTS	INTERPRETATION
POSITIVE	A serum should be considered positive for *T. pallidum* specific IgM antibodies when the Capture Index is **≥ 1.1**. Interpretation needs to be done with care as indicated in section 15, Limitations of the assay.
NEGATIVE	A serum should be considered negative for *T. pallidum* specific IgM antibodies when the Capture Index is **< 0.9**. Interpretation needs to be done with care as indicated in section 15, Limitations of the assay.
EQUIVOCAL	A serum may be considered equivocal, if the *T. pallidum* IgM Capture Index is between **0.9 and 1.1**. In such case it is advised to confirm the results by testing that serum again in duplicate. In the case the repeated result is again equivocal, a second serum should be tested and judged for a change in result (as expressed in C.I.)

15. LIMITATIONS OF THE PROCEDURE

- Appropriate medical decisions are only possible if the medical traceability is ensured. The product is intended for professional use as an aid in the diagnosis of active Syphilis infections.
- Bacterial contamination or repeated freeze-thaw cycles of the specimens may affect the absorbance values of the samples with consequent alterations of IgM antibody to *T.pallidum* levels.
- Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration clinical history, symptomatology, as well as serological data. Serological data, however, have restricted value in immunosuppressed patients.
- The performance characteristics mentioned in section 16 are acquired with the utmost care. However, a negative result does not totally exclude a recent *T.pallidum* infection. Therefore results need to be interpreted with caution.
- To estimate (primary or recurrent) *T.pallidum* infections by serology it is advised to test serum pairs. The second serum of a pair can be drawn 14-21 days after the first serum is obtained. Each serum pair should be tested at the same day and in the same test to allow interpretation of significant antibody level differences. It is advised to perform a combination of IgM and IgG testing.
- This test is suitable only for investigating single samples, not sample pools.
- In a clinical evaluation this *Treponema pallidum* IgM ELISA showed reactivity with some of the following interferent sera: ANA-IgM, Epstein Barr Virus IgM, Borrelia IgM and Parvo-B19 IgM.

16. PERFORMANCE

When the kit is employed according to the instructions given, and the appropriate equipment is used in optimal conditions, the following performances could be reached. The performance of the kit can be expressed by different parameters namely assay precision, analytical specificity, diagnostic specificity and diagnostic sensitivity.

16.1. Assay precision

Different samples containing different levels of the parameter determined, were assayed to assess repeatability and reproducibility of the assay (within- and between-assay variability). The assay precision computed on these samples gives coefficient of variation values lower then 11%.

16.2. Analytical specificity

Analytical specificity may be defined as the ability of the assay to detect specific analyte in presence of potentially interfering factors in the sample matrix (e.g. anticoagulants, haemolysis, effects of sample treatment. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not significantly affected by either anticoagulants (EDTA), slight heamolysis or freezing. Next to this, sera marked positive for IgM to various infectious agents were tested: EBV (n=5), CMV (n=5), Borrelia (n=10), ANA (n=10), Rheumafactor (n=10), Cardiolipin (n=10) and Parvo B19 (n=6).

No reactivity was shown with Rheumafactor positive sera. In some samples marked positive for the other parameters, reactivity was detected.

16.3. Diagnostic specificity

Diagnostic specificity was assessed internally by testing 100 samples originating from pregnant women of which the *T. pallidum* particle agglutination assay was negative (TPPA titer <1:80) and from 90 donor samples. Samples with an equivocal result (n=2, in pregant women) were considered negative. Diagnostic specificity is the probability of the assay procedure of scoring negative in samples of non-infected cases:

$$\textit{Diagnostic specificity} = \frac{TN}{TN + FP}$$

TN = True Negatives, FP = False Positives

Out of 100 samples of the pregnant women and expected to be negative, the kit proved to be true negative in all 100 cases, giving a diagnostic specificity of 100% in this group. In the group of donor samples 1 sample was found positive, giving a diagnostic specificity of 99% in this group.

16.4. Diagnostic sensitivity

Diagnostic sensitivity was assessed by testing 83 samples selected from patients with active or recent Syphilis as documented with the presence of IgM by 19S IgM FTA Abs assay and other clinical and laboratory data. The patients were documented as primary (n=11), secundary (n=17), tertiary (n=2), reinfections (n=5) and undocumented *T. pallidum* infections (n=48). Diagnostic sensitivity is the probability of the assay procedure of scoring positive in samples of infected cases:

$$\textit{Diagnostic sensitivity} = \frac{TP}{TP + FN}$$

TP = True Positives, FN = False Negatives

Samples with an equivocal result (n=3) were considered negative. In this group of 83 samples 76 were marked positive by 19S IgM FTA Abs test for *Treponema pallidum* IgM of which 74 samples were tested true positive in the assay and 2 false negative, therefore the diagnostic sensitivity is 97.4%.

16.5. Patients with latent Syphilis

The *Treponema pallidum* IgM reactivity in patients with Syphilis in the latent stage were tested in 52 samples, including 19 follow up samples. The *Treponema pallidum* IgM µ-capture ELISA detected reactivity in 11 samples, whereas 19S IgM FTA Abs detected reactivity in 28 samples. The agreement between the two assays was 58%.

16.6. Patients with cured Syphilis

The *Treponema pallidum* IgM reactivity in patients with a cured Syphilis were tested in 52 samples. The *T.pallidum* IgM assay detected reactivity in 3 samples. Thus in 94% of samples from cured Syphilis the *Treponema pallidum* IgM µ-capture ELISA agreed with the clinical status.

17. TRACABILITY OF CONTROLS

The level of the cut-off control as presented in this kit, represents the level as used in the clinical trials as shown above. This is organised such that a manufacturer's working reference is maintained to which manufacturer's product reference is calibrated. This manufacturer's product reference is used for validating kit performance. In this way the sensitivity and specificity of each lot represents that as shown above.

18. LITERATURE

1. Primary evaluation of the Meddens Diagnostics *Treponema pallidum* IgM and *Treponema pallidum* IgG ELISA (May 1997) Scientific Product Support, Meddens Diagnostics, SPS TP001.
2. Hagendorn. Prof.dr. July 2003. The evaluation of the clinical performance of the Meddens Diagnostics *T.pallidum* IgM assay. Sci3entific Product Support TP005.
3. Pinsky NA, Huddleston JM, Jacobson RM, Wollan PC, Poland GA. 2003. Effect of Multiple Freeze-Thaw Cycles on Detection of Measles, Mumps, and Rubella Virus Antibodies. Clin.Diagn.Lab. mmunol 10(1): 19-21.

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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