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User's Manual

Syphilis (TPA) IgM ELISA Kit

REF **DEIA2539NS**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Syphilis (TPA) IgM Enzyme Immunoassay Kit provides materials for the qualitative and semiquantitative determination of IgM-class antibodies to Treponema pallidum in human serum and plasma. This assay is intended for research use only.

General Description

Spirochetes are motile bacteria with a periplasmatic axial filament. All pathogenic species belong to the family Treponemataceae, which includes the three genera: Treponema, Borrelia, and Leptospira. The Treponema are motile bacteria, 5-15µ in length and 0.2µ in width, containing about 10 flexible, undulating, spiral shaped rods. Treponema pallidum, the causative agent of Syphilis, is transmitted by direct contact, usually through sexual intercourse. Syphilis along with Gonorrhoea, Chancroid and Lymphogranuloma venereum, designated as a venereal disease, or VD, is an acute and chronic infectious disease. After an incubation period of 12-30 days, the first symptoms to appear are chancres, soon followed by syphilitic ulcers which then spontaneously disappear in a few weeks. During this first stage (primary syphilis) the Treponema pallidum propagates in related lymph nodes to be distributed to the whole body stream. Three further stages of disease follow which are classified as secondary, tertiary, and quaternary syphilis. Treatment with antibiotics at the earliest disease stage and prophylactic measures are ways to prevent epidemics. For this purpose, antenatal and donor blood screenings are mandatory in most of countries around the world.

Principles of Testing

The CD Syphilis (TPA) IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA)

This ELISA is using a RF-Sorbent.

The Rheumatoid factor (RF) is a special autoantibody form. These are auto antibodies, which are directed against the Fc fragment of human lgG.

The RF autoantibodies are mostly class lgM, but may also be class IgA, IgG or lgE Rheumatoid factors are associated with rheumatoid arthritis. But they can also be detectable In other diseases(e.g. tuberculosis, salmonellosis, syphilis, etc.,) and even In healthy individuals. In about 5% of all healthy people, elevated RF values can be found; the titer Increases with increasing age.

The use of anti-human lgG antibodies in the RF-sorbent prevents false positive or false negative results.

Samples are diluted with Sample Diluent and additionally incubated with IgGRF-Sorbent, containing hyperimmune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors. This pretreatment avoids false negative or false positive results.

Microtiter wells as a solid phase are coated with Treponema pallidum antigen. Pretreated patient specimens and ready-for-use controls are pipetted into these wells. During incubation Treponema pallidum-specific antibodies of positive specimens and controls are bound to the immobilized antigens.

After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate

binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.

The intensity of this color is directly proportional to the amount of Treponema pallidum-specific IgM antibody in the specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells: 12×8 (break apart) strips, 96 wells; Wells coated with Treponema pallidum antigen. (incl. 1 strip holder and 1 cover foil).

2. Sample Diluent: 1 vial, 100 mL, ready to use, colored yellow; pH 7.2 ± 0.2.

3. IgG-RF-Sorbent: 1 vial, 6.5 mL, ready to use, colored yellow; Contains antihuman IgG-class antibody.

4. Pos. Control: 1 vial, 2.0 mL, ready to use; colored yellow, red cap.

5. Neg. Control: 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.

6. Cut-off Control: 1 vial, 2.0 mL, ready to use; colored yellow, black cap.

7. Enzyme Conjugate: 1 vial, 20 mL, ready to use, colored red, antibody to human IgM conjugated to horseradish peroxidase.

8. Substrate Solution: 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).

9. Stop Solution: 1 vial, 14 mL, ready to use, contains 0.2 mol/l H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns

10. Wash Solution: 1 vial, 30 mL (20× concentrated for 600 mL), pH 6.5 ± 0.1 see "Reagents Preparation".

* Contains non-mercury preservatives

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620nm ±10 nm) DAR 800.
2. Calibrated variable precision micropipettes.
3. Incubator 37°C.
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionized or (freshly) distilled water
7. Timer
8. Absorbent paper

Storage

When stored at 2°C to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil

bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

Specimen Collection And Preparation

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay.

DO not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

1. Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

2. Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

3. Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 3 days at 2°C to 8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

3. Specimen Dilution

Prior to assaying each patient specimen is first to be diluted with Sample Diluent. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with IgG-RF-Sorbent

a. Dilute each patient specimen 1+50 with Sample Diluent; e.g. 10 µL of specimen +0.5 mL of Sample Diluent. Mix well.

b. Mix well the IgG-RF-Sorbent before use.

c. Dilute this prediluted sample 1+1 with IgG-RF-Sorbent e.g. 60 µL prediluted sample + 60 µL IgG-RF-Sorbent. Mix well.

d. Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.

e. Take 100 µL of these pretreated samples for the ELISA.

Please note: Controls are ready for use and must not be diluted!

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

1. Wash Solution

Dilute Wash Solution 1+19 (e.g. 10 mL + 190 mL) with fresh and germ-free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.

Consumption: ~ 5 mL per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.

The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

2. Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

3. Damaged Test Kits

In case of any severe damage to the test kit or components, CD has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

Assay Procedure

General Remarks

1. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
7. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
8. During 37°C incubation cover microtiter strips with foil to avoid evaporation.

