ELISA-VIDITEST anti-EBNA-1 EBV IgM

ODZ-002

Instruction manual

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1. TITLE

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

2. INTENDED USE

ELISA-VIDITEST anti-EBNA1 EBV IgM is intended for in vitro diagnosis of EBV- associated diseases. The test is used for the determinantion of immunological response to EBV infection and may aid in the diagnosis of acute or convalescent phase of the infectious mononucleosis (IM) and in lymphoproliferative disorders in haematological or immunocompromised patients.
In addition to this assay, serological examination of EBV includes detection of IgM and IgG anti-VCA EBV (ELISA-VIDITEST anti-VCA EBV IgM, ELISA-VIDITEST anti-VCA EBV IgG) and determination of IgG anti-EBNA-1 antibody (ELISA-VIDITEST anti-EBNA-1 IgG). Determinations of IgG (IgM) anti-EA, IgA anti-VCA, and of IgG anti-VCA avidity (ELISA-VIDITEST anti-EA (D) EBV IgG, IgM, ELISA-VIDITEST anti-VCA EBV IgA, ELISA-VIDITEST anti-VCA EBV IgG avidity) can be used as supplementary tests.

3. TEST PRINCIPLE

ELISA-VIDITEST anti-EBNA-1 EBV IgM assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with 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 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.

4. KIT COMPONENTS

Component	Quantity
ELISA break-away strips coated with specific antigens STRIPS Ag	1 microplate
1.3. mL Calibrator, r.t.u. [1)] CAL	1 vial
1.3 mL Positive control serum, r.t.u. CONTROL +	1 vial
1.3 mL Negative control serum, r.t.u. CONTROL -	1 vial
15 mL Anti-human IgM antibodies labeled with horseradish 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
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	

[1)] ready to use

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

a. Distilled or deionised water for diluting of the Wash buffer concentrate.
b. Appropriate equipment for pipetting, liquid dispensing and washing.
c. 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 Control sera in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum samples 1:100 in Dilution buffer** (DIL) (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). 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** (WASH 10x) 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. Pipette Controls 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. Fill the next two wells with 100 µl of Calibrator (CAL), the next well fill with 100 µl of Negative control serum (CONTROL -). It is appropriate to apply also Positive control serum (CONTROL +). 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 Calibrator in triplet, and Negative control and samples 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. 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 four times with 250 µl/well of Wash buffer (see point d of this paragraph).
g. Dispense 100 µl of TMB substrate (TMB) into each well. **Incubate 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.
h. 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 few times to ensure complete mixing of the reagents.
i. Measure the absorbance at **450 nm with a microplate reader within 20 minutes**. It is recommended to use a reference reading at 630 nm.

Figure 1: Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL											
B	CAL											
C	CAL											
D	CONTROL -											
E	S1											
F	S2											
G	S3											
H	S…											

8. PROCESSING OF RESULTS

Begin the processing with subtraction of the absorbance of the DIL well (background absorbance) from the absorbances in all other wells.

8.1. Processing of results for the Qualitative interpretation

1. Compute the mean absorbance of the two wells with Calibrator (CAL). (If the CAL was applied in three parallels and one absorbance is different from the mean in more than 20%, then exclude the deviating well from the calculation and compute a new absorbance mean
with using the other two wells)
2. Compute the cut-off value by multiplying the mean absorbance of CAL by correction factor. **The correction factor value determined for the particular Lot of the kit is stated in the 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. The samples with absorbance in the range of 90-110% of cut-off value are equivocal (see note in par. 8.2)

8.2 Processing of using Positivity index (semiquantitative interpretation)

Determine Positivity Index for each serum sample and cerebrospinal fluid samples 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

Table 1: Semiquantitative interpretation of the results:

Positivity index	Interpretation
< 0.90	Negative
0.90 – 1.10	+/-
1.11 – 2.50	+
2.51 – 5.00	++
5.01 – 8.00	+++
> 8.00	++++

Example of calculation:
CAL absorbances = 1.014; 1.099; 1.123
Mean absorbance of CAL = 1.079
Correction factor CAL = 0.16
Cut-off value = 1.079 x 0.16 = 0.173
Sample absorbance = 0.550
Sample Positivity Index = 0.550 / 0.173 = 3.18

Note : An equivocal 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. INTERPRETATION OF THE 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: IgM autoantibodies to collagen, keratin and actin could cross-react with EBNA-1. It can simulate positive reaction of IgM and IgG antibodies to EBNA-1 together. Presence **of revmatoid factor could interfere with IgM detection. We recommend using the RF sorbent for sorption of IgG antibodies and RF).** This solution is not included in the kit. You can order it individually.

The result anti-EBNA-1 EBV IgG positive and anti-EBNA-1IgM positive is anomalous and can indicate an autoimmune disease.

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

10.1 Validity of the test

The test is valid if:

a. The background absorbance (the absorbance of the Dilution buffer (DIL)) is less than 0.100.
b. Absorbance of Calibrator (CAL) mean should be in range that is stated in enclosed Quality control certificate.
c. Absorbance of control sera should be 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.

Example of absorbance (A) range of positive serum samples was (n=12):
0.496 – 2.009

10.2.1 Intraassay variability

The coefficient of intraassay variability is max. 5%. It is measured for each particular Lot as absorbance of minimum 12 parallel wells for the particular microtitrate plate.

Example:

(n = number of parallels at the same microtitration plate, CV- variation coefficient)

n	A	±σ	CV
16	0.876	0.038	4.3 %
16	1.759	0.080	4.6 %

10.2.2 Interassay variability

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the absorbances of the same serum sample in several consecutive tests.

