IVD

ELISA-VIDITEST
anti-*Borrelia* recombinant IgG + VlsE (CSF)

Cat. No. ODZ-172
Instruction manual

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1. TITLE

ELISA-VIDITEST anti-*Borrelia* recombinant IgG + VlsE (CSF) – 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 or plasma, 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 (CSF) is a solid-phase immunoanalytical test. The polystyrene strips are coated with the mixture of recombinant antigens. Anti-borrelia antibodies in serum 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 recognised by animal anti-human IgG antibodies labelled with horseradish peroxidase. The presence of labelled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.

4. KIT COMPONENTS

ELISA break-away strips coated with specific recombinant antigens	STRIPS Ag	1 microplate
1.3 mL	CONTROL - 4 AU/mL[1)], r.t.u. [2)]	1 vial
1.3 mL	Standard B 15 AU/mL, r.t.u.	1 vial
1.3 mL	Standard 2 60 AU/mL, r.t.u.	1 vial
1.3 mL	Standard 1 220 AU/mL, r.t.u.	1 vial
1.3 mL	Standard E 850 AU/mL, r.t.u.	1 vial
15 mL	Anti-human IgG antibodies labelled with horseradish peroxidase, r.t.u (Px-conjugate) CONJ	1 vial
125 mL	Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
125 mL	Dilution buffer, r.t.u. DIL	1 vial
15 mL	Chromogenic substrate (TMB substrate), r.t.u. TMB	1 vial
15 mL	Stop solution, r.t.u. STOP	1 vial
Sealable pouch for unused strips		
Instruction manual		

Quality control certificate
[1)] artificial units/ml, [2)] ready to use

Dilution buffer DIL is intended only for ELISA-VIDITEST anti-*Borrelia* recombinant kits and IT IS NOT COMPATIBLE with other ELISA-VIDITEST kits produced by VIDIA spol. s r.o.

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 620-690 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/plasma and cerebrospinal fluids) and the Standards in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum/plasma samples 1:100 (101x) in Dilution buffer** and mix (e.g. 5 µL of serum/plasma sample + 500 µL of Dilution buffer). **Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 ul of cerebrospinal fluid sample + 75 ul of Dilution buffer). Dilute synovial fluid samples 1:80 in Dilution Buffer (e.g. 5 ul of synovial fluid sample + 400 ul of Dilution buffer).** Do not dilute the Standards, they are ready to use.
d. 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.
e. Do not dilute Px-conjugate, 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. Chose the method you intend to use (qualitative, semiquantitative or quantitative analysis, see below and/or paragraph 8) and pipette Standards and samples according to the pipetting schemes (page 3). Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. In case of choosing the qualitative or semiquantitative method, fill two wells with 100 µl/well of Standard 1 (serves as calibrator and also as the positive control) and then pipette 100 µl of Control -. In case of quantitative method, pipette all Standards as singlets (Control -, Standard B, Standard 2, Standard 1, Standard E). 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 1 in triplet).
c. **Incubate 60 minutes (±5 min) at room temperature**.
d. 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.
e. Mix well the bottle with Px-conjugate and add 100 µL of Px-conjugate r.t.u. into each well.
f. **Incubate 60 minutes (±5 min) at room temperature**.
g. Aspirate and wash four times with 250 µl/well of Wash buffer.
h. Dispense 100 µl of TMB substrate into each well.
i. Incubate for **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.

j. 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.

k. Measure the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use a reference reading at 620-690 nm.

Figure 1: Pippetting scheme

Qualitative and semiquantitative analysis

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL											
b	STANDARD 1											
c	STANDARD 1											
d	CONTROL -											
e	S1											
f	S2											
g	S3											
h	S…											

Quantitative analysis

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S3										
b	CONTROL -	S…										
c	STANDARD B											
d	STANDARD 2											
e	STANDARD 1											
f	STANDARD E											
g	S1											
h	S2											

8. PROCESSING OF RESULTS

Regardless of the method chosen, begin the processing with subtraction of the absorbance of the DIL well (background absorbance) from the absorbances in all other wells.

8.1. Processing of results for Qualitative interpretation

1. Compute the mean of Standard 1 absorbance from the two corresponding wells. If you applied Standard 1 into 3 wells and if any of the three Standard 1 absorbances falls out of the range +/- 20% of the mean absorbance then exclude the deviating well from the calculation and compute a new Standard 1 mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the Standard 1 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 90% of the cut-off value are considered negative and samples with absorbances higher than 110% 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 1 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 tables:

a) **serum or plasma**

Positivity Index	Interpretation
< 0.90	negative
0.90- 1.10	+/-
1.11 - 2.70	+
2.71 - 4.30	++
4.31 - 6.00	+++
> 6.00	++++

b) **cerebrospinal fluid**

Positivity Index	Interpretation
< 1.00	negative
1.00 - 1.30	+/-
> 1.30	+

c) **synovial fluid**

Positivity Index	Interpretation
< 1.00	negative
1.00 - 1.30	+/-
> 1.30	+

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample tesing. 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.

