ELISA-VIDITEST anti-VCA EBV IgG (CSF)
Quantitative assay

Cat. No.: ODZ-084

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-VCA EBV IgG (CSF) – ELISA kit for the detection of IgG antibodies to viral capsid antigen of Epstein-Barr virus in human serum and cerebrospinal fluid.

2. INTENDED USE

ELISA-VIDITEST anti-VCA EBV (CSF) is intended for in vitro diagnostic procedures in EBV induced and EBV associated diseases, such as infectious mononucleosis (IM) and the chronic active EBV infection. The test is also useful in the diagnosis of Burkitt's lymphoma, nasopharyngeal carcinoma, carcinoma of the Waldeyer's ring and in characterisation of opportunistic lymphomas (oligo- and polyclonal). The other use of the test is in characterisation of chronic fatigue syndrome, in neuroinfections and immunosupression which is frequently associated with EBV reactivation.

3. TEST PRINCIPLE

ELISA-VIDITEST anti-VCA EBV IgG (CSF) is an enzyme linked immunosorbent assay. The wells of polystyrene strips are coated with specific antigen containing immunodominant epitopes of the VCA complex. Anti-VCA antibodies if present in serum/CSF samples bind to the immobilized antigen. The antibodies that were bound to the antigen are in the next step of the assay detected with anti-human (IgG) antibodies labelled with horseradish peroxidase. The amount of the bound detection antibodies is measured by addition of a chromogenic substrate. A serum sample without anti-VCA antibodies causes only a mild change of colour which, if occurs, may be attributed to the background of the reaction.

Measurement of the intrathecal synthesis of anti-VCA antibodies reveals the antibody production within the central nervous system. Such measurements require quantification of the antibody response in paired serum and CSF samples, taken at the same time of the disease. The precise quantification is only possible if the sample reactivity is within the linear range of the ELISA calibration curve, therefore it is recommended to measure the presence of anti-VCA antibodies in two different serum dilutions (see. ASSAY PROCEDURE 7.2). The calculation of the intrathecal synthesis requires knowledge of albumin and the total IgG concentration in both, the serum and the CSF sample. The calculation is done according to Reiber's equation (see PROCESSING OF RESULTS 8.2).

4. KIT COMPONENTS

ELISA	8-well break-away strips coated with specific antigen STRIPS Ag	1 microplate
1.3 mL	STANDARD A 5 AU (Arteficial units), r.t.u.*	1 vial
1.3 mL	STANDARD B 18 AU, r.t.u.	1 vial
1.3 mL	STANDARD C 50 AU, r.t.u.	1 vial
1.3 mL	STANDARD D 150 AU, r.t.u.	1 vial
1.3 mL	STANDARD E 600 AU, r.t.u.	1 vial
13 mL	Anti-human IgG antibodies-Px conjugated (Px-conjugate), r.t.u. CONJ	1 vial
125 mL	Wash buffer, 10x concentrated WASH 10x	1 vial
100 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

Zip lock plastic bag
Instruction manual
Certificate of quality

*A.U./mL = artificial units per mL
** r.t.u. = ready to use

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT

Distilled/deionised water for diluting the concentrated Wash buffer, equipment for pipetting, dispensing and washing, test tubes for diluting samples, microplate cover or seal, 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 prior use.

c. Dilute **serum samples 101x** with Dilution buffer (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). In case of measurements of the **intrathecal synthesis,** prepare two **serum dilutions** (**101x and 404x**), e.g. mix 50 µL of 101x diluted serum sample with 150 µL of Dilution buffer. Dilute **cerebrospinal fluid samples 2x (1:1)** in Dilution buffer (e.g. 100 µL of cerebrospinal fluid sample + 100 µL of Dilution buffer).

d. Do not dilute Standards, they are ready to use.

e. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with distilled/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 concentrate in a water bath set to 32-37°C. The 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

7.1 Detection of serum antibodies only:

a. Allow the vacuum sealed strips to reach room temperature before opening. It prevents moist condensation within the wells. Withdraw the needed number of strips and put the remaining strips and the desiccant device in the provided plastic bag.

