IVD

ELISA-VIDITEST anti-HHV-6 IgM

ODZ-345

Instruction manual

PRODUCER: VIDIA spol.s r.o., Nad Safinou II 365, 252 50 Vestec, Czech Republic,
Tel.: +420 261 090 565, Web: www.vidia.cz, E-mail: **info@vidia.cz**

1. TITLE

ELISA-VIDITEST anti-HHV-6 IgM – ELISA kit for detection of IgM antibodies to HHV-6 in human serum.

2. INTENTED USE

The kit is intended for serological diagnosis of diseases associated with HHV–6 infection, such as exanthema subitum, acute respiratory illnesses, diarrhoea with fever and febrile seizures in infants, heterophile antibody-negative infectious mononucleosis in children, also interstitial pneumonia, encephalitis, meningitis, hepatitis and aplastic anemia in immunodeficient patients. The presence of IgM anti-HHV-6 antibody indicates ongoing or recent active infection. The test does not differentiate between HHV-6 subtype A and B. The test is intended for HHV-6-specific IgM determination in human sera.
The test should be supplemented with the determination of HHV-6-specific IgG antibodies (ELISA-VIDITEST anti-HHV-6 IgG).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-HHV-6 IgM assay is a solid-phase immunoanalytical test. The strips are coated with native HHV-6 antigen. The anti-HHV-6 antibodies, if present in the sera tested, 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 attached labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react - a mild change in colour, if present, may be attributed to the reaction background.

4. KIT COMPONENTS

ELISA strips coated with native antigen STRIPS Ag	1 microplate
1.3 mL Negative control serum r.t.u. [1)] NC	1 vial
1.3 mL Calibrator, r.t.u. CAL	1 vial
1.3 mL Positive control serum r.t.u. PC	1 vial
13 mL Anti-human IgM antibodies labelled with horseraddish peroxidase (Px-conjugate) r.t.u. . CONJ	1 vial
55 mL Wash buffer 10x concentrated WASH 10x	1 vial
60 mL Dilution buffer anti-HHV6 r.t.u. DIL	1 vial
13 mL Chromogenic substrate (TMB substrate) r.t.u. TMB-BF	1 vial
13 mL Stop solution r.t.u. STOP	1 vial
2 mL RF-sorbent 25 x concentrated RF SORB 25x	1 vial
Instruction manual	
Certificate of quality	

[1)] ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Dilution Buffer DIL is intended only for ELISA-VIDITEST anti-HHV-6 IgG, IgM kits. Chromogenic substrate TMB-BF is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-BF and not with other Chromogenic substrates TMB, TMB-O.

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT

1. Distilled or deionised water for dilution of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.
3. Spectrophotometer/colorimeter (microplate reader – wavelenght 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES

a. Allow all kit components to reach room temperature.
b. Mix Dilution buffer r.t.u., Px-conjugate and Chromogenic substrate r.t.u well.
c. **Mix samples, Positive and the Negative control in order to ensure homogeneity** and mix all solution well prior use.

Prepare Dilution buffer PLUS (DIL PLUS): dilute RF sorbent RF SORB 25x 25x by Dilution buffer DIL (e.g. 1 mL RF sorbent + 24 mL Dilution buffer. (Prepare only an amount necessary for the run, do not store).

d. **Dilute serum samples 101x in Dilution buffer PLUS** and mix (5 µL of serum sample + 500 µL of Dilution buffer Plus) and leave 10 min. at a room temperature. Diluted sera may form an opalescent solution. A precipitate does not interfere with the test performance.
e. Prepare Wash buffer by diluting the Wash buffer concentrate WASH 10x 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 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 month at room temperature.
f. **Do not dilute** Negative and Positive control, Px-conjugate, TMB-BF substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE

Manufacturer will not be held responsible for results if manual is not followed exactly.

a. Allow the vacuum-closed aluminium bag with strips to reach a room temperature. Withdraw the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
b. Pipette 100 µL of Dilution buffer, Controls, and serum samples to the wells according to the pipetting scheme in Figure 1: fill the first well with Dilution buffer Plus (DIL PLUS), the next two wells with Calibrator CAL, next one well with Positive control serum PC and the following one well with Negative control serum NC. Fill the remaining wells with diluted serum samples (S1, S2, S3...). It is satisfactory to apply samples and controls as singlets, however, if you want to minimize laboratory error then apply the CAL in triplet, controls and samples in doublets.

   Incubate **60 minutes (±5 min)** at room temperature.
c. Aspirate the liquid from the wells into a collecting bottle containing 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 some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
d. Add 100 µL of Px-conjugate r.t.u. CONJ into each well. Incubate **60 minutes (±5 min)** at room temperature.
e. Aspirate and wash four times with 250 µL/well of Wash buffer.
f. Dispense 100 µL of TMB-BF substrate into each well. Incubate for **20 minutes (+/-30 seconds)** at room temperature.
g. **The time measurement must be started at the beginning of TMB-BF dispensing.**

Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB-BF substrate.

h. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB-BF 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.

