Anti-VZV ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2650-9601 M	Varicella zoster virus (VZV)	IgM	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against varicella zoster virus (VZV) in serum or plasma for the diagnosis of Varicella zoster virus infections.

Application: The determination of specific antibodies is the method of choice for confirmation of suspected infections (Varicella) or reactivations (Zoster) with corresponding clinical symptoms. The determination of IgG and IgM also indicates an acute infection; in the case of reactivations, specific IgA antibodies have an important diagnostic value. In both cases however, antibodies of both class IgA and IgM may be present, so that the determination of avidity for antibodies of class IgG can be of great importance. The determination of the immune status in early pregnancy and the investigation of successful immunisation is possible by means of VZV-IgG antibodies.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with VZV antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. Sample buffer containing IgG/RF-absorbent (anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro determination
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The controls used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with green coloured sample buffer. For example: add 10 µl sample to 1.0 ml sample buffer and mix well. Incubate the mixture for at least **10 minutes** at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	P 6	P 14	P 22								
B	pos.	P 7	P 15	P 23								
C	neg.	P 8	P 16	P 24								
D	P 1	P 9	P 17									
E	P 2	P 10	P 18									
F	P 3	P 11	P 19									
G	P 4	P 12	P 20									
H	P 5	P 13	P 21									

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Calculate the ratio according to the following formula:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of the calibrator}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: **negative**
Ratio ≥0.8 to <1.1: **borderline**
Ratio ≥1.1: **positive**

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along the serological findings.

Test characteristics

Calibration: As no international reference serum exists for the detection of IgM class antibodies against VZV, results are provided in the form of ratios which are a relative measure of the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the ratios of the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrator is subject to the same influences, with the result that such variations will be largely com-pensated in the calculation of the result.

Antigen: The antigen source is provided by inactivated cell lysate of MRC-5 cells infected with the strain “VZ-10” of varicella zoster virus.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The detection limit of the Anti-VZV ELISA (IgM) is approximately ratio 0.04.

Cross reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Cross reactions with other herpes viruses cannot be excluded. Sera from patients with infections caused by various agents were investigated with the EUROIMMUN Anti-VZV ELISA (IgM).

Antibodies against	n	Anti-VZV ELISA (IgM)
Borrelia	10	0%
CMV	19	5%
EBV-CA	21	29%
HSV Pool	5	20%
Masern virus	13	0%
Mumps virus	12	0%
Rubella virus	10	0%
Toxoplasma gondii	14	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a con-centration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-Assay Variation, n = 20		
Serum	**Mean value (Ratio)**	**CV (%)**
1	2.2	11.0
2	3.3	4.2
3	3.4	7.6

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (Ratio)**	**CV (%)**
1	1.9	11.9
2	3.3	9.2
3	3.3	10.4

Specificity and sensitivity: A panel of 191 clinically characterised patient samples (INSTAND, Labquality, MQ Schweiz) was examined using the EUROIMMUN ELISA. The test shows a specificity of 96.3% and sensitivity of 98.1%, respectively.

n = 191		**INSTAND, Labquality, MQ Schweiz**		
		positive	**borderline**	**negative**
EUROIMMUN Anti-VZV ELISA (IgM)	**positive**	53	0	5
	borderline	0	0	1
	negative	1	2	129

Study: 181 patient samples from clinically characterised VZV patients were examined with the EUROIMMUN Anti-VZV ELISA (IgM) and Wampole ELISA IgM (FDA-approved assay). Based on this study the clinical specificity for the EUROIMMUN ELISA was 100% at a sensitivity of 100%.

n = 181		**Wampole ELISA IgM**		
		positive	**borderline**	**negative**
EUROIMMUN Anti-VZV ELISA (IgM)	**positive**	17	0	0
	borderline	0	2	1
	negative	0	0	161

Reference range: The levels of anti-VZV antibodies (IgM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of ratio 1.0, 0.6% of the blood donors were anti-VZV positive (IgM).

