VZV-IgM-ELA Test PKS medac

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VZV-IgM-ELA Test PKS medac

Enzyme immunoassay with Pipetting Control System for the detection of IgM antibodies to Varizella-Zoster virus (VZV) in serum

Cat. no.: 101-PKS

FOR IN VITRO DIAGNOSTIC USE ONLY

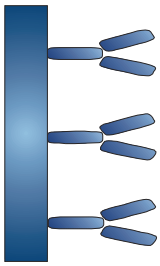
INTRODUCTION

Varicella-Zoster virus (VZV) belongs to the family of *Herpesviridae.* It consists of a double-stranded DNA genome, nucleocapsid, tegument and envelope. Primary infection predominantly occurs in childhood (varicella). In immunocompetent individuals the symptoms are usually moderate. In immunosuppressed patients the infection can cause serious complications (central nervous system involvement, pneumonia, secondary bacterial infections).
The seroprevalence in adult individuals is about 95 %. It is characteristic for VZV to persist lifelong in the sensory nerve ganglia of the dorsal root establishing latent infection in neuronal cells. An endogenous reactivation of the virus may cause the secondary illness herpes zoster. Varicella can be prevented by vaccination. The commercially available vaccines are highly efficient in preventing the disease.

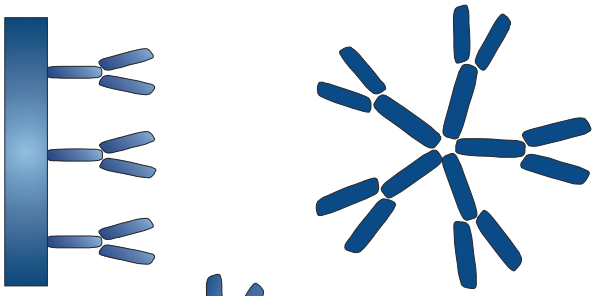
Varicella and herpes zoster are usually diagnosed by the typical clinical signs. Laboratory diagnostic investigation may become essential in cases with atypical illness or during pregnancy. The detection of antibodies against VZV is predominantly used to confirm immunity or success of vaccination. It is further useful for the confirmation of suspected VZV disease. The detection of a VZV-specific intrathecal antibody synthesis in serum-CSF pairs is a component of differential diagnosis in acute infection or chronic disease of the central nervous system.
A positive VZV IgM result confirms a primary infection or may be caused by an endogenous reactivation of the latently persisting VZV. A VZV-IgM result has always to be interpreted in conjunction with VZV IgG, IgA and clinical symptoms.

The VZV-IgM-ELA Test PKS medac detects IgM antibodies in serum directed against VZV. The test can be carried out fast and easily. The µ-capture and ELA principle allow a highly specific and sensitive diagnostic interpretation.

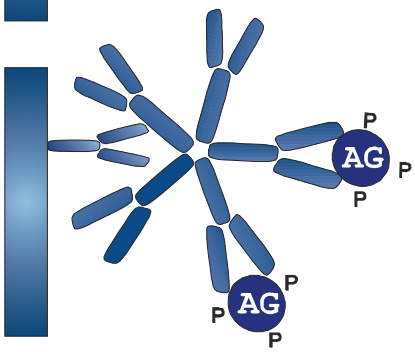
TEST PRINCIPLE



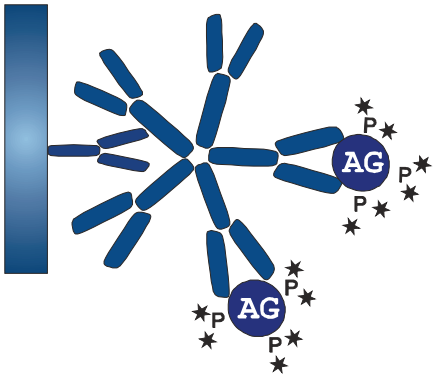
The plate is coated with anti-human IgM immunoglobulin.



IgM of the specimen is selectively bound to the wells.



The VZV-specific IgM antibodies bind to the peroxidase-labelled VZV-IgM-ELA (AG = antigen, P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- ☞ No unspecific reactions and no false positive results caused by rheumatoid factors.
- ☞ No blocking of IgM antibodies by high IgG titer.
- ☞ The Pipetting Control System allows to monitor visually each pipetting step through colour changes.
- ☞ The breakable microwell stips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.

KIT CONTENTS
Cat. no.: 101-PKS

1. **MTP**
   Microplate: 12 x 8 wells (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with goat anti-human IgM immunoglobulin, BSA and pH indicator, ready to use.

2. **CONTROL -**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

3. **CONTROL +**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycin sulfate.

4. **WB**
   Wash buffer: 1 bottle with 100 ml, PBS/Tween (10x), pH 7.2 - 7.4, contains ProClin™ 300.

5. **VIR-DIL**
   Sample diluent: 1 bottle with 110 ml, PBS/Tween/BSA, pH 7.2 - 7.4, ready to use, contains ProClin™ 300.

6. **ANTIGEN-DIL**
   Antigen diluent: 1 vial with 14 ml Tris/NaCl/Tween, ready to use, contains ProClin™ 300.

7. **ANTIGEN**
   VZV-IgM-ELA (Enzyme Labelled Antigen): 4 vials with 3.0 ml each, lyophilised, contains FCS.

8. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

9. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Antigen diluent	opened	2...8 °C	6 weeks
VZV-IgM-ELA	ready to use	2...8 °C	7 days
		$\leq$ -18 °C *	6 weeks
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date

* Aliquote and do not freeze again after thawed once.

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate reader with filters for 450 nm and 620 - 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate

The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated below point 1.

Note: The microplate wells have a light green colour. Possibly occurring greenish brown stains inside the wells are due to the production process and do not influence the test performance.

3.2. Wash buffer

Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

3.3. VZV-IgM-ELA

Reconstitute the lyophilised antigen 60 min before use with 3.0 ml **antigen diluent** each. Mix gently and take care that particles sticking to the closure are also dissolved.

After reconstitution the VZV-IgM-ELA has a red colour and is ready to use.

The ready-to-use VZV-IgM-ELA can be stored at 2 - 8 °C for 7 days or at ≤ -18 °C for 6 weeks (see 1.).

Do not mix test specific reagents (microplate, controls, VZV-IgM-ELA, antigen diluent) from different kit lots. In contrast to that sample diluent, wash buffer, TMB-substrate and stop solution are generally exchangeable in all virological ELISA medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum.

4.2. Pretreatment of sera, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain any red blood cells.

4.3. Sera have to be diluted 1:100 with sample diluent. They can be diluted further for titer determination.

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be prewashed.

5.2. Leave well A1 empty as blank (see 6.A.). Add 50 µl each of the diluted sample as well as negative control and positive control in duplicates to the wells.

After pipetting the samples (pH neutral or basic fluid) the wells turn blue/green. A missing colour change in one well indicates that no sample or control has been added.

If necessary, the microplate wells can be kept up to 30 min at RT before proceeding.

5.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.4. Prepare the VZV-IgM-ELA (see 3.3.).

5.5. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.6. Add VZV-IgM-ELA (coloured red) to each well (except A1).

50 µl of the VZV-IgM-ELA have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of the VZV-IgM-ELA have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.7. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.8. After incubation wash microplate wells again (see 5.5.).

5.9. Add 50 µl of TMB-substrate to each well (also A1) and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.10. Stop the reaction by adding 100 µl of stop solution to each well (also A1). Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the stop solution.

5.B. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Sample
Negative control	-	50 µl	-	-
Positive control	-	-	50 µl	-
Sample	-	-	-	50 µl
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer				
VZV-IgM-ELA	-	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer				
TMB-substrate	50 µl	50 µl	50 µl	50 µl
Incubate for 30 minutes at 37 °C in the dark				
Stop solution	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 - 650 nm)				

*) manual/automatic procedure (see 5.6.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 - 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* The mean OD value of the **negative control** has to be **< 0.150.**
  The mean OD value of the **positive control** has to be **> 0.600.**

* **Cut-off = mean OD value of the negative control + 0.140**

* **Grey zone = Cut-off ± 10 %**

  Repeat the run if the results do not meet the specification.

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with OD values below the lower limit of the grey zone are reported as **NEGATIVE**.

* Samples with OD values within the grey zone are reported as **EQUIVOCAL**. These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with OD values exceeding the upper limit of the grey zone are reported as **POSITIVE**.

* The results should always be interpreted in connection with clinical data of the patients as well as the VZV IgG, the VZV IgA results and additional diagnostic parameters.

  In case of suspected primary VZV infection during pregnancy and negative VZV IgM result, further diagnostic, e.g. PCR, has to be performed.

* Cross reactions, caused by antibodies against other herpes viruses, ANA and heterophilic antibodies cannot be excluded in single cases.

* An influence of very high lipid concentrations cannot be excluded.

* High concentrations of hemoglobin do not influence the test results.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SENSITIVITY AND SPECIFICITY

150 sera were measured during the diagnostic evaluation. The results were correlated with the diagnostical characterization of the lab Prof. Dr. Enders and Colleagues, Stuttgart.
The results are shown in the following table:

		Pre-definition (Prof. Enders)		
		negative	cut-off	positive
VZV-IgM-ELA Test PKS medac	negative	**99**	**0**	**4**
	cut-off	**0**	**0**	**1**
	positive	**1***	**0**	**45**

*sample from patient with confirmed acute VZV infection

Sensitivity = 90.0 %
Specificity = 99.0 %

Concordance: 96.0 %

7.B. PRECISION

Sample	**Intra-assay variation**				**Sample**	**Interassay variation**			
	mean OD	**SD**	**CV (%)**	**n**		**mean OD**	**SD**	**CV (%)**	**n**
NC	0.048	0.003	6.3	22	**NC**	0.094	0.017	18.1	11
BC	0.556	0.021	3.8	22	**BC**	0.621	0.045	7.2	11
PC	0.995	0.029	2.9	22	**PC**	1.083	0.051	4.7	11
N° 1	0.081	0.004	4.9	22	**N° 6**	0.104	0.014	13.5	11
N° 2	0.309	0.018	5.8	22	**N° 7**	0.370	0.030	8.1	11
N° 3	0.406	0.019	4.7	22	**N° 8**	0.567	0.033	5.8	11
N° 4	0.767	0.039	5.1	22	**N° 9**	0.889	0.058	6.5	11
N° 5	1.426	0.087	6.1	22	**N° 10**	1.542	0.062	4.0	11

NC = negative control; BC = weak positive control (not included in the kit);
PC = positive control

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents) should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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