ELISA-VIDITEST anti-VZV IgG

REF ODZ-168 | Σ 96 tests | 2°C 10°C 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum

Processing: Manual

CE IVD

VIDIA

ELISA-VIDITEST anti-VZV IgG

ODZ-168

Instruction manual

PRODUCER: VIDIA spol. s r.o.., Nad Safinou II 365, 252 50 Vestec, Czech Republic, Tel.: +420 261 090 565, Web: www.vidia.cz, E-mail: info@vidia.cz

1. TITLE

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

2. INTENDED USE

ELISA-VIDITEST anti-VZV IgG is intended for in vitro diagnosis of VZV-associated diseases, namely varicella and herpes zoster. The test can also be utilized for the differential diagnosis of neuroinfections, infections of the eye and of exanthematous diseases of the skin.
The measurements should be supplemented with the detection of IgM and IgA antibodies to VZV or with the determination of IgG avidity (ELISA-VIDITEST anti-VZV IgA, ELISA-VIDITEST anti-VZV IgM, ELISA-VIDITEST anti-VZV IgG and IgG avidity).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-VZV IgG is an enzyme linked imunosorbent assay. Reaction wells are coated with the native VZV antigen. Anti-VZV antibodies from serum samples bind to the immobilized antigens. The other serum antibodies do not bind and are washed away. The anti-VZV antibodies are then recognized by anti-human IgG antibodies labeled with horseradish peroxidase (Px-conjugate). The presence of the labeled antibodies bound to the anti-VZV antibodies is revealed by an enzymatic reaction with a chromogenic substrate that changes the color of the solution within the well. Negative sera do not react and the mild change in color, if present, may be attributed to the reaction background.

4. KIT COMPONENTS

ELISA 8-well break-away strips coated with a specific antigens STRIPS Ag	1 microplate
1.3 mL Standard A=negative control serum, r.t.u.[1)] ST A/NC	1 vial
1.3 mL Standard D=calibrator, r.t.u. ST D/CAL	1 vial
1.3 mL Standard E=positive control serum, r.t.u. ST E/PC	1 vial
13 mL Anti-human IgG antibodies labelled with horseraddish 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

Instruction manual
Quality Control Certificate
[1)] r.t.u. 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

Distilled or deionised water for diluting of the Wash buffer concentrate. Appropriate equipment for pipetting, liquid dispensing and washing. Thermostat (set at 37°C) for ELISA plate incubation.
Spectrophotometer/colorimeter (microplate reader – wavelenght 450 nm).

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 and Standards in order to ensure homogeneity** and mix all solutions well prior use. **Dilute serum samples 101x in Dilution buffer** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). **Do not dilute** the Controls (Standards), they are ready to use.
c. **Prepare Wash buffer** by diluting the Wash buffer concentrate ten times (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.
d. **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 bag to reach room temperature. Withdraw an adequate number of strips and put the remaining strips into the provided plastic bag and seal it with the desiccant kept inside.
b. Pipette Standards and samples according to the pipetting scheme (page 3, Fig. 1). Start with filling the first well with 100 µL of Dilution buffer DIL to estimate the reaction background. Then fill a doublet of wells with 100 µL of ST D/CAL, next well with Positive control serum ST E/PC and another one well with Negative control serum ST A/NC. Fill the remaining wells with 100 µL of the diluted samples (S1, S2, S3,...). Standard D serves as a calibrator. It is sufficient to test samples as a single well measurement, however, if you wish to minimize the laboratory error apply ST D/CAL in triplet and the controls and the samples in doublets.
   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 as in step "c".
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 for a few times to mix the reagents.
h. Measure the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use the 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	**ST D/CAL**	**S…**										
c	**ST D/CAL**											
d	**ST E/PC**											
e	**ST A/NC**											
f	**S1**											
g	**S2**											
h	**S3**											

8. PROCESSING OF RESULTS

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL well) from the absorbances of 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 mean of the Standard D ST D/CAL. (In the case Standard D was applied as triplet, it is possible to exclude an outlying well that may occur. Identify the outlying well as the well which absorbance is different from the mean in more than 20% of the mean and simply exclude the well from the calculation and compute the mean using the two other wells)
2. Compute the **cut-off value** by multiplying the Standard D ST D/CAL mean with the correction factor. **The correction factor value for this lot is stated in the Quality control certificate**. Serum 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. Serum samples with absorbance within the range of 90-110% of the cut-off value are equivocal (grey-zone, see 8.2.).

