VIROTECH VZV IgG/IgM ELISA
(VZV IgG/IgM ELISA)

Order No.: EC110.00 Color Coding: silver/transparent

VZV IgA-Set

Order No.: EC110.08

VZV IgG Liquor/CSF Standards

Order No.: EC110L60

VZV IgM Liquor/CSF Standards

Order No.: EC110L80

VZV IgA Liquor/CSF Standards

Order No.: EC110L40

VZV IgG Liquor/CSF AI Ctrl-Set

Order No.: EN110L65

FOR IN VITRO DIAGNOSIS ONLY

VIROTECH Diagnostics GmbH
Löwenplatz 5
D- 65428 Rüsselsheim

Tel.: +49-6142-6909-0
Fax: +49-6142-966613
http://www.virotechdiagnostics.com

CE

Freigabedatum: 27.04.2020 13:04
REV 18 / VIROTECH VZV IgG/IgM/IgA ELISA GB

Contents

1. Intended Use

The VIROTECH VZV IgG/IgM ELISA + VZV IgA-Set is intended for the semiquantitative and qualitative detection of IgG-, IgM- and IgA-antibodies against VZV in human serum. It is intended for differentiation respectively check of seronegativity, primary infection, past infection, reactivation and to determine if vaccination is necessary respectively has been successful.

2. Diagnostic Relevance

The species Varicella zoster virus (VZV), also called human (alpha) herpesvirus 3, is combined with a few other herpesviruses of similarly structure into the higher genus of Varicellovirus (1).
Humans are the only known reservoir for VZV. Varicellas are extremely contagious; after exposure, over 90 out of 100 susceptible, i.e. seronegative persons become ill (2). Referring to STIKO (ständige Impfkommission Deutschland = Institute for Vaccination Protection Germany), samples with >100 IU/l are seropositive (5).The virus is endemic in the population and there is a particular seasonal increase in the incidence of transmission – in winter and spring in temperate latitudes. Transmission is airborne by virus-containing droplets. Spread is also possible as a contact infection through virus-containing vesicle contents or crusts. The contagiousness of Herpes zoster is lower.
The incubation period can be 8-28 days and is usually 14-16 days. The infectiousness commences 1-2 days before the exanthem appears and ends 7 days after the last efflorescences appear.
The VZV can cause two different clinical syndromes: varicella (chickenpox) with exogenous new infection and Herpes zoster (shingles) with endogenous reactivation.

Varicella:
After an uncharacteristic prodrome (1-2 days before the start of illness) the disease begins with an itchy exanthem and increased temperature which can be over 39°C for 3-5 days. The severity of the lesions can vary greatly. Varicella usually follows a benign course in otherwise healthy persons and heals in the normal case without scars. The importance of chickenpox derives particularly from the possible complications: bacterial superinfection of the skin lesions, varicella pneumonia, CNS manifestations (for CSF-diagnostic we recommend the VIROTECH VZV CSF standards IgG, IgM and IgA), foetal varicella syndrome and severe neonatal chickenpox.

Herpes zoster:
Herpes zoster can develop only in individuals who have had previous VZV infection or previous immunisation with live vaccine. The virus persists in spinal or cranial nerve ganglia and then leads to Herpes zoster when reactivated. It occurs mainly in persons with weakened immunity and in elderly persons. Herpes zoster is characterised by unilateral vesicular eruptions within a dermatome with severe pain.
Direct laboratory diagnosis can be carried out by PCR, the finding of antigen and virus isolation.
Specific antibodies can be detected indirectly from serum or, in the case of meningitic forms, from CSF using serological methods (ELISA, IFA). In the case of herpes zoster, the specific IgA antibodies have a high diagnostic reliability. In contrast, IgM antibodies can be absent (2).
Although chickenpox often follows a typical course so that it can be diagnosed purely clinically, in some of the cases virological differential diagnosis becomes necessary to distinguish it from other exanthematous diseases. It is not infrequently mistaken for HSV (3).
Laboratory diagnosis is employed to identify atypical and severe forms of illness in patients with immunoinsufficiency, to detect CNS infections from CSF, to elucidate infections during pregnancy and in the neonate, to distinguish other vesicle-producing dermatoses (herpes simplex) and to establish the state of immunity in patients at risk because of VZV infections (4).

