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IF-VIDITEST anti-EA(D) EBV IgG

ODZ-058

Instruction manual

1. Title:

IF-VIDITEST anti-EA(D) EBV – kit for immunofluorescence detection of IgG antibodies to Epstein-Barr virus (EBV) diffuse component of early antigen (EA(D)).

2. Intended use:

The kit is intended for the detection of IgG anti-EA(D) EBV antibodies. It is used for the diagnostics of EBV-induced or -associated diseases, such as infectious mononucleosis, chronic active EBV infection, Burkitt`s lymphoma, carcinomas of Waldayer's ring, opportunistic lymphomas (oligo- or polyclonal) and nasopharyngeal carcinoma. The kit may also be used for the characterization of the chronic fatigue syndrome and of immunodeficiency conditions during which EBV is frequently activated.

3. Test principle:

Human lymphoma line cells Raji is latently infected with EBV and do not produce EA under physiological conditions. It is possible to induce an abortive virus replication by specific chemical compounds. The replication of virus begins by synthesis of an early antigen. The induced cells are then fixated on the surface of glass slides.

Human antibodies to EA(D), if present in a tested sample, bind to the EA (D) within the cells. Antibodies that do not react with the antigens are washed away. The bound antibodies are detected with anti-human IgG antibodies labelled with fluorescein-isothiocyanate (FITC conjugate). A fluorescent microscope is used to observe specific fluorescence.

4. Reagents:

5x2	Microscopic slides coated with the cells
1x0,1 ml	Positive control serum IgG (lyophilised)
1x0,1 ml	Negative control serum (lyophilised)
1x0,25 ml	Anti-IgG FITC –conjugate (lyophilised)
1x5,0 ml	Mounting medium

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Material Safety Data Sheet

5.Material Required but not provided with the kit :

Phosphate buffered saline solution (PBS) pH=7,2 for the dilution of samples and for washing of slides; distilled/deionised water for reconstitution of lyophilised reagents; a moist chamber (a plastic box with lid and with a moisten absorbent material at the bottom); test tubes and pipettes to dilute and to dispense samples and the FITC-conjugate on the test slides; and to dispense a slide washing dish and a slide rack; cover slips 50 x 25 mm; a pen that writes on glass, fluorescent microscope.

6.Preparation of reagents

Reconstitute control sera with 0.1 mL of distilled water and further dilute this solution 10x with PBS. Dilute serum samples 10x with PBS if screening your samples, prepare a set of serum dilutions in PBS if you intent to do the serum titration test, which is more important. Prepare dilutions e.g.: 10x, 20x, 40x, 80x, 160x, etc.

Reconstitute Anti-IgG FITC –conjugate (contains Evans blue) in 0,25 mL of distilled water and dilute further 20x with PBS, i.e. add 4,75 mL of PBS).

You will need about 320 µl of FITC –conjugate for one slide (30 µl per one well). Store the reconstituted (undiluted) FITC-conjugates frozen at –18 to –28°C.

7. Assay procedure:

a. Let all the components to reach room temperature. Remove slides from their plastic packing, return the unused slide into the plastic package and store sealed at –18 to –28°C.

b. Place the slides into a moist chamber and pipette approximately 30 µL of the diluted samples in each well on the slide. Pipette positive control serum in the first well, negative control serum in the second well and the samples in the other wells. Close the chamber with a lid and incubate 60 minutes ± 5 minutes at room temperature

Slides must remain moist throughout the incubation!

c. Aspirate the liquid from wells into a bottle containing a disinfectant. Insert slides into a slide rack and put them in slide washing dish containing PBS.

Change the PBS for a fresh PBS after **5 ± 1 minute** – repeat 3x.

d. Remove the slides from the rack and clean off carefully any remaining droplets of PBS, carefully without scratching the wells surface.

e. Pipette the diluted anti-IgG FITC-conjugate in wells and place the slide in the moist chamber. **Incubate 60 minutes ± 5 minutes at room temperature**.

Slides must remain moist throughout the incubation!

f. Aspirate the conjugate from the wells and wash the slides 3x with PBS (3x for **5 ± 1 minutes**) and dip the slides once in distilled water. Set the slides vertically on and absorbent pad and let them dry at room temperature.

g. Apply two drops of mounting medium upon the glass slide and place carefully the cover slip to prevent trapping air bubbles.

h. Read the results immediately using a fluorescent microscope or store them in the dark at +2 to +10°C. The fluorescent signal is clearly visible for at least one week if stored properly.

8. Processing of results :

View the slides by a fluorescence microscope in the blue excitation light. When results are positive, cells exhibit brilliant green fluorescence - it can be the whole cytoplasm, mostly in the submembrane part. The fluorescence, which is limited only for the cell nucleus may indicate the presence of autoantibodies and could not be considered as a positive result. In negative sample these cellular structures are olive green to dark red. Weak membrane fluorescence caused by the presence of the Fc receptor should not be also considered as a positive result. Slides incubated with the negative control serum should be a negative if the test was performed correctly. Slides with the positive control serum contain mostly 20% of positive cells, never less than 3%.(number of positive cells in percentage can be found in the certificate)

The screening test uses dilution 1:10 and evaluates the presence/absence of antibodies in the sample. The titration test identifies the highest serum dilution with the positive findings as the sample titre.

Anti-EA(D) antibodies appear after primary infection (mostly little later than IgM anti-VCA antibodies) in about 50 % cases of infectious mononucleosis. They may be also present in EBV reactivation. High titers of anti-EA(D) antibodies are often found in patient with EBV positive nasopharyngeal carcinoma. But the anti-EA(D) antibodies absence does not exclude active infection EBV and therefore this test must be completed with another examination (determination of anti-VCA EBV and anti-EBNA IgG and IgM antibodies).

9. 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 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramines.

Work with the conjugate with Evans blue carefully. 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.

10. Warning:

a. The producer guarantees the use of the kit as an integral set. Combining the kit components from different lots of the kit is not recommended.

b. Work aseptically to prevent microbial contamination of sera and reagents.

c. Take care not to cross-contaminate samples during the dilution and storage. Prevent contamination with reagents that are known to be destructive for the fluorescence.

d. Anti-IgG FITC –conjugate and controls contain preservative ProClin 300® (see MSDS).

11. Storage and expiration:

Store the kit and the kit reagents at-18 to +28°C, in a dry place and protected from the light.

Store serum samples and conjugates at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.

Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find any damage at any part of the kit, please inform the manufacturer.

Expiration date is indicated on the kit label and on all reagent labels.

12. References:

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