VIROTECH Liquor/CSF
feste Standardkurve/Permanent Standard Curve

VIROTECH Borrelia + VlsE IgG ELISA Order-No.: EC022G00
VIROTECH Borrelia IgM ELISA Order-No.: EC022M00
VIROTECH Borrelia + VlsE IgG Europe Order-No.: EC024G00
VIROTECH CMV IgG/IgM ELISA Order-No.: EC113.00* (only for IgG)
VIROTECH EBV IgG/IgM ELISA Order-No.: EC102.00 (only for IgG)
VIROTECH FSME/TBE IgG/IgM ELISA Order-No.: EC117.00
VIROTECH HSV 1 (gG1) IgG/IgM ELISA Order-No.: EC130.00 (only for IgG)
VIROTECH HSV 2 (gG2) IgG/IgM ELISA Order-No.: EC131.00 (only for IgG)
VIROTECH HSV Screen IgG/IgM ELISA Order-No.: EC108.00 (only for IgG)
VIROTECH Masern/Measles IgG ELISA Order-No.: EC105G00
VIROTECH Masern/Measles IgM ELISA Order-No.: EC105M00
VIROTECH Mumps IgG/IgM ELISA Order-No.: EC106.00 (only for IgG)
VIROTECH Rubella Liquor/CSF IgG ELISA Order-No.: EC109L00
VIROTECH VZV IgG/IgM ELISA Order-No.: EC110.00
VIROTECH VZV IgA-Set Order-No.: EC110.08

FOR IN-VITRO DIAGNOSIS ONLY

Virotech Diagnostics GmbH
Waldstrasse 23 A2
63128 Dietzenbach, Germany

Tel.: +49(0)6074-23698-0
Fax.: +49(0)6074-23698-900
www.goldstandarddiagnostics.com

CE CE
0483

Freigabedatum: 26.04.2022 20:56
REV 17 / VIROTECH Liquor/CSF feste Standardkurve/Permanent Standard curve EN

Contents

1. Intended Use

The fixed OD-values for the single standards are intended to be used to create a calibration curve (permanent standard curve) which is used for detection of CNS-own antibody synthesis by parallel examination of Serum-CSF-pairs. The pathogen-specific quotient of CSF and serum is calculated. The ratio between this pathogen-specific antibody quotient and the total immunoglobulin quotient is called antibody-index (AI).

2. Diagnostic Relevance

During acute infections of the central nervous system (CNS) and also during chronical inflammatory processes (e.g. multiple sclerosis) pathogen-specific antibodies are developed in the CNS. In the first case those are antibodies against the causative pathogen, in the second case a poly-specific intrathecal immune response against possibly several pathogens without actual pathogen presence is possible (1, 2).
Bacterial infections of the CNS are mainly distinguished by highly pathologic and quite characteristic CSF-results. The diagnostic detection of viral CNS infections in cerebral-spinal fluid (CSF) is, depending on stage of infection and individual status of immunity, possible in two ways: by direct antigen detection or by detection of pathogen-specific antibodies. It is well known that culture of viral pathogens – unlike bacterial pathogens - is complicated, alternative pathogen-specific testings are bound to high methodical efforts. The pathogen-specific antibody detection usually takes effect earliest 6 days after onset of the disease, but is routinely used in CSF-diagnosis by now (3).

The antibodies detected in the CSF may either diffuse out of the plasma into the CSF-area or be result of a local synthesis (intrathecal antibody production). The specific antibody-index (AI), describing the relation between the specific antibody quotient and the total immunoglobulin quotient serves for the clarification of a CNS infection. A local antibody-synthesis is present, if the pathogen-specific antibody quotient of a certain antibody-class is bigger than the corresponding total-immunglobulin-quotient (Q_{total}). (Please refer to our CSF diagnostic booklet for further information).

3. Test Principle

The antibody searched for in the human serum and CSF 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.
The extinction (OD) of the color solution is directly proportional to the concentration of the analysed pathogen-specific IgG-, IgM- respectively IgA-antibody in Serum and CSF. For the detection of CNS-own antibody-synthesis it is necessary to proceed a quantification of the antibody concentrations that are available in extinctions initially. This is the intended use of the permanent standard curve. It allows the conversion of determined OD-values to arbitrarily defined non-dimensional measurement units (wME). For this, the arrays of standard sera with graded pathogen-specific antibody-concentration are provided. Those standard sera serve for the creation of a reference curve, that can be made manually or by using a suitable programme and allows the conversion of the detected OD-values into arbitrary defined non-dimensional measurement units (wME). By allocating of the obtained measurement units (wME) with the nephelometrical measured Sera- and CSF-Total-IgG-, IgM- respectively IgA-concentrations the so-called antibody index (AI) is detected (refer to the calculation of the AI in point 8.3). This antibody index mentions the searched pathogen-specific antibody quotient as a multiple respectively as a fraction of the respective Total-Immunoglobuline-Quotient. The value is therefore independent from the condition of the individual cerebral barrier-function. The antibody-index allows the conclusion to the presence and dimension of a CNS-own synthesis of pathogen-specific antibodies.
This method is not valid in case of a poly-specific intrathecal immunoglobuline-synthesis, as then the toal-IgX-quotient is no longer suitable as barrier parameter and has to be replaced by the so called Limes value (refer to calculation of the Limes Quotient in point 8.3.4 B).

4. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.
Please follow the user manual of the corresponding serum ELISA test kit!

4.1 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.

Consider the following for the serum samples:

The samples can be stored for 1 week at 2-8°C.

Always prepare patient-dilution freshly. Maximum shelf life 6h at 2-8°C

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).

Consider the following for the CSF samples

The samples can be stored for 1 week at 2-8°C.

Always prepare patient-dilution freshly. Maximum shelf life 6h at 2-8°C

If the CSF samples are to be stored for an extended period, it is best to aliquot them and then to freeze them at -80°C. This avoids repeated thawing.

1. Vein- and lumbar puncture should always be performed at approx. the same time.
2. Only optically clear and uncelled and not inactivated CSF may be used.
3. Do not use haemolytic or microbiologically contaminated or turbid CSF.
4. The use of deepfrozen CSF is possible if after thawing the conditions of items 2 and 3 are fulfilled.

4.2 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 permanent standard curve is parameter-specific and must only be used with the plate lot numbers it is assigned to. The quality control certificate assigned to the permanent CSF-standard curve gives notice on the allowed combinations of plates- and standard curves.

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. **IgM-Diagnostic: Pre-absorbance with RF-SorboTech**
   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 and CSF with RF-SorboTech** (VIROTECH adsorbent).

4.3 VIROTECH ELISA Test Procedure

- CSF/serumpairs are principally to be analysed next to each other in the same determination row on one test plate
- We recommend a double insertion for blank, controls, patient sera and CSF-samples.
- To minimize matrix effects as much as possible, a working-dilution of 1:2 for CSF and 1:404 for serum is used. It is recommended for IgM Diagnostics in general that a start be made with a dilution of 1:101 followed, if necessary, (- the 100wME measurement point exceeded) by a 1:404 dilution. In the case of IgG, IgM and IgA diagnostic testing, the use of two dilutions is generally recommended for CSF and serum, e.g. CSF 1:2 and 1:4; serum 1:101 and 1:404, in order to rule out testing with antibody excess.
- For the IgM-diagnostic please consider the pre-absorbance with RF-SorboTech.

1. For each test run pipette **100µl** each of the **ready to use dilution buffer** (blank) the **diluted CSF**-samples and the **sera**.
   Working dilutions of the serum samples:
   IgG: 1:404; (e.g. 5µl serum + 500µl dilution buffer (1:101 dilution), then dilution 1:4, e.g. 100µl of 1:101 dilution + 300µl

dilution buffer).
IgM: 1:101; (e.g. 10µl serum + 1ml dilution buffer/RF-SorboTech.)
IgA: 1:404; (e.g. 5µl serum + 500µl dilution buffer (1:101 dilution), then dilution 1:4, e.g. 100µl of 1:101 dilution + 300µl dilution buffer).
Working dilution of **CSF: 1:2;** e.g. **150µl CSF + 150µl 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 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

4.4 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.

5. Test Evaluation

5.1 Test function control

To ensure the optimal function of the testkit, you have to score the ranges of the AI- cotrols in the instruction sheet.

Otherwise (without the AI-controls), you have to verify the test run with the serum quality – controls.

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls shall be below the OD-values mentioned in the quality control certificate; the OD-values of the positive controls and of the cut-off controls shall be above the OD-values mentioned in the quality control certificate.

b) VIROTECH Units

The VIROTECH Units (VE) of the cut-off control are defined as 10 VE. The calculated VE of the positive controls must meet the ranges of the quality control certificate.

The test has to be repeated in case those pre-given requirements are not fulfilled (OD-values, VE).

