

DRG® Treponema Pallidum (Syphilis) IgG recombinant (EIA-3798)



Revised 11 June 2007

RUO in the USA

1. INTRODUCTION

Spirochetes are motile bacteria with a periplasmic 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.

Species	Disease	Symptoms	Mechanism of Infection
Treponema pallidum	Syphilis	Skin eruption of various appearances with mucous patches Formation of gumma, cardiovascular lesions Lesions in central nervous system	Transmission by direct contact: - acquired syphilis: sexual transmission - congenital syphilis: transmission through the placenta where the fetus is transfected

Infection may be identified by:

- Microscopy: darkfield examination in primary stages by demonstrating motile treponemes in a channel
- Serology: Detection of antibodies utilizing lipoidal antigens (RPR, VDRL) or TPHA, Fluorescent Treponemal Antibody Absorption Test (FTA-ABS)

2. INTENDED USE

The DRG Treponema pallidum IgG ELISA (recombinant) is intended for the qualitative determination of antibodies against Treponema pallidum antigens in human serum. This assay offers increased diagnostic specificity and sensitivity by employing purified recombinant antigens. The assay can be used for diagnosis of Treponema pallidum infections in health care institutions and for screening in blood banks.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of antibodies against Treponema pallidum is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with Treponema pallidum recombinant antigens r15, r17, r44 to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Protein A conjugate is added. This conjugate binds to the captured Treponema pallidum-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Treponema pallidum specific

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antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS**4.1. Reagents supplied**

- **Treponema pallidum Coated Wells:** 12 breakapart 8-well snap-off strips coated with recombinant Treponema pallidum antigen; vacuum sealed, in resealable aluminium foil.
- **IgG Sample Diluent***:** 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2 ; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.):*** 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Treponema pallidum Protein A Conjugate**:** 1 bottle containing 20 ml of peroxidase Protein A; coloured red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Treponema pallidum Positive Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.
- **Treponema pallidum Cut-off Control***:** 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.
- **Treponema pallidum Negative Control***:** 1 bottle containing 2 ml; coloured yellow; ready to use; blue cap.

* contains 0.01 % Kathon after dilution

** contains 0.2 % Bronidox L

*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

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It is very important to bring all reagents, samples and controls to room temperature (20...25°C) before starting the test run!

6.1. Coated Snap-off Strips

The ready to use breakapart snap-off strips are coated with recombinant Treponema pallidum antigen. Store at 2...8°C. The strips are vacuum sealed. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Treponema pallidum Protein A Conjugate

The bottle contains 20 ml of a solution with Protein A, horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. Protein A is an immunoglobulin Fc-binding protein with a molecular weight of 42,000 Dalton. The solution is ready to use. Store at 2...8°C. *After first opening stability until expiry date when stored at 2...8°C.*

6.3. Controls

The bottles labelled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2...8 °C.*

6.4. IgG Sample Diluent

The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2...8 °C.*

6.5. Washing Solution (20xconc.)

The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for at least four weeks if stored at 2...8°C. *Crystals in the solution disappear by warming up to 37 °C in a water bath.*

6.6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged. After first opening stability until expiry date when stored at 2...8 °C.*

6.7. Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C. *After first opening stability until expiry date.*

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. *Positive and negative controls are ready to use and must not be diluted.*

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RUO in the USA**8. ASSAY PROCEDURE****8.1. Test Preparation**

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. 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 substrate blank, |
| 1 well (e.g. B1) | for the negative control, |
| 2 wells (e.g. C1+D1) | for the cut-off control and |
| 1 well (e.g. E1) | for the positive control. |

It is recommended to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to $37^{\circ} \pm 1^{\circ}\text{C}$.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour \pm 5 min at $37 \pm 1^{\circ}\text{C}$.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
5. Dispense 100µl Treponema pallidum Protein A Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature. Do not expose to direct sunlight.**
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Any blue colour developed during the incubation turns into yellow.

Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in DU by 2.

11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

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8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

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.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **lower than 0.100**.
- **Negative control** in B1: Absorbance value **lower than 0.200**.
- **Cut-off control** in C1 and D1: Absorbance value **between 0.250 and 0.900**.
- **Positive control** in E1: Absorbance value equal to or greater than the cut-off value.

9.2. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

$$\text{Cut-off} = 0.43$$

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative

→ **grey zone**

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in DRG Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = [\text{DRG-Units} = \text{DU}]$$

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ DU (DRG Units)}$

Cut-off:	10	DU
Grey zone:	9-11	DU
Negative:	<9	DU
Positive:	>11	DU

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RUO in the USA**10. SPECIFIC PERFORMANCE CHARACTERISTICS****10.1. Precision**

Interassay	n	Mean	Cv (%)
Pos. Serum	4	2.444	5.1
Intraassay	n	Mean	Cv (%)
Pos. Serum	8	2.621	1.5%

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is >98 %.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is >98 %.

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromised patients and newborns serological data only have restricted value. It is recommendable testing positive results with an alternative test system.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use. In the United States, this kit is intended for Research Use Only.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.

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- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

WARNING: In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. LITERATURE

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