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, E and F. 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 for 30 (+/- 2) minutes at laboratory temperature**.

d. Aspirate the contents of the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of the diluted Wash buffer. Avoid well to well cross-contamination. Empty the wells by inverting the plate and tapping it against a pile of adsorbent papers.

e. Add 100 µL of Px-conjugate r.t.u. into each well.

f. **Incubate for 60 (+/-5) minutes at room temperature.**

g. Aspirate and wash as in step "d".

h. Dispense 100 µL of TMB substrate into each well. **Incubate for 10 (+/-5 sec.) minutes at room temperature**. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation.

i. Stop the reaction by adding 100 µL of Stop solution. Use the same regular pipetting rhythm as mentioned above to ensure the same reaction time in all wells. Check the wells for bubbles and remove them by tapping gently the microplate for a few times.

j. Determine the absorbance at 450 nm in a microplate reader **within 20 minutes**. It is recommended to use a reference reading at 620 - 690 nm.

7.2 Measurement of the intrathecal synthesis – parallel detection of serum and CSF antibodies

a. Allow the vacuum sealed strips to reach room temperature before opening. It prevents moist condensation within the wells. Withdraw the needed number of strips and put the remaining strips and the desiccant device in the provided plastic bag

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. As a technical hint we recommend to pipette diluted samples into a clean microplate (without any antigen) and then use a multichannel pipette to transfer the diluted samples into the VCA coated microplate.

c. **Incubate 30 minutes (+/-5 min) at room temperature.**

d. Aspirate the contents of the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of the diluted Wash buffer. Avoid well to well cross-contamination. Empty the wells by inverting the plate and tapping it against a pile of adsorbent papers.

e. Add 100 µL of Px-conjugate r.t.u. into each well.

f. **Incubate for 60 (+/-5) minutes at room temperature.**

g. Aspirate and wash as in step "d".

h. Dispense 100 µL of TMB substrate into each well. **Incubate for 10** (+/-5 sec.) **minutes** at room temperature. The time measurement must be started at the beginning of TMB dispensing. Pipette in a regular rhythm or use a suitable dispensing instrument. Cover the strips with an aluminium foil or keep them in the dark during the incubation.

i. Stop the reaction by adding 100 µL of Stop solution. Use the same regular pipetting rhythm as mentioned above to ensure the same reaction time in all wells. Check the wells for bubbles and remove them by tapping gently the microplate for a few times.

j. Determine the absorbance at 450 nm in a microplate reader **within 20 minutes**. It is recommended to use a reference reading at 620 - 690 nm

Pipetting scheme:

Fig.1: Detection of serum antibodies only

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

Fig.2: Measurement of the intrathecal synthesis – parallel detection of serum and CSF antibodies

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S1 (404x)										
b	ST A	S2 (404x)										
c	ST B	CSF1 (2x)										
d	ST C	CSF2 (2x)										
e	ST D	...										
f	ST E											
g	S1 (101x)											
h	S2 (101x)											

8. PROCESSING OF RESULTS

8.1. Measurement of serum antibodies only

a. Subtract the DIL absorbance from the absorbances in all other wells (Blank difference data).
b. Construct the calibration curve by plotting the A.U./mL of Standards (x-axis) versus the absorbances of Standard wells (y-axis). It is recommended to use x-axis in logarithmic scale.
c. For each absorbance on the y-axis find the corresponding value on the x-axis. It is possible to use a variety of software applications for fitting the standard curve and calculation of the unknowns, e.g. Winliana, KimQ (4PL logistic) or MS Excell (linear regression – suitable only for the linear part of the calibration curve).

Interpretation of results:

Concentration (A.U.)	Interpretation
< 18	negative
18.0 - 24.0	+/-
>24.0	positive

Note: Range between 18 – 24 AU/mL is a grey zone. In such case it is recommended to repeat the assay. If the result of sample is in the grey zone, again use an alternative diagnostic method or initiate taking another blood sample 1-2 weeks later.

