ELISA-VIDITEST anti-VZV IgM

Cat. No. ODZ-197

Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II 365, Vestec, 252 42 Jesenice, Czech Republic, Tel.: +420 261 090 565, Web: www.vidia.cz, E-mail: info@vidia.cz

1. TITLE:

ELISA-VIDITEST anti-VZV IgM - ELISA kit for detection IgM antibodies to varicella zoster virus (VZV) in 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 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. CONTROL -	1 vial
1.3 mL Positive control serum, r.t.u. CONTROL +	1 vial
1 mL RF sorbent[2], 51x concentrated RF SORB 51x	1 vial
13 mL Anti-human IgM antibodies labelled with horseradish peroxidase r.t.u. (Px-conjugate) CONJ	1 vial
125 mL Wash buffer, 10x concentrated WASH 10x	1 vial
125 mL Dilution buffer, r.t.u. DIL	1 vial
13 mL Chromogenic substrate (TMB sbstrate), r.t.u. TMB-O	1 vial
13 mL Stop solution, r.t.u. STOP	1 vial
Sealable pouch for unused strips	
Instruction manual	
Quality control ceetificate	

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

Chromogenic substrate solution (TMB-O) r.t.u. is intended only for some ELISA-VIDITEST and IS NOT INTERCHANGABLE between 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 – wavelenght 450 nm).

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 1:50 by Dilution buffer (i.e. 0.1 mL RF sorbent + 5 mL Dilution buffer. (Prepare only an amount necessary for the run, do not store.)

d. **Dilute serum samples 1:100 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. 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.

f. 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. Pipette Calibrator, Controls and samples according to the pipetting scheme (page 3, fig 1). Start with filling the first well with 100 µl of Dilution buffer Plus (DIL-plus) to estimate the reaction background. Fill the next two wells with Calibrator (CAL). The next well fill with Negative control serum (CONTROL -). The remaining wells fill with diluted tested sera (S1...). It is also suitable to apply Positive control serum (CONTROL +) for the test control. 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 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 Px-conjugate r.t.u (CONJ) into each well.

e. **Incubate 60 minutes (±5 min) at room temperature**.

f. Aspirate and wash four times with 250 µl/well of Wash buffer.

g. Dispense 100 µl of TMB substrate into each well.

h. Incubate **10 minutes (+/-5 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.

i. 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.

j. 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**	**S5**										
b	**CAL**	**S…**										
c	**CAL**											
d	**CONTROL -**											
e	**S1**											
f	**S2**											
g	**S3**											
h	**S4**											

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.

8.1. Processing of results for the Qualitative interpretation

a) 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).

b) 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**.

c) 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 0.9-1.1 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:

a) Compute the cut-off value (see the previous paragraph)
b) Compute the Positivity Index according to the following formula:

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

c) Express the serum reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of the results.

Positivity index	Interpretation
< 0.9	negative
0.90 - 1.10	+/-
1.11 - 2.00	+
2.01 - 3.00	++
> 3.00	+++

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. 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 Dilution buffer plus) is less than 0.150
b. The mean absorbance value of Calibrator is in the range **stated in the Quality control certificate for this kit lot**.
c. OD values of control sera should be:

absorbance of (CONTROL +) > absorbance of CAL > absorbance of CONTROL -

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%
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, SD = standard deviation):

N	Mean Absorbance	SD	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	
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.

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

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:

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.

References:

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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.

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14. FLOW CHART:

Step 1	**Prepare reagents and samples** ↓ **Incubate the samples in Dilution buffer Plus 10 min. at room temperature**
	↓
Step 2	**Dispense 100 µL/well of Dilution buffer Plus, Calibrator, Controls 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-O substrate** ↓ **Incubate 10 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**

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