ELISA-VIDITEST anti-TBEV IgM

REF ODZ-194 Σ 96 tests 2°C 10°C 2°- 10 °C

Type of determination: IgM antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum/Plasma/Cerebrospinal fluid

Processing: Manual

CE IVD

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ELISA-VIDITEST anti-TBEV IgM

ODZ-194

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-TBEV IgM - ELISA kit for the detection of IgM antibodies to tick-borne encephalitis virus in serum (plasma) or cerebrospinal fluid.

2. INTENDED USE

ELISA-VIDITEST anti-TBEV IgM is intended for in vitro diagnosis of tick-borne encephalitis virus-associated diseases (encephalitis, meningoencephalitis). The diagnostic kit can be also utilized for differential diagnosis of neuroinfections and for monitoring of antibody response after vaccination against TBEV.

This assay can be supplemented with detection of IgG anti TBEV antibodies and determination of anti-TBEV IgG avidity (ELISA-VIDITEST anti-TBEV IgG, ELISA-VIDITEST anti-TBEV IgG avidity).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-TBEV IgM is a solid-phase immunoanalytical test. The polystyrene strips are coated with a native antigen containing immunodominant epitopes of TBEV. Anti- TBEV antibodies from serum samples bind to the immobilized antigens. The other serum antibodies do not bind and are washed away, those that formed complexes with the antigens are later on recognized by animal anti-human IgM antibodies labeled with horseradish peroxidase. The presence of the labeled 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 antigens	STRIPS Ag	1 microplate
1.3 mL	Calibrator, r.t.u.[1)] CAL	1 vial
1.3 mL	Negative control serum, r.t.u. NC	1 vial
1.3 mL	Positive control serum, r.t.u. PC	1 vial
13 mL	Anti-human IgM antibodies labeled with horseradish peroxidase (Px-conjugate) r.t.u. CONJ	1 vial
55 mL	Wash buffer, 10x concentrated WASH 10x	1 vial
60 mL	Dilution buffer, r.t.u. DIL	1 vial
13 mL	Chromogenic substrate (TMB substrate), r.t.u. TMB	1 vial
13 mL	Stop solution, r.t.u. STOP	1 vial

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[1)] ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Chromogenic substrate TMB is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB and not with other Chromogenic substrates TMB-O, TMB-BF.

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 – wavelenght 450 nm).
d. Thermostat (set at 37°C) for ELISA plate incubation.

6. PREPARATION OF REAGENTS AND SAMPLES

a. **Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.**
b. **Vortex samples (sera (plasma), cerebrospinal fluids) and Control sera in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum (plasma) samples 101x in Dilution buffer** DIL (5 µL of serum sample + 500 µL of Dilution buffer). **Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 µL of cerebrospinal fluid sample + 75 µL of Dilution buffer).** Do not dilute the Positive and Negative control sera, they are ready to use.
d. Prepare **Wash buffer** by diluting the Wash buffer concentrate **10x** (WASH 10x) with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 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 month if stored at room temperature.
e. Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE

Manufacturer will not be held responsible for results if manual is not followed exactly.

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 100 µL of Dilution buffer, Standards, Negative control and serum samples to the wells according to the pipetting scheme in Figure 1: start with filling the first well dilution buffer DIL, the next two wells with Calibrator CAL, next well with Positive control serum PC and another one well with Negative control serum NC. Fill the remaining wells with diluted serum samples (S1, S2, S3...). It is satisfactory to apply samples as singles, however, if you want to minimize a laboratory error then apply the Controls and samples in doublets and Calibrator in triplet. Incubate **30 minutes (±2 min) at 37°C**.
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. Mix Px-conjugate r.t.u. CONJ well and then add 100 µL of Px-conjugate into each well. **Incubate 30 minutes (±2 min) at 37°C**.
e. Aspirate and wash four times with 250 µL/well of Wash buffer (see point d of this paragraph).
f. Dispense 100 µL of TMB substrate TMB into each well. **Incubate 15 minutes (±30 seconds) at room temperature.** The time measurement must be started at the beginning of TMB dispensing. Keep the strips in the dark during the incubation with TMB substrate.

g. Stop the reaction by adding 100 µL of Stop solution STOP. 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 620-690 nm.

Figure 1: Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL	S4										
B	CAL	S…										
C	CAL											
D	PC											
E	NC											
F	S1											
G	S2											
H	S3											

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. If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

8.1 Processing of results for the Qualitative interpretation

Compute the mean absorbance of the two wells with Calibrator CAL. (If the CAL was applied in three parallels and one absorbance is different from the mean in more than 20% then exclude the deviating well from the calculation and compute a new absorbance mean with using the other two wells)

Compute the cut-off value by multiplying the mean absorbance of CAL by correction factor. **The correction factor value determined for the particular Lot of the kit is stated in the Quality control certificate. The correction factor is different for serum samples and cerebrospinal fluid samples.**

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. The samples with absorbance in the range of 90-110% of cut-off value are equivocal (see note in par. 8.2)

8.2 Processing of using Positivity index (semiquantitative interpretation)

Determine Positivity Index for each serum sample and cerebrospinal fluid samples as follows:

1. Compute the cut-off value (see the previous paragraph)
2. Compute the Positivity Index according to the following formula:

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

3. Express the serum reactivity according to Table 1

Table 1: Semiquantitative interpretation of the results:
a) serum samles

Positivity index	Interpretation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

b) cerebrospinal fluid samples

Positivity index	Interpretation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

*on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

Example of calculation:

CAL absorbances	= 1.970; 1.996
Mean absorbance of CAL	= 1. 983
Correction factor	= 0.10
Cut-off value	= 1.983 x 0.10 = 0.198
Sample absorbance	= 0.800
Sample Positivity Index	= 0.800 / 0.198 = 4.04

Note: An equivocal 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.

