ELISA-VIDITEST anti-EBNA-1 EBV IgG

ODZ-001

Instruction manual

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1. TITLE:

ELISA-VIDITEST anti-EBNA-1 EBV IgG – ELISA kit for detection of IgG antibodies to Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA-1) in human serum or plasma.

2. INTENDED USE:

The kit is intended for the diagnosis of EBV-induced or -associated diseases, such as infectious mononucleosis, chronic active EBV infection, Burkitt`s lymphoma, carcinomas of Waldayer's ring, opportunistic lymphomas (oligo- or polyclonal) and nasopharyngeal carcinoma. The kit may also be used for the overall characterization of the chronic fatigue syndrome and of immunodeficiency during which EBV is frequently activated.

3. TEST PRINCIPLE:

ELISA-VIDITEST anti-EBNA-1 EBV IgG assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with recombinant antigen that bears immunodominant epitopes of EBNA-1. The anti-EBNA-1 EBV antibodies, if present in the tested sera (plasma), bind to the immobilized antigens and the antibodies being in complexes with antigen are later on recognised by animal anti-human IgG antibodies labelled with horseradish peroxidase. The labelled antibodies are 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 recombinant antigen	STRIPS Ag	1 microplate
1.3 mL	STANDARD A, 4 AU/mL[1], r.t.u.[2]	1 vial
1.3 mL	STANDARD B, 20 AU/mL, r.t.u.	1 vial
1.3 mL	STANDARD C, 45 AU/mL, r.t.u.	1 vial
1.3 mL	STANDARD D, 90 AU/mL, r.t.u.	1 vial
1.3 mL	STANDARD E, 300 AU/mL, r.t.u.	1 vial
13 mL	Anti-human IgG antibodies labelled with horseradish peroxidase r.t.u. (Px-conjugate) CONJ	1 vial
125 mL	Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
100 mL	Dilution buffer, r.t.u. DIL	1 vial
13 mL	Chromogenic substrate TMB, r.t.u. TMB	1 vial
13 mL	Stop solution, r.t.u. STOP	1 vial

Sealable pouch for unused strips
Instruction manual
Quality control certificate

[1)] AU/ml, artificial units/ml, [2)] ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT:

a. Distilled or deionised water for dilution of the Wash buffer concentrate.
b. Appropriate equipment for pipetting, liquid dispensing and washing.
c. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES:

a. **Allow all kit components to reach room temperature.**
b. **Vortex samples and the Standards in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum samples 1:100 in Dilution buffer** and mix (e.g. 5 µL of serum/plasma sample + 500 µL of Dilution buffer). Do not dilute the 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:

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. Choose the proper method for data interpretation (qualitative, semiquantitative or quantitative, see below and/or paragraph 8) and pipette Standards and samples according to the pipetting scheme (page 3). Start with filling the first well with 100 µl of Dilution buffer (DIL) to estimate the reaction background. In case of choosing the qualitative or semiquantitative method, fill two wells with 100 µl/well of Standard D, next one well with 100 µL of Standard A (ST A). In case of quantitative method, pipette 100 µl all Standards (A, B, C, D, E). Fill the remaining wells with 100 µl of diluted serum samples (S1, S2, S3, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply the samples and Standards in doublets.
c. Incubate **30 minutes (±2 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. Mix the bottle with Anti-human IgG Px-conjugate and add 100 µL of the Anti-human IgG 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 the TMB substrate into each well; pipette in a regular rhythm or use an appropriate dispensing instrument.
i. Incubate for **10 minutes (+/-5 seconds)** 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 with TMB substrate.

j. 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.

k. Measure the absorbance at **450 nm with a microplate reader within 20 minutes**. It is recommended to use reference reading at 620-690 nm.

Figure 1: Pippetting scheme

Qualitative and semiquantitative method

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

Quantitative method

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

8. PROCESSING OF RESULTS:

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

8.1. Processing of results for Qualitative interpretation

1. Compute the absorbance mean of Standard D.
2. Compute the cut-off value by multiplying the mean of Standard D with a Correction factor. **The Correction factor value for particular Lot is written in enclosed Quality control certificate.**
3. 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.

8.2. Processing of results for 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 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	+/-
1.11 - 2.00	+
2.01 - 4.00	++
4.01 - 7.00	+++
> 7.00	++++

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.

