ELISA-VIDITEST anti-HHV-6 IgM

REF ODZ-345 Σ 96 tests 2°C 10°C 2°C - 10°C

Type of determination: IgM antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum isolated from venous or capillary blood

Processing possibility: Manual and/or automatic

VIDIA

IVD

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-HHV-6 IgM

2. INTENTED USE

The kit is intended for professional use for the qualitative and semiquantitative detection of IgM antibodies against Herpes virus type 6 (HHV-6) antigen in human serum isolated from venous or capillary blood and it is used 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 samples.

The test should be supplemented with the determination of HHV-6-specific IgG antibodies (ELISA-VIDITEST anti-HHV-6 IgG (CSF)) in samples or determination of intrathecal synthesis of these antibodies.

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. If relevant antibodies are present in the test samples, they bind to the immobilized antigen. The bound antibodies then in a next step react with horseradish peroxidase-labeled anti-human IgM antibodies. The amount of labeled antibodies bound is determined by the color enzymatic reaction. Negative samples do not react, a mild change in the colour of the wells is the reaction background.

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 IgM animal antibodies labelled with horseradish peroxidase (anti-IgM 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-BF, r.t.u. (TMB/H_2O_2) TMB-BF	1 vial
13 mL Stop solution, r.t.u. (0.4 M sulfuric acid) STOP	1 vial
2 mL RF-sorbent (anti-human IgG) 25 x concentrated RF SORB 25x	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-BF is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-BF and not compatible with other Chromogenic substrates used in other ELISA-VIDITEST TMB-O, TMB.

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, microtiter plate for preincubation of samples with RF sorbent in the ThunderBolt analyzer.

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-IgM Px CONJ and Chromogenic substrate TMB-BF.**

c. **Thoroughly mix test 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 for the samples).** 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. **When using the ThunderBolt analyzer, calculate that you will need 300 µL of DIL PLUS for one test sample plus a 2 ml reserve. In the case of a manual test, dilute the tested samples with diluent plus 101x (eg 5 µL sample + 500 µL diluent) and incubate for 10 min. at room temperature. Diluent plus contains anti-human IgG solutions designed to saturate IgG and rheumatoid factor (RF).** Samples, diluted in diluent plus, form an opalescent solution. The precipitate does not need to be removed before application. **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-IgM Px CONJ, Chromogenic substrate TMB-BF 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 wells with 100 µL of individual Standards and diluted test samples as follows (see Fig. 1). Fill the first well with DIL PLUS dilution buffer alone to determine the background of the reaction (BLANK).

Fill two wells with Standard D ST D/CAL, the next well with Standard E ST E/PC, the next well with Negative control serum ST A/NC, and the remaining wells with diluted test samples (S1, S2,…). Just apply

each sample to one well. To rule out a possible laboratory error, apply ST D/CAL to the three wells, test samples, and control sera in two wells. We recommend that a positive reference serum sample (internal control) be included in each test to verify the continuity and variability of the test.
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-IgM Px conjugate CONJ and pipette 100 µL of anti-IgM 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-BF 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-BF 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: Sample application scheme:

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

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.

Note: A microtiter plate for preincubation of samples with RF sorbent is required to perform the test on the ThunderBolt analyzer.

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

In case of a parallel testing of following kits: anti-HHV-6 IgM, anti-HHV-6 IgG, it is necessary to set the methods in the ThunderBolt analyzer in following order anti-HHV-6 IgM and then anti-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 PLUS (BLANK = reaction background) from the calibrator, control sera, and test 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. If you are applying the Standard D ST D/CAL to the three 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 D 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.

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 of Standard D ST D/CAL	= 1.407; 1.377
Mean OD of Standard D ST D/CAL	= 1.392
OD sample	= 1.200
Correction factor of Standard D ST D/CAL	= 0.21
Cut-off value	= 1.392 x 0.21 = 0.292
Positivity index value	= 1.200 / 0.292 = 4.11

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.

