CSF Diagnosis

by Immunoblot

VIROTECH Borrelia in vivo IgG LINE Immunoblot

(Borrelia in vivo IgG LINE-32; Borrelia in vivo IgG LINE-96)

Catalog no.: WE222G32; Catalog no.: WE222G96

VIROTECH Borrelia in vivo IgM LINE Immunoblot

(Borrelia in vivo IgM LINE-32; Borrelia in vivo IgM LINE-96)

Catalog no.: WE222M32; Catalog no.: WE222M96

VIROTECH Borrelia in vivo + TpN17 IgG LINE Immunoblot

(Borrelia in vivo + TpN17 IgG LINE-32; Borrelia in vivo + TpN17 IgG LINE-96)

Catalog no.: WE223G32; Catalog no.: WE223G96

VIROTECH Borrelia Europe IgG LINE Immunoblot

(Borrelia EU IgG LINE-32; Borrelia EU IgG LINE-96)

Catalog no.: WE224G32; Catalog no.: WE224G96

VIROTECH Borrelia Europe IgM LINE Immunoblot

(Borrelia EU IgM LINE-32; Borrelia EU IgM LINE-96)

Catalog no.: WE224M32; Catalog no.: WE224M96

VIROTECH Borrelia Europe + TpN17 IgG LINE Immunoblot

(Borrelia EU + TpN17 IgG LINE-32; Borrelia EU + TpN17 IgG LINE-96)

Catalog no.: WE225G32; Catalog no.: WE225G96

FOR IN VITRO DIAGNOSIS ONLY

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1. Uses

The IgG/IgM LINE Immunoblots are not only suitable for the serodiagnosis of Lyme borreliosis, but also for the diagnosis of neuroborreliosis in CSF [cerebrospinal fluid]. Together with prior determination of the AI by ELISA, they serve as an additional approach to determine the endogenous (intrathecal) synthesis of pathogen-specific antibodies to Borrelia burgdorferi sensu lato, and thus help to support the diagnosis of neuroborreliosis. This is particularly useful in unclear cases, in which the antibody response is (still) low (method related). The Immunoblot can then give a pathological result earlier.
The condition for the diagnostic use of these diagnostic test systems is that the total concentrations of IgM (or IgG) in the CSF and serum must be adjusted to be the same for each patient (see point 2.1). Moreover, polyspecific intrathecal synthesis must be considered (see point 2.2).
If the exclusion bands (TpN17-band in IgG and the EBV-VCA in IgM) are neglected, the Borrelia LINEs with differential diagnosis are also suitable for use in CSF diagnostic testing.

2. Test procedure

2.1 Test material and preparation of the reagents

No additional components are needed for performing the test procedure.

The recommended total IgG/IgM concentration (IgG/IgM $_{ges.}$) of the CSF and serum samples are 30 mg/l for IgG and 0.5 mg/l for IgM. Higher total IgG/IgM concentrations must be diluted appropriately.
The detection limit for the CSF Line test is 10 mg/l for IgG.
At a protein content < 30 mg/l and > 10 mg/l limited conclusions can be drawn in a negative case as the protein content is reduced. Intrathecal specific AB production cannot be ruled out in this case.
The recommended 30 mg/l should be regarded as an optimal dilution concentration as oversaturation is also avoided by this setting.
The same applies for IgM. In this case, a lower detection limit of 0.2 mg/l was established.