8.2 Processing of results for the Semiquantitative interpretation

1. Compute the cut-off value (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 the serum reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of 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: The indifferent sample reactivity interpreted as +/-, requires retesting of the sample. If the result is again indifferent (equivocal) then it is recommended to use an alternative testing method or to obtain another, different sample from the patient, usually withdrawn 1-2 weeks later.

Example of calculation:

Standard D absorbances	= 1.807; 1.704; 1.750
Standard D mean	= 1.754
Correction factor	= 0.15
Cut-off value	= 1.754 x 0.15 = 0.263
Sample absorbance	= 0.800
Sample Positivity Index	= 0.800 / 0.263 = 3.04

9. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

9.1 Validity of the test

The test is valid if:

The background of the reaction (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 ST E/PC / ST D/CAL are in the ranges stated in the **Quality control certificate** for this kit lot.

9.2 Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

9.2.1 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, σ = standard deviation)

N	Mean absorbance	±σ	CV%
16	1.335	0.050	3.8%
16	0.614	0.023	3.7%

9.2.2 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%
18	1.369	0.064	1.223 – 1.476	4.7%
18	0.463	0.060	0.337 – 0.569	12.9%

9.2.3 Recovery test

Measured values of recovery test for every Lot are between 80-120% of expected values.

9.3 Diagnostic specificity and sensitivity

The diagnostic sensitivity of the test is 98.6% and the specificity is 98.6%. Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

Serum samples	Total	Positive	Equivocal	Negative	
Positive	73	68	4	1	Sensitivity: 98.6%*
Negative	74	1	2	71	Specificity: 98.6% *

* Equivocal results were not taken in account for calculation

9.4 Interference

Haemolytic, icteric and lipaemic samples showed no influence on results up to the concentration of 50 mg/mL of hemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides.

10. SAFETY PRECAUTIONS

All components 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, i.e. autoclave for 1 hour at 121°C all reusable materials that were in contact with Standards or samples, burn the disposable ignitable materials, decontaminate liquid wastes and non-ignitable materials with 3% chloramine.

Liquid wastes containing Stop solution (0.4M sulphuric acid) should be neutralized with 4% sodium bicarbonate. Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin or mucous membranes, rinse immediately with plenty of water.

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 pipette by mouth. Do not smoke, eat or drink where specimens or kit reagents are handled. Wear disposable gloves while handling kit reagents and specimens and wash your hands thoroughly afterwards.

Avoid spilling or producing aerosol.

11. HANDLING PRECAUTIONS

Manufacturer guarantees performance of the entire ELISA kit if the procedure indicated in the Instruction manual is followed.

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

Wash buffer, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable among different ELISA-VIDITEST sets except those that explicitly state different in their Instruction manuals.

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 a sample or Px-conjugate
- Use of the identical pipette tip for different solutions

12. 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 plastic bag 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 dilutions.

Kits are shipped in cooling bags and the transport time up to 72 hours have no influence on expiration. If you find damage on any part of the kit, please inform the manufacturer.
Expiration date is indicated on the ELISA kit label and on all reagent labels.

13. USED SYMBOLS


number of tests


Conformité Européenne – product meets the requirements of European legislation

in vitro diagnostics

±σ standard deviation

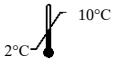
CV coefficient of variation

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

14. 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 blycoprotein 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 antipody 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

15. FLOW CHART

Step 1	**Prepare reagents 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 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
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read the absorbance at 450 nm / 620-690 nm within 10 min**

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: 02/2021