8.2 Measurement of the intrathecal synthesis – parallel detection of serum and CSF antibodies

a. Subtract the DIL absorbance from the absorbances in all other wells (Blank difference data).
b. Construct the calibration curve by plotting the A.U./mL of Standards (x-axis) versus the absorbances of Standard wells (y-axis). It is recommended to use x-axis in logarithmic scale.
c. For each absorbance on the y-axis find the corresponding value on the x-axis. It is possible to use a software for fitting the standard curve and calculation of the unknowns, e.g. Winliana, KimQ (4PL logistic) or MS Excell (linear regression – suitable only for the linear part of the calibration curve).
d. Multiply the results by the dilution factor (for sample dilution 101x – multiply the concentration in A.U./mL by 101, for 404x multiply by 401, for 2x (1:1) multiply by 2.
e. Calculate the Specific IgG antibody quotient:

$$Q_{spec} = \frac{\text{concentration of anti-VCA IgG (A.U./mL) in cerebrospinal fluid}}{\text{concentration of anti-VCA IgG (A.U./mL) in serum}}$$

f. Calculate the total IgG quotient:

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

g. Compute the Albumine quotient:

$$Q_{alb} = \frac{\text{concentration of albumin (mg/mL) in cerebrospinal fluid}}{\text{concentration of albumin (mg/mL) in serum}}$$

h. Calculate the Limit quotient Q_{lim} that shows status of the hematoencephalic barrier according to the Reiber's equation (see Reference no. 1)

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

i. Compute the Antibody Index AI:

If $Q_{total} < Q_{lim}$, then $AI = \frac{Q_{spec}}{Q_{total}}$

If $Q_{total} > Q_{lim}$ then $AI = \frac{Q_{spec}}{Q_{lim}}$

Interpretation of results (see Reference no. 2):

AI < 1.5	intrathecal antibody synthesis not found
AI 1.5 – 2.0	suspect intrathecal synthesis
AI > 2.0	intrathecal antibody synthesis proven

The automatic data processing software VIDITAB is available from VIDIA.

9. DIAGNOSTIC INTERPRETATION OF RESULTS

VCA IgM	VCA IgG	VCA IgA	EA (D) IgG	EBNA-1 IgG	EBNA-1 IgM	Stages of EBV infection
-	-	-	-	-	-	**EBV negative**
+ +	- +	+ +	- +	- -	+ +	**Primoinfection EBV (acute phase)**
+ -	+ +	+ -	+ -	+/- -	+ -	**Primoinfection EBV(post-acute phase)**
- -	+ +	+ -	- -	- +	- -	**Primoinfection EBV (convalescent phase)**
-	+	- +	-	+	-	**Eliminated infection EBV**
- +	+ +	- +	+ +	+ +	- -	**Reactivation of EBV**

VIDIA offers also the other diagnostic assays to detect the antibodies against the other EBV antigens:
ELISA-VIDITEST anti-EBNA-1 EBV IgM and IgG
ELISA-VIDITEST anti-VCA EBV IgM and IgA
ELISA-VIDITEST anti-EA (D) EBV IgM and IgG
IF-VIDITEST anti-VCA EBV IgM and IgG
IF-VIDITEST anti-EA EBV (D+R or D) IgG

10. TEST PERFORMANCE

10.1. Validity

The test is valid if:

The absorbance of the DIL well (reaction background) is less than 0.150.

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

The absorbances of Standards follow the order: ST A < ST B < ST C < ST D < ST E.

10.2. Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined by testing samples with different antibody concentrations.