Clinical significance

Varicella zoster virus (VZV), synonyme: human-pathogenic herpes virus 3 (HHV3), is the causative agent of chickenpox (varicella) after which it establishes latency and can subsequently reactivate to cause herpes zoster (shingles) [1, 2]. The virus is strictly human [3]. Chickenpox – a very contagious disease – has traditionally been regarded as a benign, inevitable disease among children (25% in 1- to 4-year-olds, 43% in 5- to 8-year-olds, 27% in 9- to 18-year-olds) with typical blister-like rash of the entire skin [1, 4, 5]. Now we know varicella can also be a serious infection, even in childhood, but especially in young and older adults and during pregnancy [6, 7, 8, 9].

Zoster is the endogenous recurrence of an earlier varicella infection or the result of a reinfection with existing residual immunity [2]. The average incidence of herpes zoster in Europe is 3 per 1000 people per year in the total population and more than 10 per 1000 people per year in those aged >80 years [10]. The entire virus genome is present in the latently infected ganglia. VZV is latent in multiple ganglia along the entire human neuraxis [1]. In zoster, the rash affects the spreading area of one or several sensitive nerve roots, especially T3-L3 and N. trigeminus [2, 3]. Central nervous system (CNS) complications can follow both primary infection and reactivation of VZV [8, 11]. The more serious manifestations arise when VZV invades the spinal cord or cerebral arteries after reactivation of the virus, causing diseases such as myelitis, focal vasculopathies, and encephalitis [7, 11].

Varicella also causes serious infections during pregnancy with severe consequences of maternal varicella for the infant [6, 12, 13]. At birth, maternal infection with the VZV poses a truly life-threatening risk to the newborn. The neonatal mortality rate is up to 20 to 30%, if the maternal VZV-infection occurs between day 4 ante partum and day 2 post partum [14]. Patients with congenital varicella syndrome (CVS) typically show clinical symptoms such as skin lesions, neurological defects, eye diseases, and/or limb hypoplasia. In rare cases, isolated manifestations in the brain or eye have been reported [15].

Antibodies against varicella zoster viruses (IgA, IgG, IgM) can be found in the serum of almost all subjects during and after a varicella infection. They can be verified by ELISA and IIFT. IgG- and IgM-antibodies against VZV are markers to confirm suspected VZV-infections, especially VZV-IgG-antibody titers during pregnancy [14, 16, 17, 18]. IgA-titers are typical for a reinfection (zoster) [19].

In addition to classic serodiagnosis of VZV, especially IgG and IgM antibodies suggestive of acute infection, measurement of VZV-IgG-avidity provides information making it possible to distinguish exactly between acute and chronic infection, as determination of avidity in other virus infections demonstrates [20]. Therefore it is of particular interest in pregnant women. Avidity describes the binding strength of specific antibody to antigen. It was found to be low in the first phase after primary infection but then to increase over time. Based on this additional information, repeated testing and unnecessary anxiety in patients can be avoided, which also is the case in the serological diagnostic of other virus infections [19, 20, 22, 23].

VZV myelitis or VZV encephalitis are diagnosed by the determination of antibodies against VZV in CSF and serum using ELISA [11]. CNS involvement results in the intrathecal synthesis of antibodies against VZV in cerebrospinal fluid (CSF). Due to the fact that specific antibodies can pass from the serum through the blood-brain barrier into the CSF by diffusion, a relative CSF/serum quotient ($CSQ_{rel.}$, synonym: antibody specificity index) [20] is determined. The quotient is calculated from the amount of specific IgG antibodies in total CSF IgG in proportion to the amount of specific IgG antibodies in total serum IgG. During conversion the CSF/serum quotient of the pathogen-specific IgG-antibody concentrations $CSQ_{path.-spec.}$ (IgG) is put into relation to the CSF/serum quotient of the total IgG concentrations CSQ_{total} (IgG) [20]. A relative CSQ result above 1.5 indicates the production of specific antibodies in the CNS and the involvement of the CNS in the disease [24, 25, 26].

Generally, a life-long immunity develops, and this is also the case after a successful protective vaccination [17, 27, 28]. A passive immunisation with specific immunoglobulins is often given to immunocompromised seronegative people, such as tumour patients and recipients of transplants, as well as seronegative pregnant women after exposure to the virus [17, 29].

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