Instruction for use
A solid-phase enzyme immunoassay kit
for the qualitative detection of IgG antibodies
to *Treponema pallidum* in human serum or plasma

Treponema pallidum IgG EIA

Catalogue number REF **K111G**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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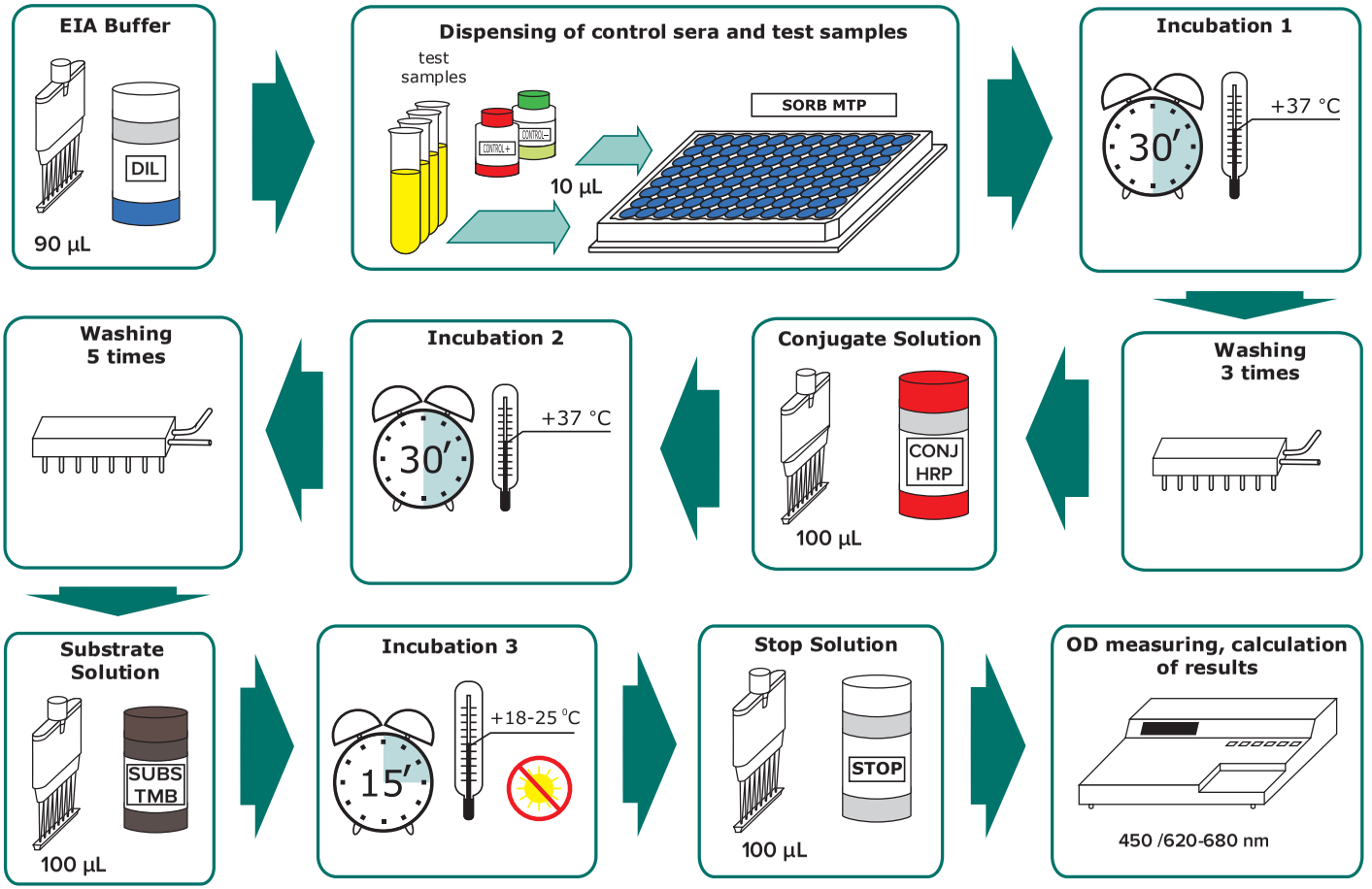
ASSAY PROCEDURE



K111G

CONTENT

Instruction for use
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Treponema pallidum IgG EIA

1. INTENDED USE

A solid-phase enzyme immunoassay for the qualitative determination of IgG antibodies to Treponema pallidum in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Syphilis is a sexually transmitted disease caused by Treponema pallidum (Tp.) bacterium belonging to the family of Spirochaetaceae. Tp is gram negative, has a cell wall and is considered strictly anaerobe, exhibiting a characteristic mobility due to periplasmic flagella.

Clinical manifestations of syphilis may be diverse, depending upon the stage of infection and the individual response, and characterized by alternating acute and latent periods. The first anti-Tp antibodies being detected from the second week after infection, belong to IgM class, their titer reaching its maximum at weeks 6-9 and then falling down.

Tp-specific IgG antibodies are produced from week 4. A successful treatment of the disease usually leads to a drop of anti-Tp IgG titer, but in some cases they may be found during a long time and detected by sensitive serological methods. Many assays have been developed for the immunological detection of the Tp infection in the past (VDRL, TPHA, RPR), but ELISA is considered to be the most sensitive.

In this test, a mixture of recombinant antigens and synthetic analogs of Tp lipoproteins with MM of 15, 17, 41-45 and 47 kDa (p15, p17, TmpA and p47, resp.) are used.

