



Sífilis

ELISA recombinante v.4.0

Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies anti-*Treponema pallidum*

SUMMARY

Syphilis is a venereal disease caused by the *Treponema pallidum*, which invades intact mucous membranes or damaged areas of the skin. Sexual contact is the most common way of transmitting this disease. However, it may be transmitted through the placenta from the mother to the fetus, or through blood transfusion.

Detection and treatment of the illness in early stages is essential to avoid severe complications as neurosyphilis, cardiovascular syphilis and congenital syphilis. The diagnosis of this disease has always been confronted with the difficulty in the detection of the etiological agent at stages when skin lesions are not yet observed, as well as with the lack of a method for the isolation of microorganisms in culture media. Diagnosis may be performed:

- by detection methods of non-specific antibodies (that use non-treponemic antigens) with visual interpretation;
- by immunoenzymatic methods (ELISA) detecting the presence of specific antibodies against *Treponema pallidum* in samples of patients at different stages of the disease.

PRINCIPLE

The microtitration wells are coated with recombinant antigens derived from the *Treponema pallidum* bacterium (p15, p17 and p47). The diluted sample is incubated in the wells. If the serum samples contain the specific antibodies, these will bound to the antigens in the well. The unbound material is eliminated by washing, after which human monoclonal anti-IgG antibodies conjugated to peroxidase are added. The conjugate will bound to the previously formed antigen-antibody complexes. The unbound conjugate is removed by washing. Tetramethylbenzidine and hydrogen peroxide solution is then added. The reactive samples develop a light blue color that changes to yellow when the reaction is stopped by adding sulfuric acid.

PROVIDED REAGENTS

Coated microtitration plate: microtitration plate with cutout strips and 96 wells coated with *Treponema pallidum* recombinant antigens.

Sample Diluent: saline buffer with surfactant. Violet color.

Concentrated Conjugate: human anti-IgG monoclonal antibody conjugated to peroxidase (10x). Red color.

Conjugate Diluent: saline buffer with proteins.

Substrate: tetramethylbenzidine and hydrogen peroxide solution.

Stopper: 2 N sulfuric acid.

Concentrated Wash Buffer: saline buffer with surfactant

(25x). Green color.

Positive Control: inactivated human serum containing anti-*Treponema pallidum* antibodies. Orange color.

Negative Control: inactivated non-reactive human serum. Yellow color.

NON-PROVIDED REAGENTS

Distilled or deionized water

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring stated volumes
- Disposable tips
- Volumetric material to prepare stated dilutions
- 37°C incubator
- Absorbent paper
- Disposable gloves
- Timer or stopwatch
- Sodium hypochlorite
- Microtitration plate wash system (manual or automatic)
- Spectrophotometer for microtitration plate reading

WARNINGS

- The reagents are for "in vitro" diagnostic use.
- All patient samples should be handled as capable of transmitting infection.
- The control sera have been tested for Hepatitis B Surface Antigen (HBsAg) and antibodies to Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) and found to be non-reactive. However, because no test method can offer complete assurance that infectious agents are absent, they should be handled as potentially infectious material.
- All materials used to perform the test must be destroyed to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for one hour at 121°C. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- Do not exchange reagents from different lots.
- Do not use reagents from other origin.
- Avoid touching the walls of the wells with the tips.
- Avoid using metal objects that may be in contact with the reagents.
- The microtitration plates should be placed in incubator. Do not open the incubator during this process. Do not use water bath.
- Avoid contact of strips with hypochlorite fumes from biohazards disposal containers or other sources, since hypochlorite affects the reaction.
- Avoid contact of the sulfuric acid (Stopper) with the skin and

eyes. R36/38: irritates eyes and skin. R34 causes burns. S24/25: avoid contact with the eyes and skin. S26: if splashing onto skin occurs, rinse the affected area with copious quantities of water and seek medical attention. S28: after contact with skin, wash immediately with plenty of water. S37/39: wear suitable gloves and eye/face protection.

- Avoid the spill of liquids and the formation of sprays.
- Do not pipette by mouth. Use disposable gloves and eye protection during handling of samples and reagents.
- All reagents and samples should be discarded according to the regulations in force.

REAGENTS PREPARATION

All the material used for reagent preparation should be clean and free from detergent and hypochlorite.

Wash Buffer: constituents of the concentrated reagent may precipitate at low temperature. In such case, bring the solution to 37°C until complete dissolution. To obtain a ready-to-use wash buffer, dilute 1 part Concentrated Wash Buffer (25x) with 24 parts distilled or deionized water. Example: 20 ml with 480 ml for one microtitration plate.