Example of calculation:

Standard D absorbances	= 1.023; 1.101
Standard D mean absorbance	= 1.062
Correction factor	= 0.18
Cut-off value	= 1.062 x 0.18 = 0.191
Sample absorbance	= 0.800
Sample Positivity Index	= 0.800 / 0.191 = 4.19

8.3. Processing of results for Quantitative interpretation

Compute the sample antibody titers in artificial units (AU/ml) as follows:

1. Construct the calibration curve by plotting the units of Standards (x-axis) to absorbance of Standards (y-axis). We recommended using logarithmic x-axis. The concentration of each Standard (A-E) is mentioned in paragraph 4 – Kit components.
2. Find the place where the absorbance of tested samples intersect calibration curve and find the corresponding values (AU/mL) on the axis x. 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.

Result interpretation:

Concentration (AU/ml)	Interpretation
<15.0	Negative
15.0 – 19.0	+/-
19.1 – 35.0	+
35.1 – 75.0	++
75.1 – 300.0	+++
>300.0	++++

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. CLINICAL INTERPRETATION OF RESULTS:

VCA IgG	VCA IgM	VCA IgA	EA (D) IgG	EBNA-1 IgG	EBNA-1 IgM	Stages of EBV infection
-	-	-	-	-	-	EBV negative
- +	+ +	+ +	- +	- -	+ +	EBV primoinfection (early phase)
+ +	+ -	+ -	+ -	+/- -	+ -	EBV primoinfection (transient phase)
+ +	- -	+ -	- -	- +	- -	EBV primoinfection (convalescent phase)
+	-	- +	-	+	-	Seropositivity without symptoms of active EBV infection
+ +	- +	- +	+ +	+ +	- -	EBV reactivation

Anomalous result of finding positive levels in both IgG and IgM anti-EBNA-1 antibody classes can be a sign of an autoimmune disease.

VIDIA produces kits for detection of antibodies to the other EBV antigens:
ELISA-VIDITEST anti-EBNA-1 EBV IgM,
ELISA-VIDITEST anti-VCA EBV IgG, IgM and IgA,
ELISA-VIDITEST anti-EA (D) EBV IgG and IgM,
IF-VIDITEST anti-VCA EBV IgG and IgM,
IF-VIDITEST anti-EA EBV (D+R or D) IgG.

10. CHARACTERISTICS OF THE TEST:

10.1 Validity of the test

The test is valid if:

a. The background of the reaction (the absorbance of the Dilution buffer) is less than 0.100.
b. The mean absorbance of Standard B is > 0.100.
c. The Standards absorbance values keep the order that: A < B < C < D< E

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	A	SD	CV%
16	0.614	0.021	3.4 %
16	1.566	0.060	3.9 %

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	A	SD	min – max	CV%
16	0.618	0.059	0.513 – 0.744	9.5%
17	1.071	0.072	0.965 – 1.214	6.9%
16	2.454	0.096	2.306 – 2.630	3.9%
16	1.404	0.062	1.303 – 1.501	4.4%

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

Diagnostic sensitivity of the test is 100 %. Evaluation was performed on blood samples tested with another commercially available diagnostic test. Samples were expected to be positive for anti-EBNA-1 EBV IgG antibodies (blood donors, patients with history of infectious mononucleosis).

Diagnostic specificity of the test is 96.4 %. Specificity was determined on blood samples from healthy EBV negative blood donors.

10.4. Limit of quantification

The limit of quantification is 3 AU/mL. The limit of quantification was defined as the lowest measurable concentration, which can be distinguished with 95% confidence from zero.

10.5. 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.

11. 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.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer.

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.

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

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

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 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 in the working concentration. 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.

References:

Dillner J. *et al.*: Antibodies against a synthetic peptide identify the Epstein-Barr virus nuclear antigen, *Proc. Natl. Acad..Sci.U.S.A.* **81**: 4652–4656, 1984.

Geltosky J.E. *et al.*: Use of synthetic peptide-based ELISA for the diagnostic of infectious mononucleosis, *J. Clin. Lab. Analysis* **1**: 153–162, 1986.

Rumpold H. *et al.*: The glycine-alanine repeating region is the major epitope of the Epstein-Barr nuclear antigen-1, *J. Immunol.* **138**: 593–599, 1987.

Smith R.S. *et al.*: A synthetic peptide for detections antibodies to Epstein-Barr virus nuclear antigen in sera from patients with infectious mononucleosis, *J. Infect. Dis.* **154**: 885–889, 1986.

Roubalová K., Horáček J., Němeček V. et al: Doporučené metody ve virologické diagnostice; Acta Hygien Epidemiol. Microbiol 2000;1, 11-12

Sumaya CV: Serologic and virologic epidemiology of Epstein Barr virus: Relevance to chronic fatigue syndrom. Rev.Infect.Dis.1991;13 (Suppl): 19-25

14. FLOW CHART:

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well Dilution buffer, Standards and samples**
	↓
	Incubate 30 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/ 620-690 nm within 20 minutes**

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