VIROTECH EBV IgG/IgM ELISA
(EBV IgG/IgM ELISA)

Order No.: EC102.00

EBV IgG Liquor/CSF Standards

Order No.: EC102L60

EBV IgG Liquor/CSF AI Ctrl-Set

Order No.: EN102L65

Color Coding: yellow

FOR IN VITRO DIAGNOSIS ONLY

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REV 18 / VIROTECH EBV IgG/IgM ELISA EN

Contents

1. Intended Use

The EBV ELISA is intended for the semiquantitative and qualitative detection of IgM- and IgG-antibodies against Epstein Barr Virus.

A diagnostic statement with regard to seronegativity, suspicion to an EBV primary infection or past EBV infection can be made when combining the two antibody classes.

2. Diagnostic Relevance

The Epstein Barr virus belongs to the family of the Herpesviridae and is transmitted mainly by saliva, when it infects the epithelial cells of the oropharynx initially and then the B-lymphocytes. The virus is the cause of infectious mononucleosis (IM) and chronic active EBV infection. There is also an association between EBV infections and Burkitt's lymphoma and also nasopharyngeal carcinomas in Africa and Asia. According to serological investigations, approx. 95% of adults are seropositive for EBV.

Primary EBV infections are normally asymptomatic but can be the cause of infectious mononucleosis in adolescents and young adults. IM is a self-limiting illness and is characterised by lymphadenopathy, fever, hepatosplenomegaly and leukocytosis with atypical lymphocytes (1-7)

The role of EBV serology consists of differentiating between or confirming seronegativity, primary infection and previous infection and in making a differential diagnosis from clinically similar symptomatic diseases (8).

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 (IgG and IgM Testkit)

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

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 correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgM-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 dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
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 to proceed 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- 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 and IgM

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.
3. 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 have 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.
4. If the measured values are below the defined borderline range, no measurable antigen specific IgM-antibodies are present in the sample. The samples are considered to be negative.

IgM	IgG	
+	+	Notice of a primary infection
+	-	
-	+	Notice of past infection
-	-	Notice of seronegativity

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. A negative ELISA result does not completely exclude an EBV infection.
3. Infective agents with similar clinical picture should be considered in differential diagnosis.
4. Crossreactivities of Epstein Barr Virus against the Herpes Virus family are known. With a positive IgM result specially crossreactivities against CMV should be excluded.
5. A negative IgM result does not exclude the possibility of a primary infection, as in some cases no IgM is built during an acute infection (IgM-nonresponder) (8,9).
6. With clinical suspicion of an EBV infection and a negative serology a second blood sample should be taken.
7. A positive EBV ELISA IgM result should be checked by specific EBV confirmation tests (VIROTECH EBV EA-D IgG ELISA, EBV EBNA1 IgG ELISA, EBV VCA IgG ELISA, EBV VCA IgM ELISA or through VIROTECH EBV IgG LINE Immunoblot / VIROTECH EBV IgM LINE Immunoblot).
8. Antibodies which were passively transmitted shortly before examination may influence the EBV serological result. This might be e.g. by blood transfusion or maternally transmitted antibodies to the infant.

10. Performance Data

10.1 Diagnostical Sensitivity

19 clinically characterized sera from patients with primary infection were tested to determine the diagnostical sensitivity (sera from Dr. Gärtner, Homburg/Saar).
Immunoblots and/or IFA were used as reference tests.

Sera Collective (n=19)		**VT EBV ELISA IgG + IgM overall result**	
		Primary Infection	**Past Infection**
Finding	**Primary Infection**	19	0
	Past Infection	0	0

The results of the VIROTECH EBV ELISA correspond with the clinical and findings in all cases.

10.2 Sensitivity

Following sera collectives were tested to determine the sensitivity:

1. sera from patients with EBV primary infection (n=20, sera from Dr. Gärtner, Homburg/Saar and proficiency testings)
2. sera from donors with past EBV infection (n=41, sera from Dr. Gärtner, Homburg/Saar)

Immunoblots and/or IFA were used as reference tests.

Sera collective (n=61)		**VT EBV ELISA IgG + IgM overall result**	
		Primary Infection	**Past Infection**
Finding	**Primary Infection**	20	0
	Past Infection	0	41

The results of the VIROTECH EBV ELISA correspond with the reference findings in all cases.

10.3 Specificity

Following sera collective was tested to determine the specificity:
20 seronegative sera (sera from Dr. Gärtner, Homburg/Saar).
Immunoblots and/or IFA were used as reference tests.

Sera collective (n=20)		VT EBV ELISA IgG + IgM overall result	
		Primary Infection	**Seronegative**
Finding	**Seronegative**	20	0
	Past Infection	0	0

The results of the VIROTECH EBV ELISA correspond with the reference findings in all cases.

10.4 Prevalence (Expected Values)

77 sera were tested to determine the prevelance rate of 95% in adults (past EBV infection) as described in literature (10).

Seracollektive (n=77)	EBV ELISA	
	IgG	**IgM**
Positive	75	0
Borderline	0	2
Negative	2	75

10.5 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 is < 9%.

10.6 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs by different persons in different laboratories.

EBV ELISA IgG

Serum	Average Value VE	Coefficient of Variation
Negative	7,8	9,0%
Positive	12,0	10,9%
Positive	24,2	11,7%

11. Literature

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Preparation of Patient Samples and Washing Solution

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

▼ IgG-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 Buffer +
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
⇩		
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)**