Conjugate: dilute 1 part Concentrated Conjugate (10x) with 9 parts Conjugate Diluent. Example: see table with concentrated Conjugate and Conjugate Diluent required volumes.

| Nº of wells | Concentrated Conjugate | Conjugate Diluent |
|-------------|------------------------|-------------------|
| 8 | 100 ul | 0.9 ml |
| 16 | 200 ul | 1.8 ml |
| 24 | 300 ul | 2.7 ml |
| 32 | 400 ul | 3.6 ml |
| 96 | 1200 ul | 10.8 ml |

Sample Diluent, Conjugate Diluent, Substrate, Stopper, Negative and Positive Controls: ready to use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable at 2-10°C until expiration date stated on the box. Do not freeze.

Concentrated Wash Buffer and Stopper: they may be stored at room temperature (2-25°C).

Wash Buffer (1x): once diluted, it is stable for 3 months at room temperature ($\leq 25^\circ\text{C}$).

Conjugate: once diluted, it is stable for 6 hours at room temperature ($\leq 25^\circ\text{C}$).

Coated microtitration plate: do not open the pouch until performing the test and until it has reached room temperature. Otherwise, the well strip surface could get moistened. Unused well strips should be kept in the pouch with the desiccant, sealed with adhesive tape and stored at 2-10°C. Test strips stored in this manner are stable for 4 months if it does not exceed the date printed on the pouch label.

SAMPLE

Serum or plasma

a) Sample collection: obtain in the usual way.

b) Additives: not required for serum. Employ plasma collected using EDTA, heparin or sodium citrate as anticoagulants.

c) Known interfering substances: no interference has been observed with bilirubin up to 30 mg/dl, ascorbic acid up to 50 mg/dl, triglycerides up to 1,500 mg/dl, hemoglobin up to 300 mg/dl. Samples containing particles should be clarified by centrifugation.

d) Stability and storage instructions: sample should be stored at 2-10°C. In case of do not perform the test within 72 hours, samples should be frozen at -20°C. Samples should not be repeatedly frozen and thawed. This may lead to erroneous results. In case of using frozen samples, they should be homogenized and centrifuged before use. Heat inactivation may affect the result. Do not use samples with microbial contamination. If samples are to be transported, they should be packaged according to local regulations for biohazard material shipment.

TEST PROCEDURE

1- Bring the reagents and samples to room temperature before opening.

2- Prepare the necessary volume of diluted wash buffer.

3- Place the required wells in the strip holder for the number of determinations to be used, including 2 wells for Positive Control (PC) and 3 for Negative Control (NC).

4- Dispense the Sample Diluent, then the sample (S) and the controls according to the following scheme:

| | S | PC | NC |
|-------------------------|--------|--------|--------|
| Sample Diluent | 100 ul | 100 ul | 100 ul |
| Positive Control | - | 20 ul | - |
| Negative Control | - | - | 20 ul |
| Sample | 20 ul | - | - |

Homogenize by loading and unloading the micropipette. When adding the sample, the Sample Diluent will change color, according to the following:

| Sample type | Without sample | Serum or plasma | Positive Control | Negative Control |
|--------------|----------------|-----------------|------------------|------------------|
| Color | Violet | Light blue | Dark orange | Green |

Warning: turbid or hemolyzed samples may change the final color without affecting the results. Color change may depend on the added sample volume and its composition. A less intense color change may be due to a lower dispensed sample volume, the sample not being in the appropriate conditions, or a low protein level.

5- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 60 ± 2 minutes at $37 \pm 1^\circ\text{C}$. At the same time, prepare the diluted conjugate (see table in REAGENTS PREPARATION).

6- The liquid from each well must be thoroughly removed after incubation. Wash 5 times according to washing instructions (see WASHING PROCEDURE).

7- Add the Conjugate:

| | | | |
|--------------------------|--------|--------|--------|
| Diluted Conjugate | 100 ul | 100 ul | 100 ul |
|--------------------------|--------|--------|--------|

To avoid evaporation cover the microplate with adhesive tape.

8- Incubate for 30 ± 2 minutes at $37 \pm 1^\circ\text{C}$.

9- Wash 5 times according to the washing instructions.