The CSF and serum are diluted with dilution/ washing buffer. The dilution factor (DF) is calculated as follows:

$$\textbf{DF (CSF)} = \frac{\text{IgG}_{\text{tot.}}\ \text{CSF (mg/l)}}{30\ \text{(mg/l)}}$$

$$\textbf{DF (CSF)} = \frac{\text{IgM}_{\text{tot.}}\ \text{CSF (mg/l)}}{\text{O.5 (mg/l)}}$$

$$\textbf{DF (Serum)} = \frac{\text{IgG}_{\text{tot.}}\ \text{Serum (mg/l)}}{30\ \text{(mg/l)}}$$

$$\textbf{DF (Serum)} = \frac{\text{IgM}_{\text{tot.}}\ \text{Serum (mg/l)}}{\text{O.5 (mg/l)}}$$

Example: IgG

$$\textbf{DF (CSF)} = \frac{60\ \text{mg/l}}{30\ \text{(mg/l)}} = 2$$

= CSF dilution: 1:2

$$\textbf{DF (Serum)} = \frac{27{,}000\ \text{mg/l}}{30\ \text{(mg/l)}} = 900$$

= Serum dilution: 1:900

Example: IgM

$$\textbf{DF (CSF)} = \frac{3.00\ \text{mg/l}}{0.5\ \text{(mg/l)}} = 6$$

= CSF dilution: 1:6

$$\textbf{DF (Serum)} = \frac{750\ \text{mg/l}}{0.5\ \text{(mg/l)}} = 1500$$

= Serum dilution 1:1500

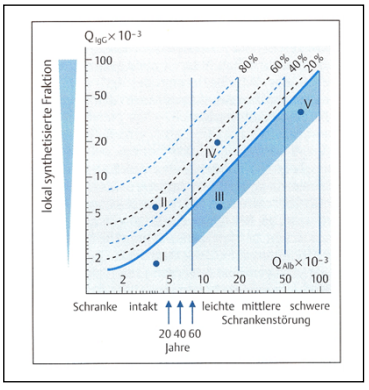
2.2 Correcting for polyspecific intrathecal IgG/IgM synthesis

If polyspecific intrathecal IgG/IgM synthesis has been demonstrated, the total IgG/IgM measured in CSF may not be used for the calculation of the dilution factor.

In such cases, the total IgG/IgM concentration may only be used for calculation of the dilution factor after subtraction of the portion of the IgG/IgM produced intrathecally.

The percentage local IgG/IgM synthesis can be read off from the REIBER quotient diagram. The absolute portion can be calculated from the following formula: IgX (loc)= (QIgX – Qlim(IgX)) x IgX Serum (mg/l)

Example: Reiber Diagram for IgG



Legend for IgG

I Normal findings

II Intrathecal IgG synthesis with intact blood-brain barrier

III Mild barrier impairment (e.g. viral meningitis)

IV Intrathecal IgG synthesis (50%) with mild barrier impairment (e.g. MS)

V Severe barrier impairment (e.g. purulent meningitis)

Example:

Total IgG concentration in CSF (IgG $_{tot.}$ Liquor) is 60mg/l and the local IgG synthesis (intrathecally produced IgG portion) is 40% (according to the Reiber diagram):

$$\text{intrathecally produced IgG portion (mg/l)} = \frac{60 \text{ mg/l} \times 40\%}{100\%} = 24 \text{ mg/l}$$

$$\text{DF (CSF)} = \frac{\text{IgG}_{tot.}\text{ CSF (mg/l)} - \text{intrathecally produced IgG portion (mg/l)}}{30 \text{ (mg/l)}} = \frac{60 \text{ mg/l} - 24 \text{ mg/l}}{30 \text{ mg/l}} = 1{,}2$$

I.e., in this case, the CSF must be diluted 1: 1.2 (e.g. 1.5 ml CSF+ 0.3 ml VP)

The total IgM concentration in CSF (IgM $_{tot.}$ CSF) is 3.5 mg/l and the portion of local IgM synthesis (intrathecally produced IgM portion) is 30% (Reiber diagram):

$$\text{intrathecally produced IgM portion (mg/l)} = \frac{3.5 \text{ mg/l} \times 30\%}{100\%} = 1.05 \text{ mg/l}$$

$$\text{DF (CSF)} = \frac{\text{IgM}_{tot.}\text{ CSF(mg/l)} - \text{intrathecally produced IgM portion (mg/l)}}{0.5 \text{ (mg/l)}} = \frac{3.5 \text{ mg/l} - 1.05 \text{ mg/l}}{0.5 \text{ mg/l}} = 4.9$$

