ELISA-VIDITEST anti-HHV-6 IgG

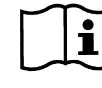
REF ODZ-235 96 tests 2°C - 10°C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative

Type of samples: Serum/Plasma isolated from venous or capillary blood

Processing possibility: Manual and/or automatic



Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II/365, 252 50 Vestec, Czech Republic, tel.: +420 261 090 565, www.vidia.cz, info@vidia.cz

1. TITLE

ELISA-VIDITEST anti-HHV-6 IgG

2. INTENTED USE

The kit is intended for professional use for the qualitative and semiquantitative detection of IgG antibodies to Herpes virus subtype 6 (HHV-6) in human serum or plasma isolated from venous or capillary blood for the 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 IgG anti-HHV-6 antibody reveals the immune status of the patient. The test does not differentiate between HHV-6 subtype A and B.

An additional test for serological testing of HHV-6 infection is the determination of IgM antibodies (ELISA-VIDITEST anti-HHV-6 IgM)

3. TEST PRINCIPLE

ELISA-VIDITEST anti-HHV-6 IgG is a solid-phase immunoanalytical test. The strips are coated with native HHV-6 antigen. If antibodies are present in the test samples, they will bind to the immobilized proteins. The bound antibodies then react in the next step with horseradish peroxidase-labeled anti-human IgG antibodies. The amount of bound labeled antibodies is determined by a color enzymatic reaction. Negative samples do not react, a slight change in the color of the wells is the background of the reaction.

4. KIT COMPONENTS

ELISA break-away strips in the handling frame coated with the specific antigen STRIPS Ag	1 x 12 pcs
1.3 mL Standard A = Negative control human serum, r.t.u. [1] ST A/NC	1 vial
2.0 mL Standard D = Calibrator (human serum), r.t.u. ST D/CAL	1 vial
1.3 mL Standard E = Positive control human serum, r.t.u. ST E/PC	1 vial
13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate) r.t.u. CONJ	1 vial
55 mL Wash buffer, 10x concentrated WASH 10x	1 vial
60 mL Dilution buffer, r.t.u. DIL	1 vial
13 mL Chromogenic substrate TMB, r.t.u. (TMB/H_2O_2) TMB	1 vial
13 mL Stop solution, r.t.u. (0.4 M sulfuric acid) STOP	1 vial

Instruction manual

Quality Control Certificate

[1] r.t.u., ready to use

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

Chromogenic substrate TMB is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB and not compatible with other Chromogenic substrates used in other ELISA-VIDITEST TMB-O, TMB-BF.

5. MATERIALS REQUIRED BUT NOT PROVIDED

Distilled/deionised water for dilution of the Wash buffer WASH 10x, pipetting equipment, equipment for liquid dispensing and strip washing, spectrophotometer/colorimeter, thermostat for incubation of the microtiter plate at 37 °C.

The test can be performed automatically using the ThunderBolt analyzer.

All instruments and devices used must have a valid function validation.

6. REAGENTS PREPARATION

a. **Allow all kit components to reach room temperature. Turn on the thermostat to 37 °C.**

b. **Thoroughly mix Dilution buffer DIL, Conjugate anti-IgG Px CONJ and Chromogenic substrate TMB.**

c. **Thoroughly mix tested samples and control sera just prior to testing. When using the ThunderBolt analyzer, the sample dilution and the whole test take place automatically (the automatically set dilution is 101x). In the case of a manual test, dilute the test samples 101x** with Dilution buffer **DIL (eg 5 µL sample + 500 µL Dilution buffer). Do not dilute** control sera and calibrator, they are in working concentration (r.t.u., ready to use).

d. Prepare a working concentration of Wash buffer WASH 10x by diluting it 10x in a suitable volume of distilled/deionized water (eg. 50 mL of WASH 10x + 450 mL H_2O). If there are salt crystals in the concentrated solution, warm it in a water bath of + 32 °C to + 37 °C and mix well before diluting. Unused wash solution in working concentration can be stored for 1 month at room temperature.

e. **Do not dilute** Conjugate anti-IgG Px CONJ, Chromogenic substrate TMB and Stop solution STOP, they are ready to use.

7. ASSAY PROCEDURE

The manufacturer is not responsible for the correct function of the kit if the assay procedure is not followed.