10- Dispense the Substrate. Transfer to a clean recipient only the required Substrate volume. Do not transfer the remaining Substrate back to the original bottle. Avoid reagent contact with oxidizing agents.

| | | | |
|------------------|--------|--------|--------|
| Substrate | 100 ul | 100 ul | 100 ul |
|------------------|--------|--------|--------|

11- Incubate for 30 ± 2 minutes at room temperature ($18-25^\circ\text{C}$), protecting from light

12- Add the Stopper:

| | | | |
|----------------|--------|--------|--------|
| Stopper | 100 ul | 100 ul | 100 ul |
|----------------|--------|--------|--------|

13- Read absorbance in spectrophotometer bichromatically at 450/620-650 nm or at 450 nm.

Note: bichromatic reading is recommended. In case the reading is monochromatic, perform a reagent blank that will have to be subtracted from all sample values.

STABILITY OF THE FINAL REACTION

Reaction color is stable for 10 minutes. Thus, results should be read within this period.

WASHING PROCEDURE

Remove the liquid from the wells by aspiration or inversion. The wells are washed with 300 ul diluted wash buffer. When filling the wells make sure not to spill. The wash solution should be in contact with the wells for 30 to 60 seconds.

Make sure no residual liquid remains after the final washing step. Perform double aspiration to remove excess buffer. If after such procedure, it still persists, invert the plate onto absorbent paper and tap it several times. Otherwise, erroneous results may be obtained.

Note: the washing procedure is crucial for the test result. If excess wash buffer remains in the wells or if the wells are not completely filled, erroneous results may be obtained. Do not let the wells air dry during the procedure. Automatic washers should be rinsed with distilled or deionized water at the end of the day to avoid obstructions due to the presence of salt in the wash buffer.

SUMMARY OF THE PROCEDURE

| STAGE | PROCEDURE | WARNINGS/OBSERVATIONS |
|----------------|---|---|
| Dilution | Prepare Wash solution (1x) | Dissolve salt crystals |
| Sample Diluent | Add 100 ul Sample Diluent in each well | |
| Samples | Add 20 ul S, PC and NC | Color change is observed when adding the sample and controls |
| Incubation | Cover the wells and incubate for 60 ± 2 minutes at $37 \pm 1^\circ\text{C}$ | In incubator |
| Washing step | Wash each well with 300 ul diluted Wash Buffer (5 times) | Time of contact of the Wash solution from 30 to 60 seconds. Completely remove the residual liquid from the wells |
| Dilution | Conjugate preparation (1x) | During incubation with the sample, dilute the Concentrated Conjugate (10x) |
| Conjugate | Add 100 ul diluted Conjugate (1x) | |
| Incubation | Cover the wells and incubate for 30 ± 2 minutes at $37 \pm 1^\circ\text{C}$ | In incubator |
| Washing step | The same as above | |
| Substrate | Add 100 ul Substrate | Transfer the required Substrate volume to be used. Do not pipette from the original bottle. Discard the remaining reagent. Avoid contact with oxidizing agents. |
| Incubation | 30 ± 2 minutos at $18-25^\circ\text{C}$ | Maintain the wells protected from light |
| Stop | Add 100 ul Stopper | |
| Reading | Read in spectrophotometer | Read within 10 minutes |

ASSAY VALIDATION CRITERIA

The assay is considered valid if the following conditions are simultaneously met:

1- The mean optical density (O.D.) of the Negative Controls should be less than or equal to 0.100.

Example:

Reading 1 = 0.014; Reading 2 = 0.018; Reading 3 = 0.019

Mean = $(0.014 + 0.018 + 0.019) / 3 = 0.017$

2- Remove any Negative Control with O.D. greater than 0.100.

3- If any Negative Control has been removed, recalculate the Negative Control mean. An assay is valid when at least two of the Negative Controls are accepted.

4- The O.D. mean of the Positive Controls should be greater or equal to 1.000.

Example:

Reading 1 = 1.697; Reading 2 = 1.774

Mean = $(1.697 + 1.774) / 2 = 1.736$

5- The O.D. mean difference of the Positive and Negative Controls should be greater or equal to 0.900.

In case one of the above conditions is not met, repeat the assay. Remember that the obtained readings will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

a) With optical instruments

The presence or absence of antibodies anti-*Treponema pallidum* is determined associating the sample absorbance with the Cut-off value.

Cut-off = NC + 0.160

NC: O.D mean of the Negative Control

Example: $0.017 + 0.160 = 0.177$

Non-reactive samples: samples with absorbances lower than the Cut-off value.

Reactive samples: samples with absorbances greater or equal to the Cut-off value.

b) Visual interpretation

If this type of interpretation is selected, every sample not presenting more color than the Negative Controls should be considered non-reactive. Otherwise, an evidently yellow sample is considered Reactive.