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents

4.1 IgG/IgM Testkit

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 1x50ml**, pH 7,2, with preservative and Tween 20
3. **VZV-IgM-Dilution Buffer (green, ready to use) 1x50ml**, pH 7,2, with preservative and Tween 20
4. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
5. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
6. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
9. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
11. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
12. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
13. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
14. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 IgA Set

1. **IgA negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
2. **IgA cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
3. **IgA positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
4. **IgA-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and

microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.

2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a proper IgM determination, it is therefore necessary to treat sera with RF - SorboTech** (VIROTECH adsorbent) **in Varicella zoster Virus IgM PBS dilution buffer (green)**. IgM controls should not be pre-adsorbed.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- IgM- and IgA-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary.
   Working dilutions for patient sera:
   for the IgG/IgA test solution: 1+100; e.g. 10µl serum + 1ml **dilution buffer (blue)**
   for the IgM test solution: use the **green dilution buffer** and carry out preabsorption with RF SorboTech.
2. After pipetting start incubation for 30 min. at 37°C (with cover).

3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends proceeding the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly, and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG-, IgA- and IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE(\text{positive control}) = \frac{OD(\text{positive control})}{OD(\text{cut - off control})} \times 10$$

$$VE(\text{patient serum}) = \frac{OD(\text{patient serum})}{OD(\text{cut - off control})} \times 10$$

9.3 Interpretation Scheme IgG, IgM and IgA

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
4. It is not possible to differ between vaccination antibodies and antibodies built after infection. Please follow vaccination management!
5. Referring to STIKO (stand. Impfkommission, Deutschland = Institute for Vaccination Protection Germany) samples with >100IU/l are considered to be seropositive. Those STIKO statement refers to an international standard. The VIROTECH VZV IgG/IgM ELISA is adjusted in IgG so that 100 IU/l correspond to 9VE (VIROTECH units).

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Cross reactions to CMV-, EBV- and HSV-positive sera may occur.

10. Performance Data

10.1 Sensitivity and Specificity

The antibody concentrations of sera collectives were determined in an ELISA of a competitor and in the VIROTECH VZV IgG/IgM ELISA and then compared to determine the sensitivity and specificity. The testing of a sera collective (47 routine sera, 66 blood bank sera, 26 pregnant women´s sera, 23 children's sera, 2 WHO standards with 4 dilutions each, 36 sera from proficiency tests, 3 other sera) in the VIROTECH VZV IgG/IgM ELISA showed a sensitivity of 99% and a specificity of 94% for IgG in relation to the previous findings.

Sera collective (n=209)		VZV IgG		
		Negative	**Borderline**	**Positive**
Finding	**Negative**	47	5	3
	Borderline	3	5	2
	Positive	1	-	143

Borderline results were not considered when calculating sensitivity and specificity.

The testing of a sera collective (60 routine sera, 63 blood bank sera, 20 pregnant women´s sera, 14 children's sera, 36 sera from proficiency testings, 17 other sera) in the VIROTECH VZV IgG/IgM ELISA showed a sensitivity of 95% and a specificity of 98% for IgM compared to the ELISA results of a competitor.

Sera collective (n=210)		VZV IgM		
		Negative	**Borderline**	**Positive**
Finding	**Negative**	133	2	3
	Borderline	5	2	2
	Positive	3	4	56

Borderline results were not considered when calculating sensitivity and specificity.

