ELISA-VIDITEST
anti-VZV IgM

REF ODZ-197 Σ 96 tests 2°C 10°C 2°- 10 °C

Type of determination: IgM antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum

Processing: Manual

CE IVD

VIDIA

IVD

ELISA-VIDITEST anti-VZV IgM

ODZ-197

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-VZV IgM - ELISA kit for detection IgM antibodies to varicella zoster virus (VZV) in human serum.

2. INTENDED USE

ELISA-VIDITEST anti-VZV IgM assay is intended for in vitro diagnosis of VZV associated diseases, namely varicella and herpes zoster. The diagnostic kit can also be utilized for differential diagnosis of neuroinfections, infections of eye and skin exanthematous diseases.

The measurements can be supplemented with the detection of IgG antibodies to VZV, with the determination of IgG avidity, eventually with the determination of antibody intrathecal synthesis (ELISA-VIDITEST anti-VZV IgG, ELISA-VIDITEST anti-VZV IgG and IgG avidity, ELISA-VIDITEST anti-VZV IgG (CSF)).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-VZV IgM assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with a native antigen. Anti-VZV 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 recognised by animal anti-human IgM antibodies labelled with horseradish peroxidase. The presence of the 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 antigen 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
2 mL RF sorbent[2], 25x concentrated RF SORB 25x	1 vial
13 mL Anti-human IgM antibodies labelled with horseradish peroxidase r.t.u. (Px-conjugate) 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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Quality control certificate	

[1] ready to use
[2] Goat anti-human IgG globulin

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.**
b. **Vortex samples (sera), Calibrator and Control sera in order to ensure homogeneity** and mix all solutions well prior use.
c. **Prepare Dilution buffer Plus DIL PLUS:** Dilute RF sorbent RF SORB 25x by Dilution buffer DIL (i.e. 1 mL RF sorbent + 24 mL Dilution buffer. (Prepare only an amount necessary for the run, do not store.)
d. **Dilute serum samples 101x in Dilution buffer Plus** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer Plus). **Mix carefully and incubate 10 min. at room temterature. Do not dilute** the Calibrator, Positive and Negative control serum, they are ready to use.
e. Prepare Wash buffer by diluting the Wash buffer concentrate 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.
f. **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. Opalescence in diluted samples does not interfere in the test performance. It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets and the calibrator CAL 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. Add 100 µL of Px-conjugate r.t.u CONJ into each well.

   Incubate 30 minutes (±2 min) at 37°C.
e. Aspirate and wash four times with 250 µL/well of Wash buffer.
f. Dispense 100 µL of TMB substrate 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: Pippetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL PLUS**	**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 PLUS 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

1. Compute the absorbance mean of the wells with Calibrator CAL. (If the CAL was applied in three parallels and the absorbance in one well is different from the mean in more than 20%, then exclude the deviating well from the calculation and compute a new absorbance mean using the other two wells).
2. Compute the cut-off value by multiplying the mean absorbance of Calibrator with a Correction factor. **The correction factor value for this lot is stated in the Quality control certificate**.
3. Serum samples with absorbances lower than the 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive. Serum samples with absorbance in the range 90% - 110% cut-off are equivocal (grey-zone, see note, 8.2.).

8.2 Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each serum sample 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 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of the results.

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.

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.

Example of calculation:

CAL absorbances	= 1.063; 0.987; 1.025
CAL mean	= 1.025
Sample absorbance	= 0.800
Correction factor	= 0.32
Cut-off value	= 1.025 x 0.32 = 0.328
Sample Positivity Index	= 0.800 / 0.328 = 2.44

9. INTERPRETATION OF RESULTS

Anti-VZV IgM antibodies can be detected especially after primary VZV infection. In the virus reactivation (herpes zoster), IgM antibody response is weak and may not be detected. In patients with polyclonal activation of immune system (infectious mononucleosis, toxoplasmosis, some autoimmune and lymphoproliferative disorders) can occur false-positive results in the test. Result of IgM anti- VZV antibody assay must be interpreted only in the context with patient´s symptoms and with the results of other complementary VZV serological tests.

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

10.1 Validity of the test

The test is valid if:

a. The background absorbance (the absorbance of the DIL PLUS) is less than 0.150
b. 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**.**

10.2.1 Intraassay variability

The coefficient of intraassay variability is max. 8%. 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	±σ	CV%
14	2.268	0.094	4.1%

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, ±σ = standard deviation):

N	Mean Absorbance	±σ	Range (min-max)	CV%
10	0.674	0.090	0.567 – 0.779	13.4%
9	0.832	0.061	0.729 – 0.927	7.3%
7	1.116	0.069	1.048 – 1.221	6.2%

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

Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

VZV status	Total	Positive	Equivocal	Negative	Evaluation
Negative	180	3	10	167	**specificity: 98.2 %**
Positive	41	37	4	0	**sensitivity: 100 %**

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, but examination of such a samples is not recommended. RF sorbent in Dilution buffer for the samples eliminates interference of rheumatoid factor in most samples. However, the samples with very high level of RF may give false positive results, so as samples from patients with infectious mononucleosis or other conditions associated with polyclonal activation of antibody production.

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.

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)).

Wash solution, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable between different ELISA-VIDITEST kits except those with different instruction in its Instruction Manual. 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 at the ELISA kit label and at all reagent labels.

14. USED SYMBOLS


number of tests


Conformité Européenne – product meets the requirements of European legislation

in vitro diagnostics

±σ standard deviation

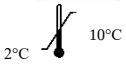
CV coefficient of variation

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. FLOW CHART

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well of Dilution buffer Plus, Calibrator, 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 r.t.u.**
	↓
	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 at room temperature in the dark
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read the absorbance at 450 / 620-690 nm within 10 min**

References:

Provost PJ, Krah DL, Kuter BJ, Morton DH et al. Antibody assays suitable for assesing immune response to live varicella vaccine. Vaccine 1991; 9: 111

Wasmuth EH, Miller WJ. Sensitive enzyme-linked immunosorbent assay for antibody to varicella zoster virus using purified VZV glycoprotein antigen. J med Virol 1990; 32: 189-93.

Leung J, Harpaz R, Baughman AL, Heath K et al. Evaluation of laboratory methods for diagnosis of varicella. Clin Infect Dis 2010; 51: 23-32.

Landry ML, Cohen SD, Mayo DR, Fong,CKY et al. Comparison of fluorescent antibody to membrane antigen test, indirect immunofluorescence assay and commercial enzyme linked immunoassay for determination of antibody to varicella zoster virus. J Clin Microbiol 1987; 25: 832-835.

Gershon AA, Steinberg SP. Antibody response to varicella zoster and the role of antibody in host defense. Amer J Med Sci 1981;282: 12-17

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

Date of the last revision of this manual: 05/2021