ELISA-VIDITEST anti-VZV IgG (CSF)

Cat. No. ODZ-087

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-VZV IgG (CSF) ELISA kit for detection IgG antibodies to varicella zoster virus in serum and cerebrospinal fluid.

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 (CSF) assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with a native antigen containing immunodominant epitopes of VZV. 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 IgG 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.

Detection of intrathecal production of antibodies (detection of specific IgG antibodies in cerebrospinal fluid produced intrathecally) is necessary for the assessment of the antibody response within the central nervous system. It requires parallel measurements of specific IgG antibodies in serum and in cerebrospinal fluid sample taken from the patient at the same time and the determination of total albumin and total IgG in both samples. The calculation of the specific antibody production is according to Reiber equation (see 8.2 – processing of the results).

4. KIT COMPONENTS

ELISA 8-well break-away strips coated with specific antigen STRIPS Ag	1 microplate
1.3 mL STANDARD A 60 mIU (international (mili-) units), r.t.u.*	1 vial
1.3 mL STANDARD B 200 mIU, r.t.u.	1 vial
1.3 mL STANDARD C 500 mIU, r.t.u.	1 vial
1.3 mL STANDARD D 1500 mIU, r.t.u.	1 vial
1.3 mL STANDARD E 10000 mIU, r.t.u.	1 vial
15 mL Anti-human IgG antibodies labelled with horseradish peroxidase, r.t.u. (Px-conjugate) CONJ	1 vial
125 mL Wash buffer, 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

*** r.t.u. = ready to use**

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. 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 and Standards in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum samples 1:101 in Dilution buffer (DIL)** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). For evaluation of the intrathecal production test two dilutions of serum samples are recommended: 101x and 404x. Dilution 404x prepare by 4x diluting the 101x diluted serum sample (e.g. 150 µL of Dilution buffer **+** 50 µL of serum sample diluted 101x). **Dilute cerebrospinal fluid samples 2x in Dilution buffer** (e.g. 100 µL of cerebrospinal fluid sample + 100 µL of Dilution buffer). Do not dilute 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.

7. ASSAY PROCEDURE

7.1 Procedure for the quantitative determination in serum:

a. Allow the antigen-coated strips to reach room temperature before opening sealed the aluminium bag in order to prevent water condensation within the wells. Withdraw the adequate number of strips and put the remaining strips in the sealable pouch and keep the desiccant inside.
b. Pipette Standards and samples according to the pipetting scheme (Fig. 1). Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 µl of Standard A, B, C, D and E. Fill the remaining wells with 100 µl of serum samples (S1, S2, S3,...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets. We recommend to include positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.
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. Add 100 µL of Px-conjugate r.t.u. 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 the TMB substrate into each well; pipette in a regular rhythm or use an appropriate dispensing instrument. **Incubate for 10 (+/-5 sec.) minutes at room temperature**. **The time measurement must be started right at the beginning of TMB dispensing.** Cover the strips and keep them in the dark during the enzymatic reaction.

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 for a few times to ensure complete mixing of the reagents.

j. Read the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use reference reading at 620 - 690 nm.

Figure 1: Pipetting scheme

QUANTITATIVE ANTIBODY DETERMINATION IN SERUM

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**	**S3**										
b	**ST A**	**S4**										
c	**ST B**	**…**										
d	**ST C**											
e	**ST D**											
f	**ST E**											
g	**S1**											
h	**S2**											

7.2 Procedure for serum and cerebrospinal fluid samples (detection of intrathecal antibodies):

a. Allow the antigen-coated strips to reach room temperature before opening sealed the aluminium bag in order to prevent water condensation within the wells. Withdraw the adequate number of strips and put the remaining strips in the sealable pouch and keep the desiccant inside.

b. Pipette Standards and samples according to the pipetting scheme (Fig 2). Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 µl of Standard A, B, C, D and E. Fill the remaining wells with 100 µl of serum samples (S1, S2, S3, ...) and cerebrospinal fluid samples. We recommend testing each serum sample in two different dilutions: 1:100 and 1:400 and each cerebrospinal fluid sample in one dilution 2x. If you wish to minimize laboratory error apply the samples in doublets. We recommend to include positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.

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. If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.

e. 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.

Incubate for **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. Read 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 2: Pipetting scheme

MEASUREMENT OF INTRATHECAL ANTIBODY PRODUCTION (SERUM AND CSF SAMPLES)

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**	**S1 (1:400)**										
b	**ST A**	**S2 (1:400)**										
c	**ST B**	**CSF1 (1:2)**										
d	**ST C**	**CSF2 (1:2)**										
e	**ST D**	**…**										
f	**ST E**											
g	**S1 (1:100)**											
h	**S2 (1:100)**											

8. PROCESSING OF RESULTS

Regardless of the method chosen, begin the processing with subtraction of the absorbance of the DB well (background absorbance) from the absorbances in all other wells.

