IVD

ELISA-VIDITEST anti-VCA EBV IgM

ODZ - 005

Instruction manual

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1. TITLE:
ELISA-VIDITEST anti-VCA EBV IgM – ELISA kit for the detection of IgM antibodies to Epstein-Barr virus (EBV) capsid antigen (VCA).

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-VCA EBV IgM assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with specific antigen that bears immunodominant epitopes of VCA complex. The anti-VCA EBV antibodies, if present in the tested sera, bind to the immobilized antigens and the antibodies being in complexes with antigen are later on recognised by animal anti-human IgM 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. Dilution buffer "plus" contains RF sorbent to saturate IgG and rheumatoid factor (RF). It is possible to prepare the "plus" buffer by mixing with Dilution buffer r.t.u. and RF sorbent according to the Instruction manual.

4. KIT COMPONENTS:

ELISA break-away strips coated with specific antigen STRIPS Ag	1 microplate
1.3 mL Calibrator CAL r.t.u.[1)]	1 vial
1.3 mL Positive control serum CONTROL + r.t.u.	1 vial
1.3 mL Negative control serum r.t.u. CONTROL -	1 vial
15 mL Anti-human IgM antibodies labelled with horseraddish peroxidase (Px-conjugate) r.t.u. CONJ	1 vial
125 mL Wash buffer 10x concentrated WASH 10x	1 vial
125 mL Dilution buffer r.t.u. DIL	1 vial
1 mL RF sorbent 51x concentrated RF SORB 51x	1 vial
15 mL Chromogenic 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
[1)] ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT:

Distilled or deionised water for dilution 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 the controls in order to ensure homogeneity and mix all solutions well prior use.
c. **Prepare Dilution buffer "plus"** (DIL PLUS) by adding 1 volume of RF sorbent to 50 volumes Dilution buffer r.t.u. – dilution 1:50 (e.g. 1 mL RF sorbent + 50 mL Dilution buffer r.t.u.). Dilute only the necessary amount for testing. **Dilute serum samples 1:100 DIL PLUS** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer "plus"). DIL PLUS contains RF sorbent to saturate IgG and rheumatoid factor (RF). Do not dilute the Control sera and Calibrator 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. Start with filling the first well with 100 µl of Dilution buffer plus (DIL PLUS) to estimate the reaction background. Fill next two wells with 100 µl/well of Calibrator (CAL) and then fill next wells with Negative control serum (CONTROL -). It is also suitable to apply Positive control serum (CONTROL +) for the test control. Fill the remaining wells with 100 µl of diluted serum samples (S1, S2, S3,...). It is sufficient to apply samples in singlets, however, if you wish to minimise laboratory error apply the samples and controls 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 4x 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. (CONJ) into each well. **Incubate 60 minutes (±5 min) at room temperature**.
f. Aspirate and wash 4x with 250 µl/well of Wash buffer.
g. 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. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
h. 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.
i. Measure 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

	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 of results with subtraction of the background absorbance (absorbance of the DB+ well) from the absorbances of all other wells.

8.1 Processing of results for the Qualitative interpretation

1. Compute the absorbance mean of the three wells with Calibrator (CAL).
2. Compute the cut-off value by multiplying the mean of Calibrator (Cal) with a Correction factor. **The Correction factor value for particular Lot is written in enclosed Quality control certificate.**
3. Serum samples with absorbances lower than the 90% cut-off value are considered negative and samples with absorbances higher than the 110% cut-off value are considered positive. The samples in 90-110% range are equivocal (see Note).

8.2 Processing of results for the 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 the serum reactivity according to Table 1 (Semiquantitative interpretation of results)

Table 1: Semiquantitative interpretation of result:

Positivity Index	Interpretation
< 0.90	negative
0.90 – 1.10	+/-
1.11 – 4.00	+
4.01 – 6.50	++
6.51 – 12.00	+++
> 12.00	++++

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:

Calibrator absorbances = 0.994; 1.010; 1.094
Mean Calibrator absorbance = 1.033
Correction factor = 0.17
Cut-off value = 1.033*0.17 = 0.176
Sample absorbance = 0.950
Sample Positivity Index = 0.950 / 0.176 = 5.40

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

Note: RF sorbent in Dilution buffer for the samples eliminates interference of rheumatoid factor in most samples.

Other kits for detection of antibodies against the other EBV antigens are available from VIDIA:
ELISA-VIDITEST anti-EBNA-1 EBV IgG and IgM;
ELISA-VIDITEST anti-VCA EBV IgG and IgA;
ELISA-VIDITEST anti-EA (D) EBV IgG and IgM;
For the confirmation can be used:
IF-VIDITEST anti-VCA EBV IgG and IgM;
IF-VIDITEST anti-EA EBV (D+R or D) IgG.

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST:

10.1 Validity of the test

The test is valid if:
The mean absorbance of Calibrator (CAL) should be in range that is written in enclosed Quality control certificate.
The absorbance of Negative control serum (CONTROL -) should be less than 0.200.
The absorbances of Control sera keep the order that: CONTROL - < CAL < (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%
16	1.335	0.050	3.8%
16	0.614	0.023	3.7%

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

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 94.7%. Samples tested were expected to be positive for anti-VCA IgM antibodies (patients in acute phase of infectious mononucleosis). The results were confirmed by another commercially available diagnostic tests.
Diagnostic specificity of the test is 96.1%. Specificity was determined on blood samples from healthy VCA EBV IgM negative blood donors.

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.

11. SAFETY PRECAUTIONS:

All ingredients of the kit are intended for laboratory use only.

Controls and Calibrator 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, Dilution buffer and RF sorbent are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.

Calibrator, Control sera, 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. 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.

14. FLOW CHART:

Step 1.	**Prepare reagents and samples**
	↓
Step 2.	**Dispense 100 µL / well Dilution buffer, Controls and samples**
	↓
	Incubate 30 minutes at room temperature
	↓
	Wash 4x (250 µL / well), aspirate
	↓
Step 3.	**Dispense 100 µL / well of Px-conjugate**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 4x (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 10 min**

References:

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