Example of calculation:

Standard 1 absorbances	= 1.231; 1.198; 1.215
Standard 1 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

8.3. Processing of results for Quantitative interpretation

Compute the sample antibody titre in artificial units (AU/ml) as follows:

1. Construct the calibration curve by plotting the absorbance of Standards (y-axis – in linear scale) to antibody concentration in artificial units (AU/ml) (x-axis – can have logarithmic scale). The antibody concentration in each Standard is mentioned in paragraph 4 – Kit components.

2. Determine the unknown antibody titre in samples from the calibration curve. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is most convenient.

Note! If the sample absorbance is out of the calibration curve (above the upper limit), repeat the test with more diluted (or less diluted) sample, e.g. 1:200 or 1:400 (1:50) for serum/plasma sample, 1:10 or 1:20 for cerebrospinal fluid sample.

Result interpretation:

a) Blood serum or plasma samples

Concentration (AU/ml)	Interpretation
< 27.0	Negative
27.0 - 33.0	+/-
33.1 - 98.0	+
98.1 - 213.0	++
213.1 - 542.0	+++
> 542.0	++++

b) Cerebrospinal fluid samples

Concentration (AU/mL)	Interpretation
< 7.0	Negative
7.0 - 9.0	+/-
> 9.0	+

c) Synovial fluid samples

Concentration (AU/mL)	Interpretation
< 19.50	Negative
19.50 - 25.50	+/-
> 25.50	+

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.

8.4 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 Intrathecal production of specific antibodies during neuroborreliosis.	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) Intrathecal production of specific antibodies during chronical neuroborreliosis	

9. DETECTION OF INTRATHECALLY PRODUCED IgG ANTIBODIES

9.1 INTRODUCTION

Detection of the intrathecal synthesis of IgG antibodies (detection of local synthesis of specific IgG antibodies in the central nervous system) is necessary for the diagnosis of the early and the late neuroborreliosis. It requires measurement of specific IgG antibodies in blood serum and in cerebrospinal fluid and determination of albumin and the total IgG level in both samples. The intrathecal synthesis of antibodies is determined as specific antibody index (AI) and calculated from the antibody concentration ratio in cerebrospinal fluid and blood serum in relation to the status of blood-cerebrospinal fluid barrier.
Note: If both serum and cerebrospinal fluid samples give negative results, do not count antibody index AI (intrathecal synthesis of specific antibodies is not expected).

VIDITAB software for the calculation of results is available on request (free).

9.2 CALCULATION OF THE ANTIBODY INDEX (AI)

9.2.1 Calculate the ratio of the total IgG concentration in cerebrospinal fluid to the total IgG concentration in serum ($Q_{total\ IgG}$) and the ratio of the cerebrospinal albumin level to the serum albumin level ($Q_{total\ alb}$).

$$Q_{total\ IgG} = \frac{\text{total IgG in CSF}}{\text{total IgG in blood serum}} \qquad Q_{total\ alb} = \frac{\text{albumin in CSF}}{\text{albumin in serum}}$$

Example of calculation:
total IgG in CSF = 0.065 g/L
total IgG in serum = 17.29 g/L
albumin in CSF = 0.272 g/L
albumin in serum = 30.64 g/L

$$Q_{total\ IgG} = \frac{0.065}{17.29} = 3.76 * 10^{-3}$$

$$Q_{total\ alb} = \frac{0.272}{30.64} = 8.88 * 10^{-3}$$

9.2.2 Calculate the limiting quotient $Q_{lim\ IgG}$ which is the amount of IgG found in cerebrospinal fluid that can originate from the systemic circulation (hyperbolic function according to Reiber et al. Clin Chem 37/7, 1153-1160 (1991)).