Procedure

Prior to commencing the assay, dilute Wash Solution, prepare samples and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least: 1 well (e.g.A1) for the Neg. Control, 2 wells (e.g. B1+C1) for the Cut-off Control and 1 well (e.g. D1) for the Pos. Control. It is left to the user to determine controls and samples in duplicate.
2. Dispense

   100 µL of Neg. Control into well A1

   100 µL of Cut-off Control into wells B1 and C1

   100 µL of Pos. Control into well D1 and

   100 µL of each preatreated sample with new disposable tips into appropriate wells.

3. Cover wells with foil supplied in the kit. Incubate for 60 minutes at 37 °C.
4. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!**
5. Dispense 100 µL Enzyme Conjugate into each well.
6. Incubate for 30 minutes at room temperature (20 °C to 25 °C). **Do not expose to direct sun light!**
7. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µL of Substrate Solution into all wells.
9. Incubate for exactly 15 minutes at room temperature (20°C to 25°C) in the dark.
10. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well. Any blue color developed during the incubation turns into yellow. **Note: Highly positive samples can cause dark precipitates of the chromogen!**
11. Read the optical density at 450/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.
12. Measurement

    Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

    Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable calculate the mean absorbance values of all duplicates.

Quality Control

It is recommended to use control samples according to state and federal regulations.

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or CD directly.

Calculation

Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

Neg. Control in A1: Absorbance value lower than 0.200

Cut-off Control in B1/C1: Absorbance value between 0.350 - 0.850

Pos. Control in E1: Absorbance value between 0.650 - 3.000

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in B1/C1).

Example: (0.44 + 0.46) / 2 = 0.45 = CO

Interpretation Of Results

POSITIVE Sample (mean) absorbance values more than 10 % above CO (Mean OD patient > 1.1 × CO)

GREY ZONE Sample (mean) absorbance values from 10 % above to 10 % below CO

repeat test 2-4 weeks later - with new samples ($0.9 \times CO \leq \text{Mean OD patient} \leq 1.1 \times CO$)

Results in the second test again in the grey zone ⇒ **NEGATIVE**

NEGATIVE Sample (mean) absorbance values more than 10 % below CO

(Mean OD patient < 0.9 × CO)

Results in CD Units

(Patient (mean) absorbance value × 10)/CO = [CD Units]

Example: (1.580 × 10)/0.45 = 35 CD Units

Result:

Cut-off value: 10 CD Units

Grey zone: 9 - 11 CD Units

Negative: < 9 CD Units

Positive: > 11 CD Units

Precision

Intra-assay

The intra-assay (within-run) precision of the CD Syphilis (TPA) IgM ELISA was determined by 20× measurements of 12 serum samples covering the whole measuring range.

Sample	Mean OD_{450}	Intra-Assay CV (%)	n
1	0,37	8,30	20
2	0,24	9,64	20
3	0,51	8,65	20
4	0,94	6,29	20
5	0,94	7,11	20
6	0,67	6,51	20
7	1,30	3,72	20
8	1,35	3,42	20
9	1,44	3,35	20
10	1,96	2,48	20
11	2,08	2,85	20
12	1,62	4,75	20

Inter-assay

The inter-assay variation of the CD Syphilis (TPA) IgM ELISA was determined with 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean OD_{450}	Inter-Assay CV (%)	n
1	1,86	2,75	40
2	1,20	3,31	40
3	1,44	2,69	40

Detection Range

The range of the assay is between 0.52 - 60 CD Units/mL.

Sensitivity

The analytical sensitivity of the CD ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.52 CD Units/mL (OD450 = 0.025).

Specificity

The antigen used for the Syphilis (TPA) IgM ELISA shows no crossreactivity to Epstein Barr Virus (VCA), Mycoplasma pneumonia, and Borrelia burgdorferi IgM antibodies.

Linearity

Three samples (serum) containing different amounts of analyte were serially diluted with sample diluent and assayed with the CD ELISA. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Serum 1	Serum 2	Serum 3
Concentration	DU/mL	42,66	34,23	44,58
Average % Recovery		97,81	104,93	92,09
Min Recovery	from	86,59	95,07	85,98
Max Recovery	to	112,41	114,17	97,53
Status Linearity (100 +/-15%)		**passed**	**passed**	**passed**

Interferences

In general, haemolytic, icteric or lipaemic samples should be avoided, but can be tolerated up to at least 4 mg/mL haemoglobin, 0.5 mg/mL Bilirubin, and 30 mg/mL triglycerides.

None of the following samples with interference factors will interfere with the ELISA: samples with rheumatoid factor, samples with pregnancy hormones, samples with tumor marker (CYFRA, CA-72-4, CA-21-1, CA-15-3), samples with HAMA, samples with ANA and samples from elderly with high amount of proteins.

Precautions

1. This kit is for in research 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.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with Stop Solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
5. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
6. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided
7. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
8. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
9. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
10. Allow the reagents to reach room temperature (21°C – 26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
11. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
12. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
13. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
14. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety

guideline or regulation.

15. Do not use reagents beyond expiry date as shown on the kit labels.
16. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
17. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. In immunocompromised patients and newborns serological data only have restricted value.