5.2 Interpretation

A reference curve is created either manually or instrumentally using the fixed IgG-, IgM- resp. IgA OD-values provided by VIROTECH Diagnostics. Thus, the OD-values of the single standards (refer to the certificate of the permanent CSF-standard

curve) have to be entered onto the ordinate (y-axis) and the corresponding antibody-concentrations in wME (arbitrary measurement units) have to be entered onto the abscissa (x-axis).
The OD-values of the Serum-CSF-Pairs may now be expressed in wME by simply looking to the curve and correspond after multiplication with the dilution factors to the concentrations of the pathogen-specific IgG-, IgM- respectively IgA-antibody in serum and CSF. To obtain plausible antibody indices, OD-values below 0,05 and wME-values below 1,5 or above 100 shall not be considered for the calculation. For OD-values, that lead to values above 100wME, a higher serum dilution than 1:404 and a higher CSF-dilution than 1:2 may be used considering the changed dilution ratio. During realisation of the CSF-diagnostic, a judgement of the 1:404 diluted patient-serum is impossible in the sense of an exceeding or fall short cut-off.

5.3 Calculation of the antibody index AI (with example)

Abbreviations:

IgX_{total} =Total IgX (IgG, IgM or IgA, mg/l) $IgX_{spec.}$ = pathogen-specific IgX (IgG, IgM or IgA)

Q = Quotient

Q_{alb} = Quotient resulting from the albumin content of CSF and albumin content of the serum (mg/l)/only necessary for calculation of Limes value!

5.3.1 $QIgX_{spec}$ (pathogen-specific antibody quotient)

Serum
- OD-values read: 0,700
- thus established concentration from the reference curve: 3,5 wME
- dilution: 1:400

CSF
- OD-values read: 0,500
- thus established concentration from the reference curve: 2,5 wME
- dilution: 1:2

$$Q\,IgX_{spec.} = \frac{IgX_{spec.\,CSF}\,(wME) \times dilution}{IgX_{spec.\,Serum}\,(wME) \times dilution} = \frac{2{,}5 wME \times 2}{3{,}5 wME \times 400} = 3{,}6 \times 10^{-3}$$

5.3.2 Q_{IgX} (Total Immunoglobulin Value: Value of the clinical chemistry)

- IgX_{CSF}= 33mg/l
- IgX_{Serum}= 10000mg/l

$$Q\,IgX_{total} = \frac{IgX_{total\,CSF}}{IgX_{total\,Serum}} = \frac{33 mg/l}{10.000 mg/l} = 3{,}3 \times 10^{-3}$$

5.3.3 Calculation of the Limes Quotient (Q_{LIM})

In case of an additional poly-specific intrathecal immunoglobulin synthesis, the Total-IgX-Quotient for the AI-determination is no longer suitable. The so called Q_{LIM} has to be used instead of the Total-IgX-Quotient. Therefore it is necessary to determine the albumin quotient additionally (value of the clinical chemistry).

Calculation of the LIMES-Value (according to Reiber):

$$Q_{LIM\text{-}IgG} = 0{,}93 \times \sqrt{Q_{alb}^{\,2} + 6 \times 10^{-6}} - 1{,}7 \times 10^{-3}$$

$$Q_{LIM\text{-}IgM} = 0{,}67 \times \sqrt{Q_{alb}^{\,2} + 120 \times 10^{-6}} - 7{,}1 \times 10^{-3}$$

$$Q_{LIM\text{-}IgA} = 0{,}77 \times \sqrt{Q_{alb}^{\,2} + 23 \times 10^{-6}} - 3{,}1 \times 10^{-3}$$

5.3.4 Calculation of the Antibody Index (AI)

A. $Q_{IgX} < Q_{LIM}$

The antibody index (AI) states the relation between the pathogen-specific antibody quotient (Q_{spec}) and the total-immunoglobulin-quotient (Q_{total}). Thus, a pathogen-specific antibody-synthesis can be detected and quantified. In this case, the total-immunoglobulin-quotient is used as barrier-parameter.

$$AI = \frac{Q\,IgX_{spec.}}{Q\,IgX_{total}} = \frac{\frac{IgX_{spec.CSF} \times dilution}{IgX_{spec.Serum} \times dilution}}{\frac{IgX_{total\,CSF}}{IgX_{total\,Serum}}} = \frac{3{,}6x10^{-3}}{3{,}3x10^{-3}} = 1{,}1$$

B. $Q_{IgX} > Q_{LIM}$

In case an additional poly-specific intrathecal immunoglobulin-synthesis is present the measured Q_{total} must no longer be used for the AI-calculation as an antibody synthesis searched for or eventually present at the same time may be falsified in its extent or even get totally unrecognizable. In these cases the so called Limes value of the immunoglobulin quotient is calculated (see formula) or graphically determined by using the albumin quotient which has to be calculated additionally. This Limes value is used instead of the immunoglobulin quotient for calculation of AI-value.

$$AI = \frac{Q\,IgX_{spec.}}{Q_{Lim}}$$

5.4 Interpretation

AI –Evaluations (4):		
AI: < 0.6	implausible	Should not theoretically occur. Occasionally found in routine work. No pathological significance. Desirable to search for errors.
AI: 0.6 – 1.3	normal:	Intrathecale antibody production is improbable.
AI: 1.4 – 1.5	borderline:	It is recommended that the sample should be retested, or that a later serum-CSF pair should be tested.
AI: >1.5	pathological:	Indicates intrathecal antibody production.

