IF-VIDITEST anti-HHV-6 IgM

REF ODZ-508 | Σ 80 tests | -18 °C to -28 °C

Type of determination: IgM antibodies

Type of evaluation: Qualitative

Type of samples: Serum/Plasma

IVD

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

IF-VIDITEST anti-HHV-6 IgM

2. INTENTED USE

The kit is intended for professional use for use for qualitative immunofluorescence detection of IgM antibodies against HHV-6 (Herpes virus type 6) in human serum or plasma. It is used for the diagnostics of diagnose diseases caused by or related to this virus, such as: in children exanthema subitum, acute respiratory disease, febrile diarrheal disease and febrile convulsions, in later age part of infectious mononucleoses without heterophilic antibodies. In patients with immunodeficiency, HHV-6 can cause interstitial pneumonia, encephalitis, hepatitis, and medullary depression.

3. TEST PRINCIPLE

T-lymphocyte cell lines line were infected with HHV-6. During infection, a cytopathic effect develops, which is manifested by the presence of large round light cells containing HHV-6 antigens and small degenerate cells, which may also contain these antigens. Coatings are prepared from the infected mixture and fixed. At first, human anti-HHV-6 antibodies, if present in the test serum or plasma bind on the HHV-6 antigen complex contained in the infected cells. The complex of antigen and human antibody is then visualised by binding an antibody against human IgM labelled with fluorescein isothiocyanate (FITC conjugate). This complex is then detected using a fluorescence microscope.

4. KIT COMPONENTS

Rasterized slides with fixed cell smears	10 x 1 slides
0.1 ml of lyophilised IgM positive human control serum	1 vial
0.1 ml of lyophilised negative human control serum	1 vial
0.25 ml of lyophilised animal anti-human IgM antibody – anti IgM FITC conjugate	1 vial
5.0 ml of mounting solution r.t.u. (90% glycerol)	1 vial
Instruction manual	
Quality Control Certificate	

5. MATERIALS REQUIRED BUT NOT PROVIDED

Phosphate buffered saline (PBS) pH 7.2 for dilution of control sera, tested samples, conjugates and for washing slides; distilled water for dissolving lyophilised kit components; wet chamber (suitable plastic box with lid, lined with damp absorbent material); tubes and pipetting equipment; haematological cuvettes for washing slides; coverslips 50 x 25 mm; pencil suitable for writing on glass; fluorescence microscope.
For the investigation of specific IgM antibodies, we recommend the use of RF sorbent (can be ordered under catalogue number OD-368).

All instruments and equipment used must have valid validation of function.

6. REAGENTS PREPARATION

Bring all components of the kit to laboratory temperature.

Prepare PBS containing RF sorbent in a 1:5 dilution, e.g. 20 µl RF sorbent + 80 µl PBS

Dilute the tested human samples 1:10 in PBS containing RF sorbent and incubate for 10 min at room temperature. We recommend removing the formed sediment by short centrifugation (e.g. 5 min at 3500 rpm or 1 min at 8000 rpm.) During the titration examination, PBS without RF sorbent can be used to prepare further dilutions.

Dissolve the control human sera in 0.1 ml of distilled water, mix and dilute in the same way as the test samples.

Note: When diluting control human sera and test samples in PBS with RF sorbent, prepare only the amount needed to perform one test. Sera and samples diluted in PBS with RF sorbent cannot be stored.

Dissolve FITC IgM conjugate in 0.5 ml distilled water and further dilute 1:10 (v/v) with PBS (i.e. add 4.50 ml PBS). You need about 50 µl of FITC conjugate per well, i.e. 400 µl per slide.

7. ASSAY PROCEDURE

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

a. Remove the slides from the packaging and place in a humid chamber. Pipette approx. 30 µl of the tested sample into the wells of the slides in the selected dilutions. Positive control serum in one well, negative control serum in the other well. It is recommended to include a positive reference serum sample (internal control) in each test to verify the continuity and variability of the test. Make sure that the droplet is always spread over the entire surface of the well and that the samples do not stick together.

   Seal the humid chamber and incubate for **120 ± 5 minutes at laboratory temperature.**

   Cell smears must remain moist throughout the incubation period.

b. Aspirate the samples into a safety collection bottle containing a suitable disinfectant (see WARNINGS), and place the slides into haematology cuvettes with PBS. After **5 ± 1 minutes,** replace the PBS with fresh – repeat the washing **3 times** in total**.** Carefully aspirate PBS from the washed slides so as not to damage the cell smears but **to keep the smears moist**.

c. Place the slides back into the moist chamber, apply anti-IgM FITC conjugate at working dilution and incubate for **60 ± 5 minutes at laboratory temperature.**

   Cell smears must remain moist throughout the incubation period.

d. Aspirate the FITC conjugate and wash the slides in PBS cuvettes **(3 times for 5 ± 1 min each)** and then briefly immerse in distilled water. Place the stained slides upright on an absorbent pad and allow to dry at laboratory temperature.

e. Apply two to three drops of mounting solution per slide and cover with a cover slip to prevent the formation of air bubbles.

f. Read off the stained slides immediately or store them in the dark at +2 °C to +10 °C.

   Immunofluorescence is clearly visible at least one week after staining.

8. TEST EVALUATION

View the stained slides in a fluorescence microscope under blue excitation light. If positive, the cells show a brilliant green fluorescence.

