ELISA-VIDITEST
anti-*Borrelia* recombinant IgG + VlsE
Cat. No. ODZ-282

Instruction manual

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1. TITLE:

ELISA-VIDITEST anti-*Borrelia* recombinant IgG + VlsE – the 3rd generation ELISA kit of high diagnostic sensitivity and specificity.

2. INTENDED USE:

The kit is intended for the detection of IgG antibodies to the pathogenic borrelia strains (***B. afzelii, B. garinii and B. burgdorferi sensu stricto***) in human serum, cerebrospinal fluid and synovial fluid. The detection of antibodies is one of the laboratory tests that help to diagnose Lyme disease (LD). The diagnosis of LD is based on the combination of clinical examination and laboratory testing. Anti-borrelia IgG antibodies are detectable 6 weeks after infection. The level of IgG antibodies usually increases with disease progression to the 2nd stage. During the 3rd stage of the disease the antibody levels in serum are high and can remain elevated for several years.
However, clinical symptoms of LD are similar to other diseases, therefore the serological methods are of use in differential diagnosis of neuroinfections, arthropathies, carditis and skin diseases.

3. TEST PRINCIPLE:

ELISA-VIDITEST anti-*Borrelia* recombinant IgG + VlsE is a solid-phase immunoanalytical test of high diagnostic sensitivity and specificity. The polystyrene strips are coated with the mixture of specific antigens. Anti-borrelia antibodies in samples bind to the immobilized antigens. The serum antibodies that do not bind are washed away and those that formed complexes with the antigens are later on recognized by animal anti-human IgG antibodies labeled with horseradish peroxidase. The presence of labeled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative samples do not react and the mild change in color, if present, may be attributed to the reaction background.

4. KIT COMPONENTS:

ELISA break-away strips **(turquoise)** coated with specific recombinant antigens		1 microplate
1.3 mL	Standard A, r.t.u. (ready to use)	1 vial
1.3 mL	Standard D, r.t.u.	1 vial
0.2 mL	Anti-human IgG antibodies labeled with horseradish peroxidase 101x concentrated (Px-conjugate)	1 vial
125 mL	Wash buffer concentrate, 10x concentrated	1 vial
125 mL	Dilution buffer (DB), r.t.u.	1 vial
15 mL	Chromogenic substrate (TMB substrate), r.t.u.	1 vial
15 mL	Stop solution, r.t.u.	1 vial

Sealable pouch for unused strips
Instruction manual
Quality control certificate

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

a. Distilled or deionised water for diluting of the Wash buffer concentrate.
b. Appropriate equipment for pipetting, liquid dispensing and washing.
c. Spectrophotometer/colorimeter/microplate reader – wavelength 450 nm (and 630 nm reference filter – recommended, not required).

6. PREPARATION OF REAGENTS AND SAMPLES:

a. **Allow all kit components to reach room temperature.**
b. **Vortex samples (sera, cerebrospinal fluids, synovial fluids) and the Standards in order to ensure homogeneity** and mix all solutions well prior use. **Dilute serum samples 1:100 (101x) in Dilution buffer** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). **Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 ul of cerebrospinal fluid sample + 75ul of Dilution buffer). Dilute synovial fluid samples 1:80 in Dilution buffer (e.g. 5 ul of cerebrospinal fluid sample + 400 ul of Dilution buffer).** Do not dilute the Standards, they are ready to use.
c. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one week if stored at +2 to +10°C.
d. **Dilute the concentrated Px-conjugate 1:100 with Dilution buffer (e.g. 0.1 mL Px-conjugate + 10 mL Dilution buffer).**

   (Note: For one microtitre plate you will need approx. 12 mL of the diluted Px-conjugate., i.e. 0,12 mL of the concentrated Px-conjugate + 12 mL of Dilution buffer).
e. Do not dilute TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE:

a. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
b. Pipette Standards and samples according to the pipetting schemes: Start with filling the first well with 100 µl of Dilution buffer (DB) to estimate the reaction background. Then fill two wells with 100 µl/well of Standard D (serves as calibrator and also as the positive control) and then pipette 100 µl of Standard A (negative control). Fill the remaining wells with 100 µl of diluted samples (S1, S2, S3,...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply the samples in doublets (Standard D in triplet). **Incubate 60 minutes (±5 min) at room temperature**.
c. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
d. Add 100 µL of diluted Px-conjugate into each well. **Incubate 60 minutes (±5 min) at room temperature**.
e. Aspirate and wash four times with 250 µl/well of Wash buffer. Aspirate and tap.
f. Dispense 100 µl of TMB substrate into each well. **Incubate 10 minutes (+/-5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing.** Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
g. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents.

h. Measure the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use a reference reading at 630 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
a	DB											
b	ST D											
c	ST D											
d	ST A											
e	S1											
f	S2											
g	S3											
h	S4…											

8. PROCESSING OF RESULTS:

Begin the processing with subtraction of the absorbance of the DB well (background absorbance) from the absorbances in all other wells.