7.1 Assay procedure for manual performance

a. Allow strips STRIPS Ag, vacuum sealed with desiccant, to reach room temperature before opening the bag, to avoid dew condensation of the plate. Prepare the required number of strips for the reaction. Seal unused strips together with the desiccant in a zipper bag or seal under vacuum.

b. Fill the wells with 100 µL of Standards and diluted serum samples according to the pipetting scheme (Figure 1). Start with filling the first well with 100 µL of Dilution buffer DIL to estimate the reaction background (BLANK). Then fill the next two wells with Standard D ST D/CAL, serves as a calibrator. Next well with Positive control serum ST E/PC and next one well with Negative control serum ST A/NC. Fill the remaining wells with diluted samples (S1, S2, S3, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply ST D/CAL in triplet and the samples and control sera in doublets. We recommend to include positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.

   Incubate **30 minutes** (+/- 2 min) **at 37 °C**.

c. Aspirate the contents of the wells into a safety collection bottle containing a suitable disinfectant (see WARNINGS). Then wash the wells 4 times with 250 µL of wash solution. Avoid overflowing the solution out of the wells. Aspirate the contents of the wells and tap the plate on an adsorbent paper.

d. Mix thoroughly the vial of anti-IgG Px conjugate CONJ and pipette 100 µL of anti-IgG Px conjugate CONJ into the wells.

   Incubate **30 minutes** (+/- 2 min) **at 37 °C**.

e. Aspirate the fluid from the wells and wash them with 4 x 250 µL of wash solution. Aspirate and tap.

f. Pipette 100 µL of Chromogenic substrate TMB solution into the wells.

   Incubate for **15 minutes** (+/- 30 sec) **in the dark at room temperature**.

   Start measuring the incubation time after pipetting the first strip of the plate. Follow this rule to avoid breaking the time interval. Pipette quickly at regular rhythm, or use a suitable dispenser. Cover the strips with foil, an opaque lid, or keep them in a dark place for the duration of the reaction.

g. Stop the reaction by adding 100 µL of Stop solution STOP. Pipette at the same rate as the Chromogenic substrate TMB so that the enzymatic reaction proceeds in all wells at the same time. Check that there are no bubbles in the wells, if so, gently tap the plate frame to remove them.

h. Measure the intensity of the colour reaction on a spectrophotometer/colorimeter at 450 nm **within 20 minutes** after stopping the reaction. We recommend using a 620-690 nm reference filter.

Figure 1: Scheme of application of samples

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
a	**DIL**											
b	**ST D/CAL**											
c	**ST D/CAL**											
d	**ST E/PC**											
e	**ST A/NC**											
f	**S1**											
g	**S2**											
h	**S…**											

7.2 Assay procedure for performance on ThunderBolt analyzer

In the ThunderBolt analyzer, sample dilution and the entire test take place automatically. Use the appropriate method listed in the **Quality control Certificate** for specific kit lot.

Before using the specific kit lot for the first time, it is necessary to set all parameters stated in the Quality Control Certificate for given kit lot.

In the case of a parallel test of anti-HHV-6 IgM and HHV-6 IgG kits, the methods must be scheduled in the order of HHV-6 IgM and then HHV-6 IgG.

The incubation conditions programmed in the appropriate software may differ slightly from the specifications given in the instruction manual for the manually performed ELISA-VIDITEST test. These conditions have been validated by the manufacturer. Validation protocols are available on request.

It is possible to perform ELISA-VIDITEST tests using other automated analyzers with an open system, but this combination must be verified by the user.

8. TEST EVALUATION

The ThunderBolt analyzer performs the data evaluation automatically.

First, subtract the absorbance of the well with Dilution buffer DIL (BLANK = reaction background) from the calibrator, control sera, and tested samples.

If the values of Control sera or tested samples are negative after background subtraction, consider them as zero value.

8.1 Qualitative orientation evaluation

1. Calculate the mean OD value of the Standard D ST D/CAL from the two wells. If you are applying three Standard D ST D/CAL wells and some of these values differ by more than 20% from the mean, do not use it for calculation and calculate the mean of the remaining two values.
2. **Determine the cut-off value** by multiplying the mean OD value of the Standard ST D/CAL by the correction factor. **The value of the correction factor is stated in the Quality Control Certificate for the given kit lot**.
3. Samples with an OD value < 90 % cut-off are negative and samples with an OD value > 110 % cut-off are considered positive.