(n= number of an independent examinations of the same serum sample)

n	A	±σ	min – max	CV
16	0.065	0.008	0.050 – 0.076	12.1 %
16	1.983	0.112	1.753 – 2.139	5.7 %
16	1.098	0.093	0.954 – 1.230	8.5 %

10.2.3 Spiking recovery test

The percentage of recovery was between 80 – 120%.

10.3 Diagnostic sensitivity and specificity

Diagnostic sensitivity of the test is 95.5%. Evaluation was performed on serum samples which were expected to be positive for anti-EBNA-1 EBV IgM antibodies (patients in acute phase infectious mononucleosis).

Diagnostic specificity of the test is 95.5%. Specificity was determined on serum samples from healthy EBV negative blood donors expected to be IgM anti-EBNA1 negative. The results obtained on VIDITEST were compared with another commercially available IVD tests.

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 100 mg/mL of triglycerides.

11. SAFETY PRECAUTIONS

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

Controls 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 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.

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

TMB substrate, Wash solution, Stop solution and Dilution buffer are compatible and interchangeable between different ELISA-VIDITEST kits except those with explicit statement 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 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 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.

Kits are shipped in cooling bags, the transport time up to 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 on the ELISA kit label and on all reagent labels.

References:

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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.

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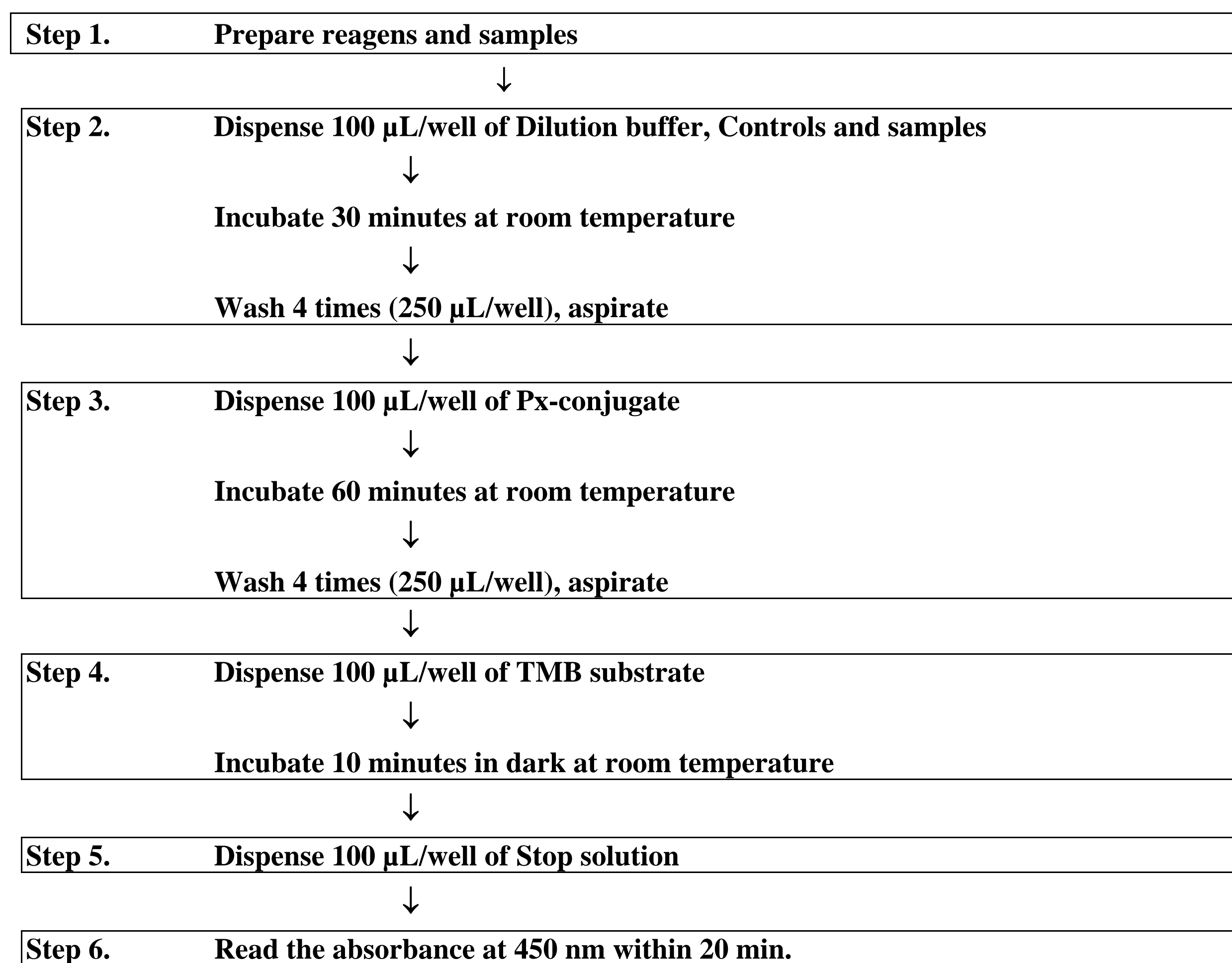
Sumaya CV: Serologic and virologic epidemiology of Epstein Barr virus: Relevance to chronic fatigue syndrom. Rev.Infect.Dis.1991;13 (Suppl): 19-25.

14. FLOW CHART:



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