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

Fig 1: Pippetting schema

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL PLUS**	**S4**										
b	**CAL**	**S…**										
c	**CAL**											
d	**PC**											
e	**NC**											
f	**S1**											
g	**S2**											
h	**S3**											

8. PROCESSING OF THE RESULTS

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

If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

8.1 Qualitative evaluation

1. Compute the mean absorbance of the two parallels of CAL. (If the CAL was applied in three parallels, and one the absorbance is different from the mean more than 20%, then exclude the deviating well from the calculation and compute a new absorbance mean using the remaining two wells).
2. Compute the cut-off value by multiplying the mean absorbance of CAL with a Correction factor. **The Correction factor of CAL is indicated on Quality Control Certificate.**
3. Assign the samples with absorbances less than the 90 % of the cut-off value as negative and the samples with absorbances higher than 110% of the cut-off value as positive.

8.2 Semiquantitative evaluation

Determination of sample Positivity Index:

1. Compute the cut-off value (see previous paragraph 8.1)
2. Compute the Positivity Index for each sample according to the following formula:

$$\text{Sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}$$

3. Express serum reactivity in accordance with data in Table 1 (Evaluation of results)

Table 1 Evaluation of results

Index value	Interpretation
< 0.90	Negative
0.90 - 1.10	+/-
> 1.10	Positive*

* on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

Note! Indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is repetitively indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient withdrawn later.

Example of calculation:

CAL absorbances	= 1.407; 1.377
CAL mean	= 1.392
Correction factor	= 0.21
Cut-off value	= 1.392*0.21 = 0,292
Sample absorbance	= 1.200
Sample Positivity Index	= 1.200 / 0.292 = 4.11

9. RESULTS INTERPRETATION

Anti-HHV-6 IgM antibodies are present transiently in response to the active infection. During the virus reactivation, they may be absent or undetectable. Infants may be seronegative during acute phase of the primary infection and their IgM response to the virus could be delayed. The kit can detect cross-reactive antibodies to HHV-7 and CMV. Sera from the patients with polyclonal activation of immune system (i.e. infectious mononucleosis, toxoplasmosis, some autoimmune or lymphoproliferative disorders) may provide false-positive results in this test. For final diagnosis, the clinical symptoms of the patient should be taken in consideration. Results from immunosupressed patients should be interpreted with caution.

10. VALIDITY, SPECITITY AND SENSITIVITY OF THE TEST

10.1 Validity of the test

The test is valid if:

The background absorbance (the absorbance Dilution PLUS) is less than 0.150.

The mean absorbance values of standards (control sera), and the ratio between the absorbance values of PC / CAL are in the ranges stated in the Quality control certificate for the lot of this kit lot.

10.2 Precision of the test

The intra-assay and the inter-assay reproducibility were determined using samples with different absorbance values. In anti-HHV-6 IgM-positive samples intra-assay and inter-assay variability coeficients (CV) did not exceed 8% and 15% of mean absorbance values, respectively.

An example of **intra-assay** variability (n= number of paralel determinations in the same test)

n	A	±δ	CV
16	0.808	0.074	5.8 %
16	2.777	0.046	1.6 %

An example **of inter-assay** variability (n= number of determinations in several independent tests)

n	A	±δ	Min.- max.	CV
5	1.798	0.166	1.6-2.039	9.2 %
5	2.631	0.19	2.286-2.807	7.2 %

10.3 Sensitivity and specificity of the test

The diagnostic sensitivity of the test is 93%. It was determined from 45 positive samples. The specificity is 94%, which was determined from 245 negative samples. Evaluation was performed by the comparing the ELISA-VIDITEST anti-HHV-6 IgM kit with other commercial ELISA and IFA CE IVD tests.

HHV-6 status	Negative	Equivocal	Positive	Sum
Seronegative	228	3	14	245
Seropositive	3	1	41	45

10.4 Accuracy of the test

10.4.1 Spiking recovery test

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

10.4.2 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. However, examination of such samples is not recommended. An adition of RF-sorbent into the dilution buffer mostly eliminates interference of rheumatoid factor. However, samples with unusually high levels of rheumatoid factor may provide false-positive results.

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 0.5 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.

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.

Standards, Negative control, Dilution buffer, Chromogenic substrate 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-BF 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

The ELISA kit should be used within three months after opening.

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 -18 to -28°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. USED SYMBOLS

 number of tests

CE Conformité Européenne – product meets the requirements of European legislation

 in vitro diagnostics

±σ standard deviation

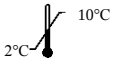
CV coefficient of variation

OD optical density

 manufacturer

 expiration

 Lot of kit

 storage at +2°C - +10°C

°C Celsius degree

% percentage

n number of tested samples

A valuea of tested sample

 read usage instructions

Cat. No. catalog number

15. FLOW CHART

Step 1	**Prepare reagents and samples**
	↓
Step 2	**Dispense 100 µL/well standards, control and samples**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3	**Dispense 100 µL/well of Px-conjugate r.t.u.**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well of TMB-BF substrate**
	↓
	Incubate 20 minutes 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**

References:

De Bolle L., Naesens L., De Clercq E., Update on Human Herpesvirus 6 Biology, Clinical Features and Therapy, Clinical Microbiology Reviews, 217-245, 2005

Fox J.D., et al. Production of IgM antibody to HHV6 in reactivation and primary infection. Epidemiol.Infect. 104: 289-296, 1990.

Date of the last revision of this manual: 02/2018

The development of this kit was supported by grant from the Ministry of Industry and Trade of the Czech Republic