OD range 0.322 – 2.852

10.2.1 Intraassay variation (n = number of parallels):

n	Mean value	Standard deviation	CV (%)
16	0.229	0.012	5.2
16	1.080	0.053	4.9

10.2.2 Interassay variation (n= number of repetitions of the testing)

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	Mean value	Standard deviation	min – max	CV (%)
18	0.430	0.042	0.364 – 0.488	9.7
18	1.127	0.092	0.962 – 1.260	8.1
18	1.500	0.126	1.347 – 1.772	8.4
19	2.341	0.089	2.231 – 2.506	3.8

10.3. Diagnostic specificity and sensitivity

The diagnostic sensitivity was measured by testing the population sample that is expected to have anti-VCA IgG antibodies (blood donors, patients with active EBV infection). The results were confirmed by other commercial test. The diagnostic sensitivity was 98.1 %.

The specificity of the test was measured by testing EBV negative healthy blood donors. The specificity of the test was 97.1%.

10.4. Dilution test

Samples having high, medium and low anti-VCA antibody titres were initially diluted 1:100 and then further diluted and analysed. The resulting values were within the range of 85-115% of the expected value.

10.5. Recovery

The analytical recovery was measured by testing samples prepared as a mixture of a sample having optical density within the linear part of the calibration curve and of a sample having optical density maximally 10 times higher or 10 times lower. The resulting values are within the range of 80-120% of the expected value.

10.6
Limit of detection

The limit of detection was calculated as the minimal concentration that was at the 95% confidence level different from the Blank. The limit of detection was 1.6 A.U./mL

10.5. 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.

11. 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.

12. HANDLING PRECAUTIONS

Manufacturer guarantees the performance of the ELISA kit.

Wash solution, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.

Avoid microbial contamination of serum samples and kit reagents.

Avoid cross-contamination of reagents.

Standards, TMB solution, Dilution buffer and Px-conjugate contain preservative ProClin 300®.

Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

Follow the assay procedure indicated in the Instruction manual. Unreliable results of the test are usually due to:

* Insufficient mixing of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique, spilling drops of the samples or Px-conjugate to the rim of wells
* Use of the same pipette tip for different solutions

13. STORAGE AND EXPIRATION

The kits are shipped cooled in cool bags. The transport time up to 72 hours has no influence on the expiration date. If you find damage to any kit component, please inform the manufacturer.

Store the kit and the reagents at +2 to +10°C, in a dry place, protected from the light. The date of expiration is indicated on the ELISA kit label and on labels of the components.

Store the unused strips in the provided plastic bag with the desiccant kept inside.

Store serum and CSF samples at temperature -18 to -28°C, undiluted in small aliquots. Avoid repeated freezing and thawing of samples. Short term storage (up to one week) of thawed/fresh serum samples is possible at +2 to +10°C. Do not store the diluted samples, always prepare fresh.

14. FLOW CHART:

Step 1.	**Prepare/dilute reagents and samples**
	↓
Step 2.	**Pipette 100 µL / well of Standards and samples**
	↓
	Incubate for 30 minutes at laboratory temperature
	↓
	Wash 4 times (250 µL / well)
	↓
Step 3.	**Dispense 100 µL / well of Px conjugate**
	↓
	Incubate for 60 minutes at laboratory temperature
	↓
	Wash 4 times (250 µL / well)
	↓
Step 4.	**Dispense 100 µL / well TMB substrate**
	↓
	Incubate for 10 minutes laboratory temperature in the dark
	↓
Step 5.	**Dispense 100 µL / well of Stop solution**
	↓
Step 6.	**Read optical density at 450 nm (ref. 620 – 690 nm) within 20 minutes**

References:

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3. Gorgievski-Hrisoho M.,Hinderer W.,Nebel-Schickel H.,Horn J.,Vornhagen R., Sonneborn H., Wolf H., Siegl G.: Serodiagnosis of Infectious Mononucleosis by Using Recombinant Epstein-Barr Antigens and Enzyme-Linked Immunosorbent Assay Technology. J.of Clinical Microbiology 28: 2305-2311, 1990

4. Bowdre J.H.: Epstein-Barr virus serology, Clin.Imunol Newsletter 1991;11, 81-85

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