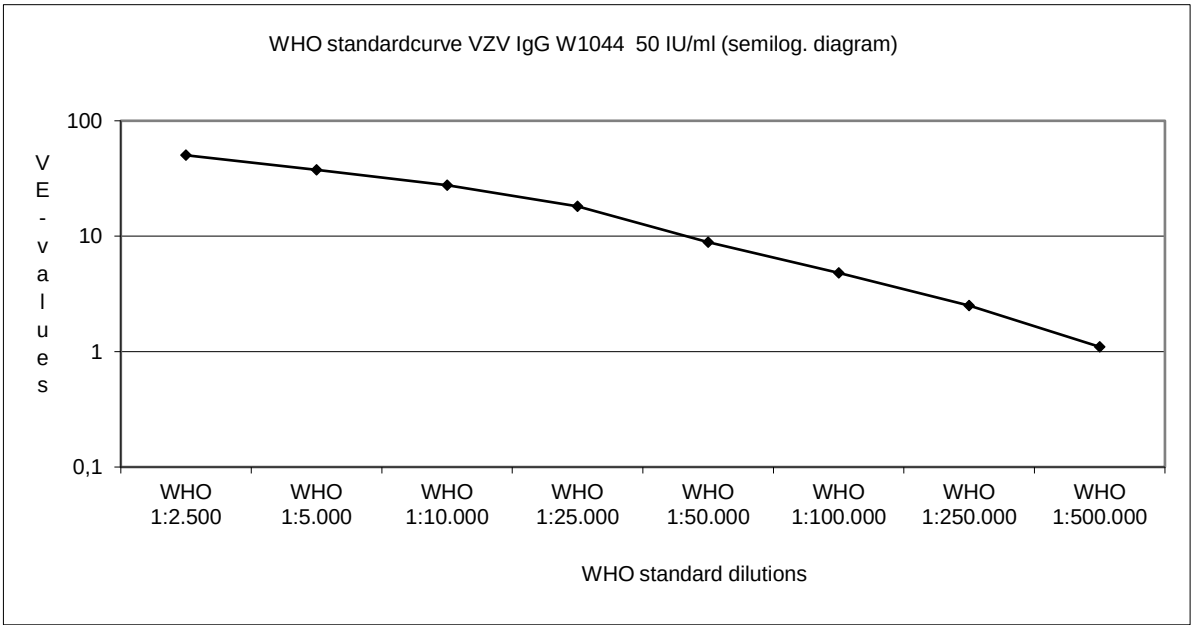
The testing of a sera collective (26 routine sera, 61 blood bank sera, 20 pregnant women´s sera, 22 children's sera, 29 other sera) in the VIROTECH VZV IgG/IgM ELISA showed for IgA in relation to the previous findings and compared to the ELISA results of a competitor a sensitivity of >99,8% and a specificity of >99,8%.

Sera collective (n=158)		VZV ELISA IgA		
		Negative	**Borderline**	**Positive**
Finding	**Negative**	107	1	-
	Borderline	10	2	-
	Positive	-	13	25

Borderline results were not considered when calculating sensitivity and specificity.

10.2 Detection Limits

The WHO standard W1044 (50IU/ml) was used in 8 different dilutions to determine the detection limits. The results show an upper detection limit of 50VE and a lower detection limit of 1VE. Thus, 9VE correspond to 100 IU/l.



10.3 Prevalence (Expected Values)

The following table shows the results of the examination of blood bank sera in IgG, IgA and IgM:

	IgG (n=120)		IgM (n=80)		IgA (n=120)	
	No.	**%**	**No.**	**%**	**No.**	**%**
Negative	4	3	79	98,8	118	98,3
Borderline	1	1	1	1,2	0	0,0
Positive	115	96	0	0,0	2	1,7

According to literature the prevalence in IgG is between 80 and 90% already in adolescents (3). In other sources a prevalence in adults of >90% (6) or >95% (2) is mentioned.

10.4 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation for IgG is < 9%.

10.5 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 12 independent test runs by different persons in different laboratories.
The obtained variation coefficient values is <15%.

11. Literature

1. Tomas Porstmann (Hrsg,), Virusdiagnostik, Diagn. Bibliothek, Band 1, Blackwell Wissenschaft, 1996, S.291.
2. RKI-Ratgeber Infektionskrankheiten – Merkblätter für Ärzte – Varizellen / Herpes zoster. (11.01.2010)
3. Brandis, Köhler, Eggers, Pulverer, Lerhbuch der Medizinischen Mikrobiologie, 7. Auflage, Fischer 1994, S.777-778
4. Tomas Porstmann (Hrsg,), Virusdiagnostik, Diagn. Bibliothek, Band 1, Blackwell Wissenschaft, 1996, S.296.
5. RKI, Mitteilung der Ständigen Impfkommission (STIKO) am RKI, Epidemiologisches Bulletin Nr. 8, 23.02.2001, S.58.
6. Mikrobiologische Diagnostik und Krankenhaushygiene, MVP, 2. Ausgabe, Stand Jan 2003, S 61.

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ IgG-/IgA-Samples – Dilution 1:101

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

▼ IgM-Samples - Dilution 1:101
Rheumafactor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffe (green) +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgM, IgA
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**