IF-VIDITEST
anti-VCA EBV

REF ODZ-060 Σ 240 tests -18°C to -28°C

Type of determination: IgG, IgM antibodies

Type of evaluation: Qualitative

Type of samples: Serum/Plasma

IVD

VIDIA

Instruction manual

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1. TITLE

IF-VIDITEST anti-VCA EBV

2. INTENTED USE

The kit is intended for professional use for use for qualitative immunofluorescence detection of IgG and IgM antibodies against the viral capsid antigen (VCA) complex of Epstein-Barr virus (EBV) in human serum or plasma.
It is used for the diagnostics of EBV-induced or EBV-related diseases such as infectious mononucleosis, chronic active EBV infection, Burkitt's lymphoma, lymphomas of Waldayer's ring, opportunistic lymphomas in immunosuppressed individuals and nasopharyngeal carcinoma. The kit will also find its application in the complex characterisation of immunodeficiency syndromes and chronic fatigue syndrome, where EBV is often activated.

3. TEST PRINCIPLE

The human lymphoma cell line is latently infected with EBV. In a certain percentage of the population of these cells, spontaneous activation of productive virus replication occurs. These cells contain viral capsid antigens (VCA). Appropriate procedures can be used to ensure that VCA is expressed in an increased number of cells in a given population. From these cells, smears are prepared and fixed in a suitable way. Human anti-VCA antibodies, if present in the serum or plasma tested, are bound to the VCA contained in these cells in an indirect fluorescence assay. The complex of antigen and human antibody is then visualised by binding an antibody against human IgG or IgM labelled with fluorescein isothiocyanate (FITC conjugate). This complex is then detected using a fluorescence microscope.

4. KIT COMPONENTS

Rasterised slides with fixed cell smears	3 x 10 slides
0.1 ml of lyophilised IgG positive human control serum	1 vial
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 IgG antibody – anti IgG FITC conjugate	1 vial
0.5 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; glass pencil; 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.

For IgG antibody testing

Dilute the tested human samples 1:10 for screening examination. For titration testing, which is much more important in EBV diagnostics, prepare a number of samples in dilutions e.g. 1:10, 1:20, 1:40, 1:80, 1:160...etc. Use PBS for all dilutions of the test samples.

Dissolve the control human sera in 0.1 ml of distilled water and dilute further with PBS at a ratio of 1:10 (i.e. add 0.9 ml of PBS).

Dissolve FITC IgG conjugate in 0.25 ml distilled water and further dilute 1:20 (v/v) with PBS (i.e. add 4.75 ml PBS). You need about 40 µl of FITC conjugate per well, i.e. 320 µl per slide.

For IgM antibody testing

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

Dilute the human samples to be tested 1:10 in PBS containing RF sorbent and incubate for 10 min at laboratory temperature. It is recommended

to remove the produced sediment by short centrifugation (e.g. 5 min. at 3,500 rpm or 1 min. at 8,000 rpm).

For titration testing, 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 manner as the test samples.

Note: When diluting control human sera and test samples into 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 40 µl of FITC conjugate per well, i.e. 320 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. If you do not use both slides at the same time, put the unused slide back into the bag immediately, close and store at -18 to -28°C.

b. Place the slides 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 (anti IgG or anti IgM) 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 follow up 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:

   60 ± 5 minutes at laboratory temperature for detection of IgG antibodies

   120 ± 5 minutes at laboratory temperature for detection of IgM antibodies

   Cell smears must remain moist throughout the incubation period.

c. 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**.

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

Cell smears must remain moist throughout the incubation period.

e. 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.

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

g. Read off the stained slides immediately or store them in the dark at +2 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. Whole cells, or parts of them such as the cytoplasm or cell nucleus, can glow. Cells that do not contain VCA or cells stained with negative serum are olive green to bright red. Weak membrane fluorescence due to the presence of the Fc receptor is not considered a positive result.

A well incubated with a negative control serum should be negative if the test is performed correctly.

A well with a positive control serum contains at least 3 % positive cells (the percentage of positive cells is indicated in the Quality Control Certificate.

In the screening test for the presence of antibodies in the sample, the sample is tested at a dilution of 1:10 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 antigen tested. For the differential diagnostics of EBV-associated diseases, titration testing of antibodies against VCA in the IgG class is important. For the detection of IgM antibodies to VCA, an orientational determination and possible titration testing of positive cases is often sufficient.

9. CLINICAL SIGNIFICANCE

Primo-infection in childhood is usually asymptomatic or with only mild symptoms common to other viral diseases. In adolescence and early adulthood, primo-infection presents as infectious mononucleosis (fever, lymphadenopathy, pharyngitis, splenomegaly, hepatomegaly). The clinical symptoms recede in the second week of the disease. However, fatigue and malaise may persist for several weeks.

Chronic active infection is characterised as a disease lasting at least 6 months from the time of primo-infection. Inflammatory involvement of various tissues and organs occurs.

10. TEST CHARACTERISTICS

The kit is designed for professional use for qualitative immunofluorescence detection of IgG and IgM antibodies against the viral capsid antigen (VCA) complex of Epstein-Barr virus (EBV) 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

The test was prepared based on the description of the standard method for the determination of IgG antibodies against VCA EBV, published in the literature. In the test of the test sample of the kit, a sensitivity of

89.00 % and a specificity of 100.00 % were found when compared with the reference method of indirect immunofluorescence used by the National Reference Laboratory.

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.

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. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
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

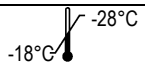
H317 May cause an allergic skin reaction.
H412 Harmful to aquatic life with long-lasting effects.
Wear protective gloves/protective clothing/ protective glasses/ face protection.
P302+P352 OF ON SKIN: Wash with plenty of water.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
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 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 to -28°C for long-term storage. Frequent freezing and thawing is not recommended. 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
°C	Celsius degree

%	percentage
	read the package leaflet
REF	catalog number

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