All samples initially reactive should be repeated by duplicate. If one or both repetitions are reactive, it should be considered reactive.

A sample initially reactive may be non-reactive in both repetitions. This may be due to:

- Cross contamination of a non-reactive well with a reactive sample.
- Sample contamination during dispensation, lack of precision in sample, conjugate and/or Substrate dispensation into the well.
- Tip re-utilization.
- Well contamination with hypochlorite or other oxidizing agents.

In certain cases a non-reactive sample may produce a falsely reactive reaction, both in the initial analysis as in its repetitions. Some probable causes of this effect may be:

- Sample contamination during collection, processing or storage.
- Presence of interfering substances, such as autoantibodies, drugs, etc.
- Ineffective dispensation and/or aspiration of the wash solution (obstructed system).

PROCEDURE LIMITATIONS

- See Known interfering substances under SAMPLE.
- Do not use pooled samples.
- Do not use other body fluids such as saliva, cerebrospinal fluid or urine.
- A negative result does not exclude the possibility of exposure or infection with *Treponema pallidum*.
- Repeatedly reactive results should be verified by a confirmatory method; according to the current the legal regulations.
- Do not use heat-inactivated samples, as they may yield false positive results.

SPECIFIC PERFORMANCE FEATURES

a) Sensitivity

Clinical Sensitivity in Performance Panels

In a study performed on different international commercial panels, the following results were obtained:

- Syphilis mixed Titer Performance Panel (PSS 201), Boston Bio-medica, Inc.: 22 out of 22 reactive samples were detected.
- Syphilis mixed Titer Performance Panel (PSS 202), Boston Bio-medica, Inc.: 18 out of 18 reactive samples were detected.
- Syphilis Qualification Panel (QSS 701), Boston Biomedica, Inc.: 5 out of 5 reactive samples were detected.
- WO2 Panel of Centers for Disease Control (CDC), 20 out of 20 reactive samples were detected.
- Performance Panel for Syphilis, Q Panel, Brazil (PP0406): 16 out of 16 reactive samples were detected.
- Performance Panel for Syphilis, Q Panel, Brazil (PP0407): 16 out of 16 reactive samples were detected.

b) Specificity

In a study performed on 3519 sera and plasma samples from different health centers, the obtained specificity was 99.85%. Over a panel of 461 plasmas from a high prevalence population, the obtained specificity was 100%.

A possible cross-reactivity was evaluated, assaying samples from 169 individuals with different clinical conditions that may be the cause of unspecific reactions for the Sifilis ELISA recombinante v.4.0 test. These conditions include patients with autoimmune diseases or infectious diseases other than Syphilis (HIV, HTLV, Hepatitis C, Hepatitis B, Chagas, others). For this population the specificity was 99.4%.

c) Precision

The test precision was evaluated following EP5-A protocol recommended by the NCCLS. The assays were performed with samples having different reactivity levels and with con-

trols. Two daily assays were performed testing each sample by duplicate during 20 days.

| | Mean (O.D.) | Intra-assay | | Total | |
|-------------|----------------|-------------|---------|-------|---------|
| | | S.D. | C.V. | S.D. | C.V. |
| Sample 1 | 0.716 | 0.082 | 11.440% | 0.137 | 19.129% |
| Sample 2 | 0.829 | 0.057 | 6.876% | 0.083 | 10.013% |
| Sample 3 | 0.389 | 0.031 | 8.020% | 0.044 | 11.311% |
| Sample 4 | 1.403 | 0.104 | 7.430% | 0.154 | 10.976% |
| (+) Control | 1.585 | 0.084 | 5.290% | 0.127 | 8.013% |
| (-)Control | 0.014 | 0.003 | 19.710% | 0.002 | 12.143% |

n = 80

WIENER LAB. PROVIDES

Kit for 96 tests (Cat. N° 1723452).

Kit for 192 tests (Cat. N° 1723552).

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SYMBOLS EXPLANATION

Policubeta **Sensib.**

Coated microtitration plate

Diluyente **Muestra**

Sample Diluent

Conjugado **Conc.**

Concentrated Conjugate

Conjugado **Diluy.**

Conjugate Diluent

Revelador

Substrate

Buf. Lavado **Conc.**

Concentrated Wash Buffer

Control **+**

Positive Control

Control **-**

Negative Control

Stopper

Stopper

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC **REP** Authorized representative in the European Community

IVD "In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL - Negative Control

REF Catalog number

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