8.1. Processing of results for Qualitative interpretation

1. Compute the mean of Standard D (ST D) absorbance from the two corresponding wells. If you applied Standard D into 3 wells and if any of the three Standard D absorbances falls out of the range +/-20% of the mean absorbance then exclude the deviating well from the calculation and compute a new Standard D mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the Standard D mean with a Correction factor. **The Correction factor value for particular Lot is written in enclosed Quality control certificate.**
3. Samples with absorbances lower than 100% of the cut-off value are considered negative and samples with absorbances higher than 120% of the cut-off value are considered positive.

8.2 Processing of results for Semiquantitative interpretation

Determine the Positivity Index for each serum sample as follows:

1. Compute the cut-off value using the Standard D mean and the Correction factor (see the previous paragraph)
2. Compute the Positivity Index for each sample according to the following formula:

$$\text{sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}$$

3. Interpret the sample Positivity indexes according to the following table:

   a) <u>serum samples</u>

Positivity Index	Interpretation
< 1	negative
1.00 - 1.20	+/-
1.21 - 3.50	+
3.51 - 6.00	++
6.01 - 8.60	+++
> 8.60	++++

b) **cerebrospibal fluid samples**

Positivity index	Interpretation
< 1.00	Negative
1.00 – 1.30	+/-
> 1.30	+

c) **synovial fluid samples**

Positivity index	Interpretation
< 1.00	Negative
1.00 – 1.20	+/-
> 1.20	+

Example of calculation:

Standard D absorbances	= 1.231; 1.198; 1.215
Standard D mean	= 1.215
Correction factor	= 0.29
Cut-off value	= 1.215*0.29 = 0.352
Sample absorbance	= 1.587
Sample Positivity Index	= 1.587/0.352 = 4.51

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

9. CLINICAL INTERPRETATION OF RESULTS:

Diagnosis (stage)	Disease	Laboratory evidence	
		essential	supporting
I. Early localized infection	Erythema migrans	IgM positive (3-6 weeks post-infection) **Often seronegative**	Skin biopsy
II. Early disseminated infection	Borrelial lymphocytoma Myocarditis Ophtalmoborreliosis Neuroborreliosis	**IgM positive, IgG positive** or IgM negative, IgG positive	Histological evidence of B-cell pseudolymphocytoma
III. Late disseminated infection	Arthritis Acrodematitis chronica atrophicans Chronical neuroborreliosis	**IgM negative, IgG positive** (high titers of IgG antibodies)	

10. CHARACTERISTICS OF THE TEST:

10.1 Validity of the test

The test is valid if:

a. The background absorbance (the absorbance of the Dilution buffer) is less than 0.100.

b. The absorbance of Standard A is less than 0.100.

c. The absorbance of Standard D is stated in **Quality control certificate**

The test is intended for the detection of IgG antibodies in human serum, cerebrospinal fluid, synovial fluid.

10.2 Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

10.2.1. Intraassay variability

The coefficient of intraassay variability is max. 5%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

Example: (N = number of parallels of the same microtitration plate, SD = standard deviation)

N	Mean absorbance	SD	CV%
16	1.335	0.050	3.8%
16	0.614	0.023	3.7%

10.2.2. Interassay variability

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (N = number of an independent examination of the same serum sample, SD = standard deviation):

N	Mean Absorbance	SD	Range (min-max)	CV%
18	1.369	0.064	1.223 – 1.476	4.7%
43	1.372	0.119	1.184 – 1.750	8.7%

10.2.3. Recovery test

Measured values of recovery test for every Lot are between 80-120% of expected values.

10.3. Diagnostic sensitivity and specificity

The diagnostic sensitivity of the test is 95% and the specificity is 99%. Evaluation was performed with a set of blood samples that comprised of: a) anti-borrelia IgG positive sera, b) anti-borrelia IgG negative sera. Results were confirmed by other commercially available diagnostic test during the internal validation testing and external validation testing.

10.4. Interference

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 80 mg/mL of triglycerides.

11. SAFETY PRECAUTIONS:

All ingredients of the kit are intended for laboratory use only.