8.2 Semiquantitative evaluation

Determine Positivity **Index** for each sample:

1. First determine the cut-off value as in the previous evaluation method (See paragraph 8.1, point 2).
2. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.
3. Read the appropriate degree of reactivity of the sample (See RESULTS EVALUATION).

RESULTS EVALUATION

Positivity index	Evaluation
< 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

Example: Obtained OD Standard D ST D/CAL	= 1.119; 1.145
Mean OD Standard D ST D/CAL	= 1.132
OD sample	= 0.800
Correction factor Standard D ST D/CAL	= 0.16
Cut-off value	= 1.132 x 0.16 = 0.181
Positivity index value	= 0.800 / 0.181 = 4.42

Note: A rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new sample from the same individual 1-2 weeks later.

9. RESULT INTERPRETATION

Anti-HHV-6 IgG antibodies are anamnestic. They persist for the whole life after the primary infection. The kit can detect cross-reactive antibodies to HHV-7. Significant increase of IgG antibodies can be caused by reactivation of the infection, but could not be always proved due to the recurrent character of the reactivations. For final diagnosis, the results of other laboratory tests and the clinical symptoms of the patient should be taken in consideration.

10. TEST CHARACTERISTICS

The kit is intended for the qualitative and semiquantitative detection of anti-HHV-6 IgG antibodies in human serum and plasma. Suitable specimens are serum and plasma (heparinised) samples obtained by standard laboratory techniques.

10.1 Validity of the test

The absorbance value of the Dilution buffer DIL (BLANK = reaction background) is stated in the Quality Control Certificate of the lot.

The OD values of the standards / control sera and the ratio of the OD values of the standards ST E/PC / ST D/CAL should be within the ranges stated in the Quality Control Certificate of the lot.

The Calibrator and Controls are human sera, and as such they may show inhomogeneity, if their value in the test is significantly different from the values stated in the Certificate of analysis (see CoA - lot characteristics), consult the results with the manufacturer.

10.2 Precision of the test

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

10.2.1 Repeatability (intraassay)

The variation coefficient of intraassay is max. 8 %. It is measured for each particular lot at least on 12 parallels of the same microtiter plate.

Example: (n = number of parallel wells on the same plate)

n	A	±σ	CV rep.
14	2.268	0.094	4.1 %

10.2.2 Reproducibility (interassay)

The variation coefficient of reproducibility is a maximum of 15 %. It is measured for each lot by comparing the wells of the same sample in several consecutive tests.

Example: (n = number of tests of a certain sample)

n	A	±σ	min – max	CVrepro
10	0.674	0.090	0.567 – 0.779	13.4 %
9	0.874	0.061	0.729 – 0.927	7.3 %
7	1.116	0.069	1.048 – 1.221	6.2 %

10.2.3 Recovery test

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

10.3 Diagnostic sensitivity and specificity of the test

Diagnostic susceptibility testing was performed by testing 248 HHV-6 positive samples characterized for other commercial kits. The diagnostic sensitivity of the test is 99 %. The specificity of the assay was determined by testing 43 HHV-6 negative samples. The final specificity of the test from the given samples was 95 %.

	Result in ELISA-VIDITEST anti-HHV-6 IgG			
HHV-6 status	Negative	Equivocal	Positive	Sum
Seronegative	40	1	2	43
Seropositive	3	0	245	248

10.4 Analytical sensitivity of the test

The analytical sensitivity of the assay is defined as the mean of the sample without analyte plus three times of the standard deviation and represents the lowest detectable antibody titer. The analytical sensitivity value is determined for each kit lot and is stated in the **Quality Control Certificate** of that kit lot.

10.5 Analytical specificity of the test

The quality of the native Herpes virus subtype 6 (HHV-6) antigen used, which recognizes specific antibodies in patient samples, ensures the high specificity and sensitivity of this assay. However, there is some degree of cross-reactivity of antibodies against other infectious agents, see Chapter 9 (RESULT INTERPRETATION) and Chapter 10.6 (Cross-reactivity).