Compute Q_{lim} using the equation:

$$Q_{lim\ IgG} = 0.93 * \sqrt{(Q_{total\ alb})^2 + 6*10^{-6}} - 1.7 * 10^{-3}$$

Example of calculation:

$$Q_{lim\ IgG} = 0.93 * \sqrt{(8.88*10^{-3})^2 + 6*10^{-6}} - 1.7 \times 10^{-3}$$

$$Q_{lim\ IgG} = 6.86 * 10^{-3}$$

9.2.3 Calculate the ratio of concentration of specific IgG antibodies in CSF to concentration of specific IgG in serum $Q_{spec\ IgG}$.

$$Q_{spec.IgG} = \frac{\text{spec.IgG CSF} * \text{sample dilution}}{\text{spec.IgG serum} * \text{sample dilution}}$$

Where spec. IgG CSF is the concentration of specific antibodies in AU/mL in cerebrospinal fluid and spec. IgG serum is the concentration of specific antibodies in AU/mL in serum.

Example of calculation:

spec. IgG CSF = 38 AU/mL, sample diluted 2 times in dilution buffer

spec. IgG serum = 10 AU/mL, sample diluted 101 times in dilution buffer

$$Q_{spec.IgG} = \frac{38*2}{10*101} = 75.2 * 10^{-3}$$

9.2.4 Calculation of antibody index AI

a) If $Q_{total\ IgG} < Q_{lim\ IgG,}$ then calculate AI using the formula:

$$AI = \frac{Q_{spec.IgG}}{Q_{total\ IgG}}$$

Example of calculation:

$Q_{total\ IgG} = 3.76 * 10^{-3}$

$Q_{lim\ IgG} = 6.86 * 10^{-3}$

$Q_{spec.IgG} = 75.2 * 10^{-3}$

$Q_{total\ IgG} = 3.76 * 10^{-3} < Q_{limIgG} = 6.86 * 10^{-3}$

$$AI = \frac{0.0752}{0.00376} = 20$$

b) If $Q_{total\ IgG} > Q_{lim\ IgG}$ compute AI using the formula:

$$AI = \frac{Q_{spec.IgG}}{Q_{lim\ IgG}}$$

Example of calculation:

$Q_{total\ IgG} = 13.5 * 10^{-3}$

$Q_{limIgG} = 6.86 * 10^{-3}$

$Q_{spec.IgG} = 75.2 \text{ x } 10^{-3}$

$$AI = \frac{0.0752}{0.00686} = 11$$

Note: Suitable software applications (e.g. EPI info 6) can be used for calculation of the specific antibody index.

VIDITAB software for the calculation of results is available on request (free).

9.3 RESULT INTERPRETATION (according to Reiber)

AI value	Interpretation
< 1.3	negative, intrathecal synthesis not proven
1.3 - 1.5	equivocal
> 1.5	positive, intrathecal synthesis proven

Note: If the sample absorbance is out of the calibration curve (above the upper limit), repeat the test with different sample dilution.

9.4 DIAGNOSTIC INTERPRETATION OF RESULTS

Diagnostic criteria for neuroborreliosis – based on EUCALB

Diagnosis (stage)	Clinical criteria	Laboratory evidence (blood serum + cerebrospinal fluid)	
		Essential	Supporting
Early neuroborreliosis	Meningoradiculoneuritis Meningitis Garin-Bujadoux-Bannwarth Syndrom	**Intrathecal synthesis of specific antibodies**	Specific oligoclonal bands in CSF, significant increase in titres of serum antibodies
Chronic neuroborreliosis (very rare)	Long standing encephalitis, meningoencephalitis encephalomyelitis radiculomyelitis	**Intrathecal synthesis of specific antibodies** Lymphocytic pleocytosis in CSF Detection of specific IgG antibodies in blood serum	Specific oligoclonal bands in CSF

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 0.150.
b. The absorbance of Standard 1 should be in range that is written in enclosed **Quality control certificate.**
c. The Standards absorbance values keep the order that: Control - < Standard B < Standard 2 < Standard 1 < Standard E.

The test is intended for the detection of IgG antibodies in human serum or plasma, 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 Limit of quantification

The limit of quantification is 2.41 AU/mL. The limit of quantification was defined as the lowest measurable concentration which can be distinguished with 95% confidence from zero.

10.5 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

Manufacturer guarantees performance of the entire ELISA kit.

Follow the assay procedure indicated in the Instruction manual.

Wash buffer, chromogenic substrate TMB and Stop solution are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.

Standards, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.

Avoid microbial contamination of serum samples and kit reagents.

Avoid cross-contamination of reagents.

Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

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/plasma 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. 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

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well of Dilution buffer, Standards and samples**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3	**Dispense 100 µL/well of Px-conjugate**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well of TMB substrate**
	↓
	Incubate 10 minutes in dark at room temperature
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read the absorbance at 450/ 620-690 nm within 10 min.**

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