1. Since a minimum of four different results (pathogen-specific CSF- and serum-antibody measurement-units, total serum- and CSF-IgG-, IgM- or IgA-value, CSF- and serum-albumine in mg/l) are considered for the calculation of the diagnostical relevant AI-value, all methodic and coincidental errors add up here. In the most unfavourable case, a continuing mistake in the same sense is possible; a double determination or better the measuring of two different sample dilutions are the best way to recognize this. For this reason, a clinically relevant limited AI value of 1,5 has established as note for a local synthesis of pathogen-specific antibodies in the CSF.
2. Normally for virusspecific antibodies of the IgG-, IgM- or IgA-class there is the same ratio between CSF and serum as it is found for the summarised IgG-, IgM- or IgA-fraction. The theoretically expected AI-value is therefore 1,0. Corresponding tests have shown, that for all virus-specific antibodies a reference-range of 0,6 – 1,5 is valid. AI-values above 1,5 may be considered as pathologic in case of sufficient analytical quality of all incoming single values and may be characterized by a CNS-own synthesis of the corresponding virus-specific antibodies.
3. AI-values below 0,6 are theoretically impossible and do normally point out an analytical mistake.
4. Only high AI-values without corresponding clinical reference do not allow a definite conclusion for an acute stage of an infectious CNS-disease. Long-time persisting and poly-specific CNS-own antibody-synthesis in particular of the IgG-class but also of the IgM-class are possible. IgM-AI-increases are usually considered as prove for florid CNS-infections. In case of doubt, the significant change of the AI-value from a second determination, similar to titer motion, is of advantage for the

judgement of an infection of the central nervous system. Such a control is mandatorily bound to a further CSF-withdrawel, taken in an adequate time interval. However, for its indication only the clinical aspects are decisive, as a rule.

5.5 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. In case of very high pathogen-specific antibody concentrations in the cerebrospinal fluid or in the serum, a risk that the antigen-concentration in the wells is insufficient to fulfill the optimum conditions for a quantitative antibody detection is present. If an antibody-excess is suspected (please consider also Heidelberg curve and total CSF-result) a second determination with higher dilution of serum respectively CSF has to follow.

Notice the detailed performance data (sensitivity and specificity) for Borrelia – and CMV – CSF diagnostic in the regular instruction sheet in addition to the serology.

6. Literature

1. Zimmermann K., Liquordiagnostik, MTA 11 (1996)4 ; 258 - 260
2. Reiber H, Lange P., Virus-spezifische Antikörper in Liquor und Serum. ELISA-Analytik und Auswertung mittels Antikörper-Index und Quotientendiagramm, Lab.med. 15: 204 (1991) 204 - 207
3. Linke E, Zimmermann K: Liquordiagnostik; hauseigene Liquorbroschüre 2003
4. Petereit, Sindern, Wick (2007): Leitlinien der Liquordiagnostik und Methodenkatalog der Deutschen Gesellschaft für Liquordiagnostik und Klinische Neurochemie, Springer Verlag, ISBN 978-3-540-39017-6

Preparation of the Patient Samples and Washing Solution

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

▼ IgG/IgA-Samples – Dilution 1:404

▼ CSF-Dilution 1:2

e.g.:
1:101: 5 µl serum/plasma + 500 µl dilution buffer
1:404: 100 µl serum diluted 1:101 +300 µl dilution buffer

150 µl CSF-sample + 150 µl Dilution Buffer

▼ IgM-Samples – Dilution 1:101/1:404

▼ CSF-Dilution 1:2

Rheumafactor-absorption with RF-SorboTech

e.g.:
1:101: 5 µl serum/plasma + 450 µl dilution buffer + 1 drop RF-SorboTech
Incubate at RT for 15 min
1:404: 100 µl serum/VP/RF-SorboTech mixture +300 µl dilution buffer

50 µl RF-SorboTech + 200 µl Dilution Buffer
225 µl RF-SorboTech-buffer mixture + 225 µl CSF-sample
Incubate at room temperature for 15 min

Testprocedure

Samples Incubation	**30 Minutes at 37°C**	**100 µl Patient Samples** blank (Dilution Buffer)
⇩		
Wash 4 times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 Minutes at 37°C**	**100 µl Conjugate** IgG, IgM, IgA
⇩		
Wash 4 times		**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)**