10.6 Cross-reactivity

In this test, lysate from infected cells is used as antigen. It contains a set of native viral proteins, some of which are structurally similar to proteins of other herpes viruses, as well as proteins from human cells. Tests of specificity did not show a significant influence of cross-reactivity of IgG antibodies against other herpes viruses on the diagnostic efficiency of ELISA-VIDITEST, however, it cannot be completely excluded, especially in samples with a high proportion of low-avid antibodies against group ß herpesviruses. Some autoantibodies can also cause false positivity, so test results must be interpreted with caution, especially in patients with autoimmune diseases, transplants, or burns. This test does not differentiate between HHV-6 type A, B and HHV-7 antibodies. In patients with different types of acute infections (CMV, EBV, measles, rubella) a parallel rise of IgG antibodies against HHV-6 was observed. However, the cause was not cross-reactivity, but polyclonal activation of the humoral immune response, or secondary reactivation of HHV-6.

10.7 Measuring range

The measuring range is determined by the measuring capability of the spectrophotometer / colorimeter used.

10.8 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. Nevertheless, such samples can only be tested with reservations.

11. WARNINGS

a. All kit components are for laboratory use only.

b. The manufacturer guarantees the usability of the kit as a whole.

c. Wash buffer WASH 10x, Chromogenic substrate TMB, Stop solution STOP, and Dilution buffer DIL are interchangeable between ELISA-VIDITEST kits, unless otherwise noted in the kit instructions.

d. Work aseptically to avoid microbial contamination of samples and reagents.

e. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.

f. The Chromogenic Substrate TMB shouldn't come into contact with oxidizing agents and metal surfaces. Because it is sensitive to light, close the bottle immediately after use. The Chromogenic substrate TMB must be clear in use. Do not use the solution if it is blue.

g. Follow the Instruction manual exactly. Non-reproducible results may arise in particular:
   * insufficient mixing of reagents and samples before use
   * inaccurate pipetting and non-compliance with the incubation times given in Chapter 7
   * poor washing technique and splashing of the edges of the wells with sample or conjugate
   * using the same tip when pipetting different solutions or swapping caps

h. Human control sera and standards used in the kit were tested for the absence of HBsAg, HCV and anti-HIV-1,2 antibodies. Treat test specimens, control sera, standards, and used strips as infectious material. Autoclave items that have been in contact with them for 1 hour at 121 °C or disinfect for at least 30 minutes with 3% chloramine solution.

i. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.

j. Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine, ...) at the concentration recommended by the manufacturer.

k. Handle Stop solution STOP carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.

l. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.

m. All reagents and packaging material must be disposed of in accordance with applicable legislation.

n. In case of suspicion of an adverse event in connection with the use of the kit, inform the manufacturer and the competent state authority without delay.

12. SAFETY PRECAUTIONS

Standard D ST D/CAL, Positive control human serum Standard E ST E/PC, negative control human serum Standard A ST A/PC, Dilution buffer DIL, and Chromogenic Substrate TMB are preserved with ProClin 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

Warning		
	H317	May cause an allergic skin reaction.
	H411	Toxic to aquatic life with long lasting effects.
	P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
	P302+P352	OF ON SKIN: Wash with plenty of water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before reuse.

The anti-IgG Px conjugate CONJ contains N-methyl-2-pyrrolidone. Therefore, the following warnings and precautions apply to this solution:

Danger		
	H360D	Warning: May damage the unborn child.
	P202	Do not use until you have read and understood all safety instructions.
	P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
	P308+P313	If exposed or concerned: Get medical advice/attention.
	P501	Dispose of contents/container in accordance with local regulations.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

It is recommended to use the kit within three months after opening.

a. Store the kit and the kit reagents at +2 °C to +10 °C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label on the kit package, the expiration date of the individual components is indicated on their package.

b. Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.

c. The kits are transported refrigerated in thermal bags, transport time up to 72 hours has no influence on expiration. If, upon receipt of the kit, you notice serious damage to the packaging of any component of the kit, inform the manufacturer immediately.