3. TEST PRINCIPLE

The detection of IgG antibodies to *Treponema pallidum* is based the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized recombinant antigens of *Treponema pallidum {p15; p17; p41 and p47}*. Murine monoclonal IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to *Treponema pallidum IgG* antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal IgG antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density (OD) in the microwell is directly related to the concentration of the measured IgG antibodies to *Treponema pallidum* in test specimen.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P111GZ	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with recombinant antigens of *Treponema pallidum IgG*, ready to use
CN111GZ	CONTROL -	**Negative Control Serum K-**	0.2 mL	1	Solution based on human serum, free of specific antibodies to *Treponema pallidum IgG*, with preservative, ready to use (yellow liquid)
CP111GZ	CONTROL +	**Positive Control Serum K+**	0.5 mL	1	Solution based on inactivated human serum pool with a high content of specific antibodies to *Treponema pallidum IgG*, with preservative, ready to use (red liquid)
T111GZ	CONJ HPR	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase, ready to use (red liquid)
S011Z	DIL	**EIA Buffer**	12 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Treponema pallidum IgG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Treponema pallidum IgG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 4 wells for Positive and Negative Control Serum (1 well for Positive Control (CP) and 3 wells for Negative Control Serum (CN)).

10.2. Dispense **90 µL of EIA Buffer** to all wells.

10.3. Dispense **10 µL of Positive and Negative Control Serum as well as 10 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Positive and Negative Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Positive and Negative Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP	SAMP5	SAMP13	SAMP21								
B	CN	SAMP6	SAMP14	SAMP22								
C	CN	SAMP7	SAMP15	SAMP23								
D	CN	SAMP8	SAMP16									
E	SAMP1	SAMP9	SAMP17									
F	SAMP2	SAMP10	SAMP18									
G	SAMP3	SAMP11	SAMP19									
H	SAMP4	SAMP12	SAMP20									

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **3 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution in the wells after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Conjugate Solution** to all wells

10.7. Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8. At the end of the incubation period, aspirate and wash each well **5 times** as described in 10.5.

10.9. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11. Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution.

11. TEST VALIDITY AND CALCULATION OF RESULTS

11.1. The test results are valid only if Positive and Negative Control Serum are within the specified ranges and if all other test parameters are also within the given assay specifications, namely:

- OD of Negative Control Serum - < 0.15;
- OD of Positive Control Serum + > 1.5.
- OD(CN) x 0,5 < OD(CN) < OD(CN) x 2.

11.2. Calculate the mean OD value of the Negative Control Serum:

meanOD(CN) = (OD1(CN) + OD2(CN) + OD3(CN))/3

If one of the OD values of the Negative Control Serum differs significantly, it should be discarded and the meanOD(CN) should be calculated using the remaining OD values of the Negative Control Serum.

11.3. Calculate the Cut-off value by adding to the mean OD value of the Negative Control Serum the coeficient 0.2.

Cut-off = meanOD(CN) + 0.2

11.4. Calculate the boundary of the «gray zone» (GZ) - the OD values that are within 10% below the Cut-off value:

Cut-off x 0.9 ≤ GZ ≤ Cut-off

11.5. Alternatively, calculate Positivity Index (PI) for each sample by dividing the OD of the sample by Cut-off value:

PI = ODsample/Cut-off

12. INTERPRETATION OF THE RESULTS

Calculation of results taking into account the OD sample:

Samples with the OD > Cut off are considered **POSITIVE**,
Samples within the «gray zone» are considered **EQUIVOCAL**,
Samples with OD < the value of the «gray zone» are considered **NEGATIVE.**

Calculation of results taking into account the PI:

If PI value > 1.0 the result is **POSITIVE**,
If PI value is between 0.9 and 1.0 the result is **EQUIVOCAL**,
If PI value < 0.9 the result is **NEGATIVE**.

Positive samples should be retested again with the «Treponema pallidum IgG - EIA» reagent kit. After repeated testing, samples are considered positive if the OD of at least one of the replicates was higher than or equal to the Cut-off. If during repeated testing the OD of the sample was below the Cut-off value, such a sample should be considered negative.

If equivocal results are obtained, it is recommended to conduct a reexamination of the sample in several replicates. If the result is equivocal again, a new sample should be obtained within 5-7 days and retested. If the result remains equivocal, the sample should be considered negative.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

No. sample	mean PI	CV, %
1	1.33	2.3
2	5.47	4.7

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

No. sample	mean PI	CV, %
1	1.32	6.3
2	5.46	3.2

13.1.2. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

13.2. Diagnostic performance characteristics

The clinical sensitivity and specificity of the assay was evaluated on a panel of 300 positive and 280 negative clinical serum samples and was found to be 100%. The positive predictive value (PPV) of the kit and the negative predictive value (NPV) were 100%. The relative sensitivity and specificity of the assay was evaluated using a panel of 360 donor sera characterized for Treponema pallidum IgG antibodies in commercial kits and was determined to be 96.8%.

14. LIMITATIONS

A positive test result indicates that the patient has IgG antibodies specific to Treponema pallidum antigens. In some cases, in the early stages of the disease, the EIA result may be negative due to the absence or ultra-low titer of antibodies below the limit of sensitivity of the test. In such cases, in the presence of symptoms of the disease, it is recommended to re-sample and analyze the sample in 7-10 days, as well as verify the result by another laboratory method, such as PCR, culture, microscopic, etc.

Both laboratory test results and clinical manifestations of the disease should be taken into account for the establishment of the diagnosis.

15. REFERENCES

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SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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