I.e., in this case, the CSF must be diluted 1: 4.9 (e.g. 1 ml CSF + 3.9 ml VP or 0.5 ml CSF + 1.95 ml VP)

2.3 Immunoblot test procedure

For the subsequent procedure, please refer to the instructions for serology of the Immunoblots VIROTECH Borrelia in vivo IgG/IgM LINE and VIROTECH Borrelia Europe IgG/IgM LINE (Chapter 8. Test Procedure).
See too Point 8 Test Procedure for CSF-Serum Pairs in abbreviated form at the end of this test instruction.
The Immunoblot- strips for testing a serum-CSF pair must come from the same blot booklet. If possible, sequential strips should be taken for a serum-CSF pair and both must be processed within a single test run.
In modification to the usual LINE test procedure, the **incubation time for serum and CSF** should be increased to **16 hours.** To prevent evaporation during this period, the incubation tray should be covered with parafilm. All other steps are identical to the normal test procedure of VIROTECH Immunoblots. During pipetting and decantation, care must be taken that there is no cross-contamination of the patient samples.
In an internal comparison test, it was shown that a shortened incubation period of 30 min is possible for serum-CSF pairs (emergency diagnosis), but the band spectrum is smaller and the band intensity is lower. A conclusion is possible only if intrathecal AB production is detectable even after this short incubation (e.g. with high-titre sera). In a negative case, a reliable conclusion is not possible and should be verified with a 16-hour incubation period. In general, a 16-hour incubation period is recommended as standard.

3. Test evaluation

The Immunoblot strips are evaluated purely visually. This is done by comparing the Borrelia-specific bands of the CSF strip with those of the serum strip.

There is **intrathecal synthesis** of Borrelia-specific antibody, **if**:

- One or several bands visible only on the CSF strip, but not on the serum strip.
- One or several bands on the CSF strip clearly more intensely visible than on the serum strip.

4. Test limits

In some patients, the ELISA result is normal (AI normal or not calculable), although neuroborreliosis is still suspected, because of continuing clinical symptoms. In these cases, the Immunoblot may be used to confirm the diagnosis of neuroborreliosis or to complement the available results.
Additional testing with the Immunoblot is recommended for all patients for whom the diagnosis of neuroborreliosis is uncertain. Nevertheless, even a negative LINE Blot result does not reliably exclude neuroborreliosis in every case. In general, the CSF diagnostic Immunoblot results should not be regarded as final and conclusive. Because of the therapeutic consequences, they should only be evaluated together with the clinical status.
The TpN-17 band in IgG and the EBV-VCA band in IgM have not been validated for use in CSF diagnostic testing.

5. IgG performance data of the VIRTOECH Borrelia in vivo IgG LINE Immunoblot / VIROTECH Borrelia in vivo TpN17 IgG LINE Immunoblot

5.1 Diagnostic sensitivity

To determine the diagnostic sensitivity, clinically characterised serum-CSF 77 pairs from patients with confirmed neuroborreliosis were tested in the VIROTECH Borrelia LINE IgG.

Serum-CSF pairs (n=77)		**Borrelia in vivo LINE IgG**		
		Negative	**Borderline**	**Positive**
Diagnostic Findings	**Neuroborreliosis**	6	0	71

Relative to the diagnostic findings, the diagnostic sensitivity for IgG was calculated as 92.2%.

5.2 Sensitivity

To determine the sensitivity, 77 serum-CSF pairs were tested in comparison to a reference ELISA (findings).

Serum-CSF pairs (n=77)		Borrelia in vivo LINE IgG	
		Negative	**Positive**
Findings (ELISA)	**Negative**	0	0
	Positive	6	71

The sensitivity for IgG is calculated to be 92.2 %.

5.3 Specificity

To determine the specificity, 31 serum-CSF pairs were tested in comparison to a reference ELISA (findings).

