Version 22-04/24

User's Manual

Human Anti-Varicella Zoster Virus (VZV/chickenpox) IgM ELISA kit

REF **DEIA-NS2307-5**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the detection of human IgM antibodies against Varicella zoster in serum or plasma.

General Description

Varicella-zoster virus (VZV) is known by many names, including: chickenpox virus, varicella virus, zoster virus, and human herpes virus type 3 (HHV-3). VZV is closely related to the herpes simplex viruses (HSV), sharing much genome homology. It is one of eight herpes viruses known to infect humans and other vertebrates. It commonly causes chicken-pox in children and adults and herpes zoster (shingles) in adults and rarely in children. As with the other herpes viruses, VZV causes both acute illness and lifelong latency. Before vaccination became widespread, acute primary infection (varicella or "chickenpox") was common during childhood--especially in temperate climates. Varicella usually is a benign and selflimiting illness, but can be more severe in adults and in individuals with cellular immunodeficiency. These individuals are at much higher risk of pneumonia and disseminated disease with visceral involvement. Zoster typically presents as a painful, localized cutaneous eruption occurring along 1 or more contiguous dermatomes. Humans are the only known natural hosts of VZV. Transmission of VZV occurs through direct contact with infectious lesions or by inoculation of aerosolized infected droplets onto a susceptible mucosal surface.

The known envelope glycoproteins (gB, gC, gE, gH, gI, gK, gL) correspond with those in HSV; however, there is no equivalent of HSV gD. The most popular test detects VZV- specific IgM antibody in blood; this appears only during chickenpox or herpes zoster and not while the virus is dormant. VZV vaccines is a chickenpox vaccine for children, adolescents and adults.

Principles of Testing

CD's Varicella zoster IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Varizella zoster antigen is bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Varizella zoster antigen takes place. After one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

Reagents And Materials Provided

1. Varizella zoster antigen coated strip plate, (8×12 strip or 96 wells), 1 plate
2. VZV IgM Calibrator A (Negative Control), 2 mL, 1 vial
3. VZV IgM Calibrator B (Cut-off standard), 2 mL, 1 vial
4. VZV IgM Calibrator C (weak positive control), 2 mL, 1 vial

5. VZV IgM Calibrator D (positive control), 2 mL, 1 vial
6. Anti-Human IgM-HRP Conjugate, (15 ml), 1 bottle
7. Sample Diluent, 60 ml, 1 bottle
8. Wash buffer (10×), 60 ml, 1 bottle
9. TMB Substrate Solution, 15 ml, 1 bottle
10. Stop Solution, 15 ml, 1 bottle
11. Complete Instruction Manual, 1

Materials Required But Not Supplied

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

Storage

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

Specimen Collection And Preparation

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent). Do not dilute the calibrators.

Reagent Preparation

1. Dilute Wash buffer 1:10 with water. (Dilute 60 ml stock with 940 ml distilled water) Store diluted buffer at 4°C for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.
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All reagents must be at room temperature prior to their use

Assay Procedure

(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:101 with the sample diluent. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. DO NOT dilute calibrators or controls. Dilute wash buffer stock (10×) 1:10 with distilled water.

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense 100 ul diluent in 1 well to be used as blank. Pipet 100 ul of calibrators, controls, and diluted samples into appropriate wells in duplicate. Cover the plate, mix gently for 5-seconds and incubate at room temp (25-28°C) for 60 min.
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, wash the wells 3 times with 300 µl of 1× wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. Add 100 µl anti-human IgM-HRP conjugate to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temp (18-26°C).
6. Wash the wells 3 times as in step 3.
7. Add 100 µl TMB substrate solution. Mix gently for 5-10 seconds. Cover the plate and incubate for 20 minutes at room temp. Blue color develops in positive controls and samples.
8. Stop the reaction by adding 100 µl of stop solution to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).
9. Measure the absorbance at 450 nm using an ELISA reader within 60 min.

NOTES:

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.

Calculation

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards. The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. The initial dilution of unknowns has been taken into consideration when reading the results from the graph. Results of unknowns of higher predilution have to be adjusted for the dilution factor. Unknowns showing concentrations above the highest calibrator have to be diluted as described in "Assay Procedure" and reassayed.

Interpretation Of Results

U/mL	Results
<8	**negative**
8-12	**equivocal**
>12	**positive**

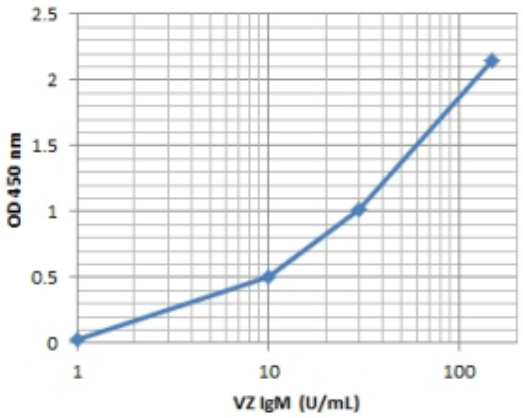
In an in-house study apparently healthy research subjects showed the following results:

Ig isotype	n	Interpretations		
		positive	**equivocal**	**negative**
IgM	56	0.0%	2%	98.0%

Typical Standard Curve

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

	U/mL	Net A450
Calibrator A (Negative Control)	1	0.013
Calibrator B (Cut off std)	10	0.438
Calibrator C (weak positive Control)	30	0.895
Calibrator D (Positive Control)	150	1.883



Typical Std Curve (do not use this for sample calculation)

Precautions

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from

different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Limitations

1. This assay is intended for research use only – not for use in diagnostic procedures. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
2. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
3. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
4. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
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