9. RESULT INTERPRETATION

IgM antibodies against HHV-6 are formed transiently after an acute infection has been overcome. In recurrent infections, specific IgM antibodies are formed only in low titters and may not be demonstrable by ELISA test. Small children can be seronegative in the acute phase of infection and develop IgM antibodies only during the recovery period. HHV-6 causes lifelong latent infection in >90% of adults. In addition to primary infection, HHV-6 IgM positivity may be due to cross-reactivity with IgM antibodies against other herpesviruses (HHV-7) or, more commonly, secondary reactivation of latent HHV-6 due to another recent active infection (especially CMV or EBV primary infection) or other inflammatory processes (i.e. autoimmune diseases). A false positive in the test can also be the result of cross-reactivity with some autoantibodies and polyreactive antibodies associated with polyclonal activation of humoral immunity (ie in pregnancy, infectious mononucleosis, toxoplasmosis, some autoimmune and lymphoproliferative diseases). In case of discrepant HHV-6 IgM results in adults, examination of a second serum sample taken 2-3 weeks later can be recommended to monitor the dynamics of anti-HHV-6 IgM and IgG antibodies. In general, HHV-6 IgM serology in adult patients is an additional test and it is not reliable for organ transplant recipients, as well as other immunodeficient patients or chronically ill adults. For final diagnosis, the test results must always be interpreted in the context of clinical symptoms and the results of other laboratory tests.

10. TEST CHARACTERISTICS

The kit is intended for the qualitative and semiquantitative detection of anti-HHV-6 IgM antibodies against Herpes virus type 6 (HHV6) in human serum. Suitable specimens are serum samples obtained by standard laboratory techniques.

10.1 Validity of the test

The absorbance value of the Dilution buffer PLUS (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.
16	0.808	0.074	5.8 %
16	2.777	0.046	1.6 %

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
4	1.798	0.166	1.600-2.039	9.2 %
5	2.621	0.190	2.286-2.807	7.2 %

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

Evaluation of the diagnostic sensitivity was performed by testing of 45 HHV-6 IgM positive samples on other commercial test. From the results it was calculated the sensitivity 93%. The specificity of the test was determined by testing of 245 HHV-6 IgM negative samples. From the results it was calculated specificity 94%. Samples with a borderline result were not included in the calculation.

	Results in ELISA-VIDITEST anti-HHV-6 IgM			
HHV-6 status	Negative	Equivocal	Positive	Sum
Seronegative	228	3	14	245
Seropositive	3	1	41	45

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 type 6 (HHV-6) antigen used, which recognizes specific antibodies in patient samples, ensures high specificity. However, there is some degree of cross-reactivity of antibodies against other infectious agents, see Chapter 9 (RESULT INTERPRETATION).

10.6 Cross-reactivity

The quality of the antigen used ensures high specificity and sensitivity of this ELISA test. However, there is some degree of cross-reactivity of antibodies against other infectious agents, see Chapter 9 (RESULT INTERPRETATION).

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. However, examination of such samples is not recommended. An addition 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. 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-BF, 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-BF 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-BF 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

The dilution buffer DIL contain the preservative ProCline 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)).
Standard D ST D/CAL, positive control serum Standard E ST E/PC, and negative control serum Standard A ST A/NC may contain the preservative ProCline 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). The presence of the preservative is indicated on the label.

Therefore, the following hazard and safety warnings 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-IgM 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.

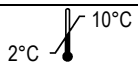
b. Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.

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
	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
	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
	read the package leaflet
REF	catalog number
	do not use if package is damaged and consult instructions for use
	do not re-use/ Single use only

15. TEST SCHEME

Step 1.	**Prepare reagents and test samples in working concentration**
	↓
Step 2.	**Apply 100 μL/well of control sera and test samples**
	↓
	Incubate 30 minutes at 37 °C
	↓
	Wash 4 times (250 μL/well), aspirate
	↓
Step 3.	**Apply 100 μL/well of anti-IgM Px conjugate**
	↓
	Incubate 30 minutes at 37 °C
	↓
	Wash 4 times (250 μL/well), aspirate
	↓
Step 4.	**Apply 100 μL/well of Chromogenic substrate TMB-BF**
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5.	**Apply 100 μL/well of Stop solution**
	↓
Step 6.	**Read the absorbance at 450 / 620-690 nm within 20 minutes**

Recommended literature:

Salahuddin S.Z., Ablashi D.V., Markham P.D., Joseph S.F., Sturzenegger S., Kaplan M. Halligan G., Biberfeld P., Wong-Stall F., Kramarsky B., Gallo R.C.; Isolation of a new virus, HBLV, in patiens with lymphoproliferative disorders. Science 234: 596-601, 1986

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.

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