8.1 Processing of results for the quantitative determination in serum samples

Compute the sample antibody titre in international (mili-) units (mIU/ml) as follows:

1. Construct the calibration curve by plotting the absorbance of Calibrators (y-axis – in linear scale) to antibody concentration in international (mili-) units (mIU/ml) (x-axis – may have logarithmic scale). The antibody concentration in each Standard (A-E) is mentioned in paragraph 4 – Kit components.
2. Determine the unknown antibody titre in the samples from the calibration curve. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.
3. Calculate the concentration of anti-VZV antibodies (mIU/mL) by multiplying the mIU value of the sample from the calibration curve with the sample dilution (i.e. mIU x 101 for 101x (1:100) dilution)

Result interpretation:

Concentration (mIU/mL)	Interpretation
< 150	Negative
150 - 200	+/-
> 200	Positive

Note 1: The quantification is accurate only within the linear part of the calibration curve. If the sample OD is outside the linear interval (OD 0.2 – 2.3) it is necessary to repeat the test with more diluted serum samples to obtain the precise quantification.

Note 2: 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.

8.2 Processing of results for estimation of the intrathecal antibody production

1. Calculate the anti-VZV IgG antibody concentrations (mIU/mL) for all tested samples by multiplying the mIU in the sample deducted from the calibration curve with the sample dilution. For sera mIU x101 and mIU x 404, for cerebrospinal fluid mIU x 2.
2. Calculate the Specific antibody quotient as follows:

   Q_{spec}= concentration of IgG anti-VZV (mIU/mL) in cerebrospinal fluid / concentration of IgG anti-VZV (mIU/mL) in serum

3. Compute the Total antibodies quotient as follows:

   Q_{total}= concentration of total IgG (mg/mL) in cerebrospinal fluid / concentration of total IgG (mg/mL) in serum

4. Compute the Albumine quotient as follows:

   Q_{alb}= concentration of albumin (mg/mL) in cerebrospinal fluid / Concentration albumin (mg/mL) in serum

5. Calculate the Limite quotient Q_{lim} that shows status of the hematoencephalic barrier according to the Reiber equation[1]

   Compute Q_{lim} using the equation:

   $$Q_{lim}(IgG) = 0.93 * \sqrt{(Q_{alb})^2 + 6*10^{-6}} - 1.7 * 10^{-3}$$

6. Compute the Antibody Index AI:

   a) If $Q_{total\ IgG} < Q_{lim,}$ then calculate AI using the formula:

   $$AI = \frac{Q_{spec}}{Q_{total}}$$

   b) If $Q_{total\ IgG} > Q_{lim}$ compute AI using the formula:

   $$AI = \frac{Q_{spec}}{Q_{lim}}$$

Result interpretation[2]:

AI < 1.5	intrathecal antibody production not found
AI 1.5 – 2.00	suspect intrathecal antibody production
AI > 2.0	inthrathecal antibody production proven

[1] according to Reiber H.: The hyperbolic function: a mathematical solution of the protein influx/CSF flow model for blood-CSF barier function. J Neurol Sci 1994; 126: 243-5.

[2] according to Reiber H, Lange P.: Quantification of Virus-Specific Antibodies in Cerebrospinal Fluid and Serum: Sensitive and Specific Detection of Antibody Synthesis in Brain Clin. Chem 1991;.37/7: 1153-1160

An automatic data processing is possible when purchasing the VIDITAB software available from VIDIA.

9. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

9.1 Validity of the test

The test is valid when:

The background of the reaction (absorbance of the Dilution buffer well) is less than 0.100.

The mean Standard D absorbance should be in range that **is written in enclosed Quality control certificate.**

The absorbance of Standards follows this order: ST A < ST B < ST C < ST D < ST E.

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. 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, σ = standard deviation)

n	Mean value	±σ	CV (%)
16	1.396	0.033	2.4 %
16	0.340	0.015	4.3 %

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 value	±σ	min – max	CV (%)
8	0.237	0.024	0.210 – 0.288	10.1
8	1.100	0.087	0.998 – 1.280	7.9
8	1.624	0.086	1.516 – 1.778	5.3

9.2.3. Recovery test

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

9.3 Diagnostic sensitivity and specificity

The diagnostic sensitivity of the test is 96.6% and the specificity is 96.9%. Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

9.4 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.

10. 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. 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 pipette by mouth. 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.

11. HANDLING PRECAUTIONS

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Follow the assay procedure indicated in the Instruction manual.
c. Wash buffer, chromogenic substrate TMB, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.
d. Avoid contamination of serum samples and kit reagents.
e. Avoid cross-contamination of reagents.
f. Standards, TMB solution, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
g. Avoid microbial contamination of serum samples and kit reagents.
h. Avoid cross-contamination of reagents.
i. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
j. 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

12. STORAGE AND EXPIRATION DATE

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 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 serum samples. Always prepare fresh.

Kits are shipped in cooling bags, the transport time of 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.

13. FLOW CHART

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well 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 nm (ref. 620 – 690 nm) within 10 minutes**

References:

Reiber H.: The hyperbolic function: a mathematical solution of the protein influx/CSF flow model for blood-CSF barier function. J Neurol Sci 1994; 126: 243-5.

Reiber H, Lange P.: Quantification of Virus-Specific Antibodies in Cerebrospinal Fluid and Serum: Sensitive and Specific Detection of Antibody Synthesis in Brain Clin. Chem 1991;.37/7: 1153-1160

Schultze D,Weder B,Cassinotti P,Vitek L, et al. Diagnostic significance of intrathecally produced herpes simplex and varicella-zoster virus-specific antibodies in central nervous systém infections. Swiss med wkly 2004;134: 700-704. (www.smw.ch)

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