9. Safety precautions:

All components of the kit are intended for laboratory use only.

Controls contain human sera that have been negative in tests 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 non-ignitable materials with 3% chloramine

Work with the FITC-conjugates that contain Evans blue carefully to avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
Evans blue is a potential carcinogen and teratogen.

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 dilution and storage. Prevent contamination with reagents that are known to be destructive for the fluorescence.
d. Controls and FITC-conjugates contain sodium azide as a preservative. FITC conjugates contain Evans blue.

11. Storage and expiry date:

Store the kit and the kit reagents at-18 to -28°C in a dry place and protected from the light. The expiration of the properly stored kit is 12 months from the date of production. Expiration date is indicated at the kit label and at all reagent labels.

Store the reconstituted Controls and the reconstituted FITC-conjugates +2 to +10°C up to one week, for longer storage make aliquots and store them at -18 to -28°C. Avoid repeated thawing and freezing.

Kits are shipped in cooling bags. 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.

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Date of last revision of the instructions: 12/2010

VIDIA

IVD

CE

IF-VIDITEST anti-VZV

Instruction manual

Producer: VIDIA s.r.o.
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252 42 Jesenice u Prahy
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www.vidia.cz

1. Title:

IF-VIDITEST anti-VZV – kit for the detection of IgG and IgM antibodies varicella-zoster virus (VZV) by indirect immunofluorescence.

2. Intended use:

The kit is intended for the detection of IgG and IgM antibodies to VZV. The use of the kit is in diagnosis of diseases induced or associated with VZV, such as varicella, shingles (herpes zoster), their complications (paralysis, neuralgia, encephalitis) and generalized infections in immunodeficient patients (pneumonia, generalized zoster).

3. Principle:

The surface of slides is coated with human embryonic lung cells infected with VZV and with non-infected cells. Human antibodies to VTV, if present in a tested sample, bind to the viral antigens present in the infected cells. Antibodies that do not react with the antigens are washed off. The bound antibodies are detected with anti-human IgG or anti-human IgM antibodies labelled with fluorescein-isothiocyanate (FITC conjugate). A fluorescent microscope is used to observe specific fluorescence. The uninfected cells serve as an internal control of the assay.

4. Kit contents:

10x Microscopic slides coated with a cell mixture
1x0,1 ml Positive control serum IgG (lyophilised)
1x0,1 ml Positive control serum IgM (lyophilised)
1x0,1 ml Negative control serum (lyophilised)
1x0,25 ml Anti-IgG FITC –conjugate (lyophilised)
1x0,5 ml Anti-IgM FITC –conjugate (lyophilised)
1x5 ml Mounting medium
Instruction manual
Certificate of quality

5. Necessary materials and equipment not included in 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; 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-conjugates 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).

Reconstitute Anti-IgM FITC –conjugate (contains Evans blue) in 0,5 mL of distilled water and dilute further 10x with PBS, i.e. add 4,5 mL of PBS).

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

7. Assay procedure:

a. Let all the components to reach room temperature (~ 15 minutes). 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 (IgG or IgM), negative control serum in the second well and the samples in the other wells. Close the chamber with a lid and incubate: a) 1 hour ± 5 minutes at room temperature to detect IgG antibodies and b) 3 hours ± 5 minutes to detect IgM antibodies.

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.

d. Pipette the diluted FITC-conjugate (20x diluted anti-IgG, 10x diluted anti-IgM) in wells and place the slide in the moist chamber. Incubate 60 ± 5 minutes at room temperature.

Slides must remain moist throughout the incubation!

e. Aspirate the liquid from 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.

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

g. 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.

Appendix to the instructions:
Sample incubation
Note!
Alternative protocol applicable only in the case of detection of class IgM antibodies!
b. Close the moist chamber and incubate slides for 2 hours ± 5 minutes at +35 to +38°C.

8. Processing of results:

View the slides in a fluorescent microscope using blue excitation wavelengths. Positive result is in case when cells show a brilliant green fluorescence in the nucleus, the cytoplasm or both. The cells that do not contain VZV antigens and the negative serum controls show themselves in olive green to dark red. A weak fluorescent signal found solely in the cytoplasm can not be considered as positive result since it might be caused by a nonspecific interaction with the cytoplasmic proteins binding Fc-fragment. Positive control serum show both the green fluorescent (infected) cells as well as negative cells (uninfected – kit internal control). Usually, there are 10-90% of positive cells found if testing class IgG antibodies, the number is lower in case of testing class IgM antibodies.

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.

The immune status (a contact with VZV in history) of an individual can be evaluated by the IgG screening test. Primary acute infection show either positive screening test for IgM antibodies or seroconversion in paired sera collected 1-2 weeks apart. Acute infections cause elevation in IgG antibody titers in paired sera. The shingles in most patients show positive for IgM antibodies and also elevation of IgG antibodies in relation to the time interval from the beginning of the skin symptoms.