Serum-CSF pairs(n=31)		Borrelia LINE IgG	
		Negative	**Positive**
Findings (ELISA)	**Negative**	26	5
	Positive	0	0

Relative to the findings, the specificity for IgG was calculated as 83.9%.

6. IgG performance data of the VIRTOECH Borrelia Europe IgG LINE Immunoblot / VIROTECH Borrelia Europe TpN17 IgG LINE Immunoblot

6.1 Diagnostic sensitivity

To determine diagnostic sensitivity, 15 clinically characterised CSF/serum pairs with established neuroborreliosis were tested in the Borrelia Europe LINE IgG.

Serum-CSF pairs (n=15)		Borrelia Europe LINE IgG		
		Negative	**Borderline**	**Positive**
Diagnostic finding	**Neuroborreliosis**	0	0	15

The diagnostic sensitivity for IgG/IgM was therefore calculated as >99.9%.

6.2 Sensitivity

To determine sensitivity, 15 CSF/serum pairs were compared to the Borrelia-LINE (findings) as reference Immunoblot.

Serum-CSF pairs (n=15)		Borrelia Europe LINE IgG	
		Negative	**Positive**
Finding (Immunoblot)	**Negative**	0	0
	Positive	0	15

The sensitivity for IgG/IgM was calculated as >99.9%.

6.3 Specificity

To determine the specificity, 9 serum/CSF pairs with suspected neuroborreliosis were compared with the Borrelia LINE as reference immunoblot (finding).

The serum/CSF pairs give an AI in the normal range in ELISA, which initially failed to confirm the suspicion of neuroborreliosis. In 4 of 9 cases, testing with the Borrelia Europe LINE IgG gave bands in CSF which were enhanced to a greater or lesser extent relative to serum - a finding which can only be explained by intrathecal synthesis of the corresponding antibodies. These 4 serum/CSF pairs were also recognised as pathological with the Borrelia LINE IgG. If the clinical suspicion remains, these findings suggest that a follow-up control should be recommended, as the suspected neuroborreliosis may either have run its course or be in the process of development.

7. IgM Performance data of the VIROTECH Borrelia in vivo IgM LINE Immunoblot/ VIROTECH Borrelia Europe IgM LINE Immunoblot

7.1 Sensitivity

To determine the sensitivity, 9 serum/CSF pairs were tested in comparison with a Borrelia CSF ELISA as reference (finding).

Serum-CSF Pairs (n=9)		**Borrelia in vivo LINE IgM Europe LINE IgM**	
		Negative	**Positive**
Finding (ELISA)	**Negative**	0	0
	Positive	0	9

7.2 Specificity

To determine the specificity, 10 serum/CSF pairs with suspected neuroborreliosis were tested in comparison with ELISA as reference (finding).

These serum/CSF pairs give an AI in the normal range in ELISA. 9 out of 10 evaluated serum/CSF pairs gave a positive finding on testing with the Borrelia LINE, compared with 8 of 10 serum/CSF pairs with the Borrelia Europe LINE. These positive findings could only be explained by accompanying intrathecal synthesis of the corresponding antibodies. If the clinical suspicion remains, these findings suggest that a follow-up control should be recommended,

8. Test Procedure Scheme

Test plan for serum-CSF pairs (short form):

Serum/ CSF incubation	**16 hours (covered)**	1.5 ml of the calculated serum-CSF dilution with the same total IgG/IgM concentration (30 mg/l for IgG, 0.5 mg/l for IgM). (Caution: Allow for polyspecific intrathecal IgG/IgM synthesis!)
Wash	**3 x 5 minutes**	With 1.5 ml dilution and washing buffer
Conjugate incubation	**30 minutes**	With 1.5 ml working dilution (1 + 100)
Wash	**3 x 5 minutes** **1 x 1 minute**	With 1.5 ml dilution and washing buffer With distilled or deionised water
Substrate incubation	**10 ± 3 minutes**	With 1.5 ml substrate solution
Stop	**3 x without interim incubation**	With 1.5 ml distilled or deionised water