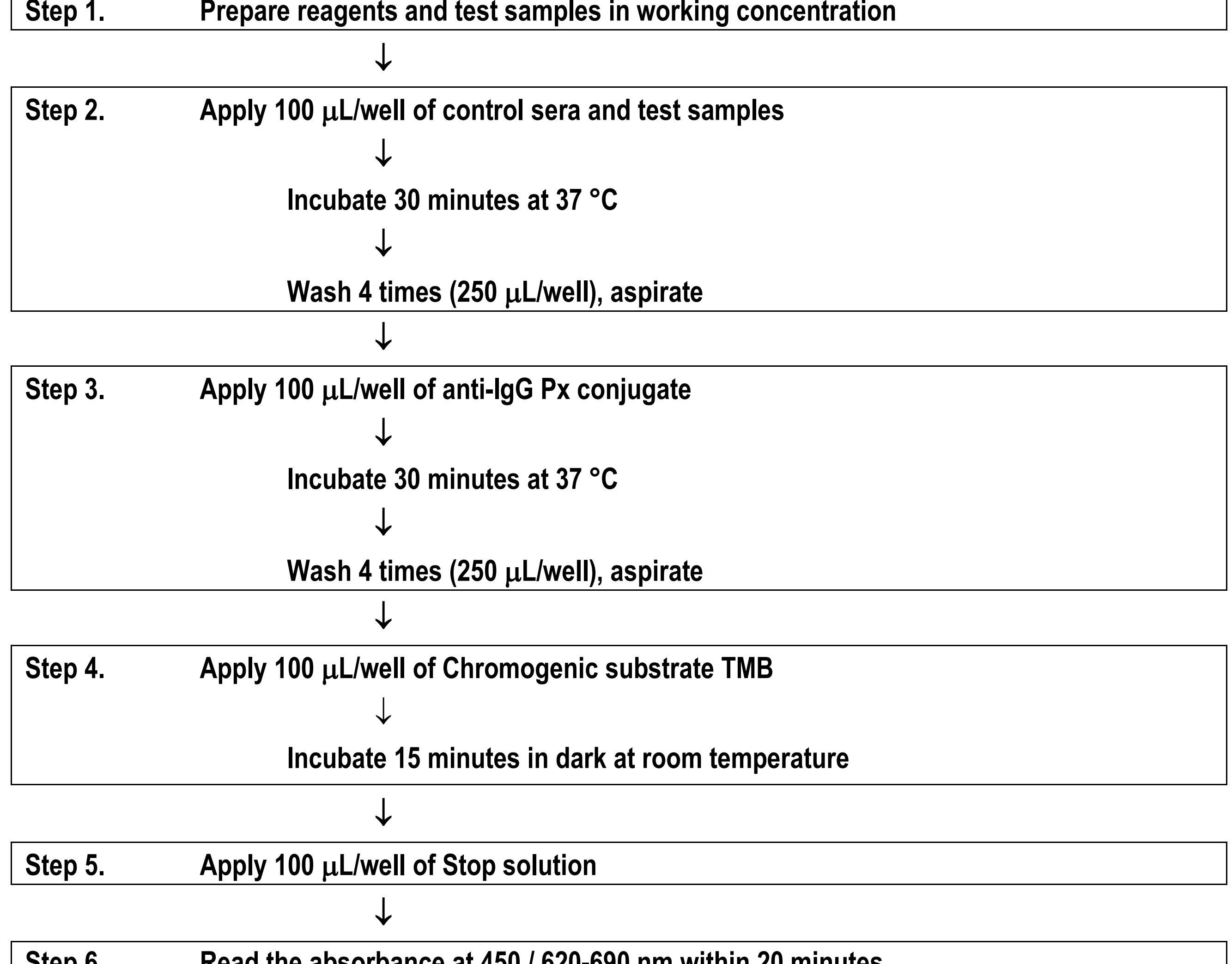
d. Store unused test samples undiluted, aliquoted and frozen at -18 °C to -28 °C. Frequent freezing and thawing is not recommended. If you store samples at + 2 °C to + 10 °C, then test them within one week.

e. Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

14. USED SYMBOLS

Symbol	Explanation
Σ	number of tests
CE	Conformité Européenne – product meets the requirements of European legislation
IVD	diagnostics *in vitro*
±σ	standard deviation
CV	coefficient of variation
OD	optical density
	manufacturer
	expiration
LOT	lot of kit
2°C 10°C	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
i	read the package leaflet
REF	catalog number

15. TEST SCHEME



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

Date of the revision of manual: 03. 03. 2025