ELISA-VIDITEST anti-*Borrelia* recombinant IgM (CSF)

Cat. No. ODZ-281

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-*Borrelia* recombinant IgM (CSF) – the 3rd generation ELISA kit of high diagnostic sensitivity and specificity

2. INTENDED USE

The kit is intended for a detection of IgM 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 IgM antibodies are detectable 3 weeks after infection with its maximum during the sixth week. Subsequently, the titre of IgM antibodies decreases and the IgG antibodies prevail. The detection of anti-borrelia antibodies is very important at the early stage of the disease since the typical symptoms are present only in a certain proportion of patients (e.g. erythema migrans is present in 50% of patients). The clinical symptoms of LD are similar to the symptoms in other diseases, therefore the serology is also of use in differential diagnosis of neuroinfections, arthropathies, carditis and skin diseases.
During the interpretation of the test results it has to be taken into account that the titres of antibodies at early stage of the disease is rather low and it can be influenced by administration of antibiotics. Seronegativity in a small percentage of patients and the crossreactivity of antibodies elevated due to the other diseases has to be considered. Therefore, it is necessary to compare the test results with clinical data (*adapted from the text at the web page of the Czech National Reference Laboratory for Lyme disease*).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-*Borrelia* recombinant IgM (CSF) assay 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 IgM 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	Standard 1 r.t.u.[1)] STANDARD 1	1 vial
1.3 mL	Standard 2 r.t.u. STANDARD 2	1 vial
1.3 mL	Negative control serum r.t.u. CONTROL -	1 vial
15 mL	Anti-human IgM antibodies labelled with horseradish peroxidase r.t.u. (Px-conjugate) CONJ	1 vial
125 mL	Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
100 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)] ready to use

Dilution buffer (DIL) r.t.u. is intended only for ELISA-VIDITEST anti-*Borrelia* recombinant and IS NOT INTERCHANGABLE between other ELISA-VIDITEST kits produced by VIDIA spol. s r.o.

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT

a. Distilled or deionised water for dilution 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 and the Standards and Negative control 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 cerobrospinal fluid samples 1:1 in Dilution buffer** (e.g. 75 µL of cerebrospinal fluid sample + 75 µL of Dilution buffer). **Dilute synovial fluid samples 1:80 in Dilution buffer** (e.g. 5 µL of synovial fluid sample + 400 µL 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. Mix each solution well prior 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 scheme (page 3): Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. Then fill two wells with 100 µl/well of Standard 1 (serves as calibrator) and then pipette 100 µl of Negative control (CONTROL -). Fill the remaining wells with 100 µl of diluted samples (S1, S2, S3, ...). It is also suitable to apply positive control serum (STANDARD 2) for the test control. It is sufficient to apply samples as singlets, however, if you wish to minimise the laboratory error apply the samples in doublets (or triplets for Standard 1).
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 Px-conjugate r.t.u. (CONJ) well and then add 100 µL of Px-conjugate 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.

Pippeting 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	**….**											

8. PROCESSING OF RESULTS

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 Standard 1 absorbance mean of the two corresponding wells. If you applied Standard 1 as triplet and if any of the three Standard 1 absorbances is different from the mean in more than 20% 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/plasma sample, cerebrospinal fluid samples and synovial fluid samples 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. Express a sample reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1 - Semiquantitative interpretation of results

a) serum or plasma

Positivity Index	Interpretation
< 0.90	negative
0.90 – 1.10	+/-
1.11 – 3.50	+
3.51 – 5.70	++
5.71 – 7.90	+++
> 7.90	++++

b) cerebrospinal fluid

Positivity Index	Interpretation
< 1.00	negative
1.00 – 1.40	+/-
> 1.40	+

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 test. 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	= 1.054; 1.089; 1.015
Standard 1 mean	= 1.053
Correction factor	= 0.14
Cut-off value	= 1.053*0.14 = 0.147
Sample absorbance	= 0.712
Sample Positivity Index	= 0.712/0.147 = 4.84

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 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	

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 OD of CONTROL - mean is less than 0.150.
c. The absorbance of Standard 1 should be in range that is written in enclosed **Quality control certificate.**

The test is intended for the determination of specific IgM antibodies in human serum or plasma, cerebrospinal fluid and 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)

Example:

N	A	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%
18	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 99% and the specificity is 97%. Evaluation was performed with a set of blood samples that comprised of: a) anti-Borrelia IgM positive sera, b) anti-Borrelia IgM 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 40 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. Expiration date is indicated at the ELISA kit label and at all reagent labels.

Store unused strips in the sealable pouch and keep the desiccant inside.

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.

Store undiluted serum/plasma samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -18 to -28°C. Avoid repeated thawing and freezing.

Do not store diluted samples. Always prepare fresh.

14. FLOW CHART

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well 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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