Evaluate the tested samples as positive if the glowing cells show a cytopathic effect. These cells are two to more times larger than other cells. A positive cell glows either whole or the nucleus, the cytoplasm can also

glow, in which aggregated clusters are sometimes visible. Small degenerate cells can sometimes glow, but don't evaluate them. Negative cells that do not contain HHV-6 antigens are olive green to bright red.

The well incubated with the negative control serum should be negative if the test is performed correctly, i.e. the cells should be red to olive green.

The well incubated with the positive control serum contains at least 3 % positive cells, but usually around 20 % (the percentage of positive cells is stated in the Quality Control Certificate.), which glow with brilliant green fluorescence.

In the screening test, the presence of antibodies in the sample is tested at a 1:10 dilution and it is determined whether the sample shows a positive reaction at this dilution. The titration test determines the highest dilution of the sample that still shows a positive reaction. The reciprocal value of this dilution is given as the antibody titre against the tested antigen. The screening test examines only the immune status of an individual.

Determination of IgM antibodies proves acute HHV-6 infection without the need to examine a second sample, or it is possible to perform a titration examination of detected positive cases.

9. CLINICAL SIGNIFICANCE

Herpes virus type 6 causes, for example, in children exanthema subitum, acute respiratory disease, febrile diarrheal disease and febrile convulsions, in later age part of infectious mononucleosis without heterophilic antibodies. In patients with immunodeficiency, HHV-6 can cause interstitial pneumonia, encephalitis, hepatitis, and medullary depression.

10. TEST CHARACTERISTICS

The kit is designed for professional use for qualitative immunofluorescence detection of IgM antibodies against HHV-6 in human serum or plasma.

Samples of serum or plasma (heparinized) taken in a standard laboratory manner are suitable for testing.

10.1 Diagnostic sensitivity and specificity of the test

Determination of diagnostic sensitivity was performed by testing 18 HHV-6 IgM positive samples characterized on another commercial kit. From these results, a sensitivity of 94.44 % was calculated. The specificity of the test was determined by testing 33 HHV-6 IgM negative samples. The specificity of 96.97 % was calculated from the results. Samples with equivocal result were not included in the calculation.

HHV-6 status	Negative	Positive	Total	
IgM - negative	32	1	33	Specifity 96.97 %
IgM - positive	1	17	18	Sensitivity 94.44 %

10.2 Measuring range

The starting point for dilution is 1:10, samples can be further serially diluted, i.e. 1:20, 40, 160, 320, etc. There is no upper limit to the range.

10.3 Reproducibility (Intraassay)

Reproducibility was tested on more than 20 wells within the batch. The intensity of immunofluorescence is indicated from "negative" (i.e. no fluorescence) to "++++" (strongest fluorescence). For the quantitative evaluations of the results, the maximum deviation was one level of intensity.

10.4 Repeatability (Interassay)

Repeatability was measured on more than 15 different batches. The intensity of immunofluorescence is indicated from "negative" (i.e.no fluorescence) to "++++" (strongest fluorescence). For the quantitative evaluations of the results, the maximum deviation was one level of intensity.

10.5 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.

10.6 Cross reactivity

IgM antibodies to HHV6 are formed transiently after acute infection. Young children may be seronegative in the acute phase of the infection and develop IgM antibodies only during the recovery period. The assay can also detect cross-reactive antibodies to HHV7 and CMV. Samples from patients with polycolonal activation of the immune system (infectious mononucleosis, toxoplasmosis, some autoimmune and lymphoproliferative diseases) may give false positive results in the test. To determine the diagnosis, the test results must always be interpreted in the context of clinical signs and the results of other laboratory tests.

11. WARNINGS

a. All kit components are for laboratory use only.
b. The manufacturer guarantees the usability of the kit as a whole. Combining components of different batches of kits is not recommended.
c. Work aseptically to avoid microbial contamination of samples and reagents.
d. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with fluorescence quenching agents.
e. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices.
f. Wear protective work equipment (clothing, rubber gloves, face shield) and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
g. Human sera (control) used in the kit were tested for the absence of HBsAg, anti-HIV-1,2 and HCV antibodies. Treat test samples, control sera and used slides as infectious material. Autoclave items that have come into contact with them for 1 hour at 121 °C or disinfect with a 3% chloramine solution for at least 30 minutes.
h. 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.
i. Handle FITC conjugate with Evans Blue with care to avoid staining the skin or mucous membranes or affecting the eye. If this happens, wash the affected area with sufficient amount of running water.
j. All reagents and packaging material must be disposed of in accordance with applicable legislation.
k. 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 conjugate and control human sera 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.
	H412	Harmful 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.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

a. Store the kit and its components in a dry and dark place at a temperature of -18 °C to -28 °C. 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.

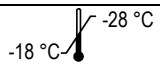
b. Store unused dissolved control human sera and conjugate at -18 °C to -28 °C for long-term storage. Avoid frequent freezing and thawing. If you store human sera and conjugates at + 2 °C to + 10 °C, then test them within one week

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*
	manufacturer
	expiration
LOT	lot of kit
	storage at -18°C to -28 °C
	read the package leaflet
REF	catalog number
°C	Celsius degree
%	percentage

Date of the revision of the manual: 30.06.2022