The presence of rheumatoid factor may interfere with the determination of IgM. We recommend you to saturate the sample by RF sorbent before the test. The RF sorbent is diluted in the dilution solution 25x. ELISA-VIDITEST RF sorbent 2 ml is not included in the kit and can be ordered from the producer separately.

9. INTERPRETATION OF THE RESULTS

Detection of anti-TBEV antibody			Interpretation
IgG	IgM	IgG Avidity	
-	-	-	Seronegative, sensitive to primoinfection
+	-	high	Anamnestic antibody (past infection, response to vaccination) Infection in vaccinated person* (in this case follow up of IgG dynamics in second serum sample withdrawn 1 week later is recommended)
-	+	-	Suspect acute infection – early phase, examination of the second serum sample withdrawn 1 week later is recommended
+	+	low	Acute infection
+	-	low	Suspect acute or recent infection
+	+	high	Suspect recent infection, infection in vaccinated person or unspecific IgM reactivity (follow up of IgM and IgG dynamics in second serum sample withdrawn 2 weeks later is recommended)

***Note:** Result of IgM anti- TBEV antibody assay must be interpreted only in the context with patient´s symptoms and with the results of other complementary serological tests.

ELISA-VIDITEST anti-TBEV IgM may detect cross-reactive antibodies against other flaviviruses, i.e. dengue virus, West Nile, yellow fever or Japanese encephalitis viruses. Detection of IgG anti-TBEV does not ensure protective immunity against TBEV infection. Presence of protective antibodies must be confirmed by virus-neutralization test.

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

10.1 Validity of the test

The test is valid if:

The background absorbance (the absorbance of the Dilution buffer) is less than 0.150.
The mean absorbance values of standards/ control sera, and the ratio between the absorbance values of PC / CAL are in the ranges stated in the **Quality control certificate** for this kit lot.

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.

Absorbance (A) range of anti-TBEV IgM-positive serum samples was (n=19): 0.448 – 3.483

10.2.1 Intraassay variability

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot as absorbance of minimum 12 parallel wells for the particular microtitrate plate.

Example:

(n = number of parallels at the same microtitration plate, CV- variation coefficient)

n	A	±σ	CV
14	2.480	0.060	2.4 %

10.2.2 Interassay variability

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

(n= number of an independent examinations of the same serum sample)

n	A	±σ	min – max	CV
5	1.140	0.079	1.035 – 1.235	6.9 %
6	0.502	0.061	0.420 – 0.566	12.2 %

10.3 Diagnostic sensitivity and specificity

Diagnostic sensitivity was evaluated using 28 IgM anti-TBEV-positive serum samples from patients with serologically and clinically confirmed tick-borne encephalitis. Diagnostic sensitivity was 100% and the results agreement with comparative commercial IVD test was 100%

Diagnostic specificity was evaluated using 97 serum samples from healthy blood donors and was found 97.9%. The results agreement with comparative commercial IVD test was 96.7%.

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 50 mg/mL of triglycerides. However, examination of such samples is not recommended. Rheumatoid factor-positive samples did not provide false positive results, although in samples with very high level some false positivities cannot be excluded. ELISA-VIDITEST RF sorbent is not included in the kit and can be ordered from the producer separately.

11. SAFETY PRECAUTIONS

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

Controls 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.

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.

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

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.

Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)).

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

The ELISA kit should be used within three months after opening.

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. 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 on the ELISA kit label and on all reagent labels.

14. USED SYMBOLS

 number of tests

 Conformité Européenne – product meets the requirements of European legislation

 in vitro diagnostics

±σ standard deviation

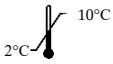
CV coefficient of variation

OD optical density

 manufacturer

 expiration

 Lot of kit

 storage at +2°C - +10°C

°C Celsius degree

% percentage

n number of tested samples

A valuea of tested sample

 read usage instructions

 catalog number

15. REFERENCES

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Holzmann H:Diagnosis of thick-borne encephalitis. Vaccine 2003; S1: S1/36-S1/40.

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16. FLOW CHART

Step 1	**Prepare reagens and samples**
	↓
Step 2	**Dispense 100 µL/well of Dilution buffer, Controls and samples**
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3	**Dispense 100 µL/well of Px-conjugate**
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well of TMB substrate**
	↓
	Incubate 15 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.**

The development of this kit was supported by grant from Ministry of Industry and Trade of the Czech Republic.

Date of the last revision of this manual: 03/2021