Standards contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

12. HANDLING PRECAUTIONS:

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Wash buffer, chromogenic substrate TMB, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.
c. Avoid microbial contamination of serum samples and kit reagents.
d. Avoid cross-contamination of reagents.
e. Standards, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
f. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
g. Use validated device only for pipetting and absorbance measuring.
h. Follow the assay procedure indicated in the Instruction manual.

   Variations in the test results are usually due to:

   * Insufficient mixing of reagents and samples
   * Inaccurate pipetting and inadequate incubation times
   * Poor washing technique or spilling the rim of well with sample or Px-conjugate
   * Use of identical pipette tip for different solutions

13. STORAGE AND EXPIRATION:

Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.

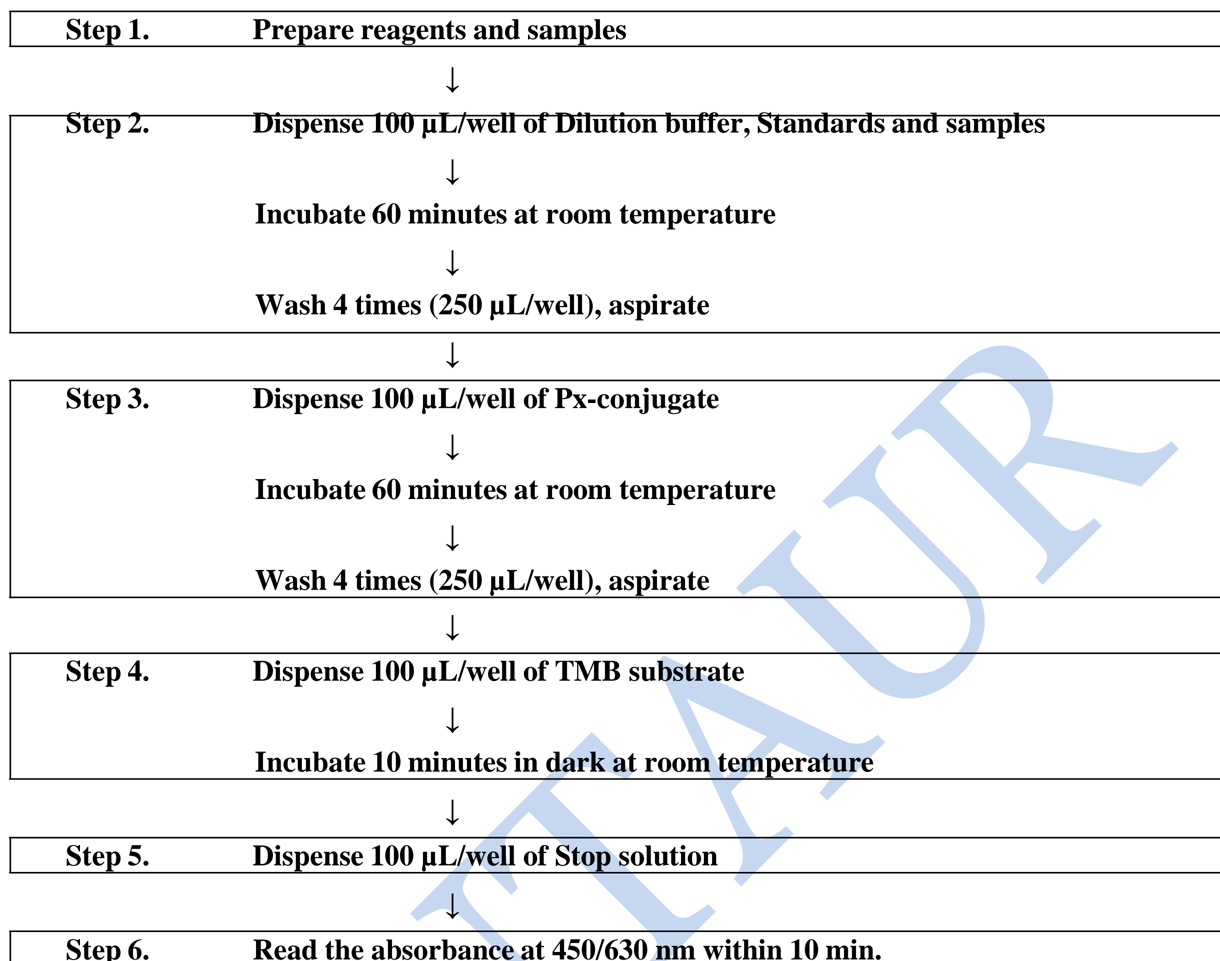
Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.

Do not store diluted samples and a diluted Px-conjugate. Always prepare fresh.

Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

14. FLOW CHART:



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