

VIROTECH Borrelia Europe IgG LINE Immunoblot

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

Order No.: WE224G32; WE224G96

VIROTECH Borrelia Europe IgM LINE Immunoblot

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

Order No.: WE224M32; WE224M96

VIROTECH Borrelia Europe + TpN17 IgG LINE Immunoblot

(Borrelia EU + Tpn17 IgG LINE-32; Borrelia EU + TpN17 IgG LINE-96)

Order No.: WE225G32; WE225G96

FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

LINE Immunoblot Testkit for the qualitative detection of *Borrelia (B.) burgdorferi* sensu lato specific IgG- respectively IgM-antibodies in human serum.

Aside from its use in the serodiagnosis of Lyme borreliosis, the IgG Line Immunoblot is also suited for the diagnosis of neuroborreliosis in the CSF. Please order separate instructions for the use in serodiagnosis.

2. Diagnostic Meaning

The Lyme-Borreliosis is a systematic disease caused by the infection with the spirochaeta *B. burgdorferi* (40,41). The transmission of the spirochaete to humans is effected by the bite of an infected tick. In Europe the tick *Ixodes ricinus* has been identified as main vector (25). The following human pathogenic *B. burgdorferi* species are currently recognised in Europe. They are comprised under the term *Borrelia burgdorferi* sensu lato (s.l.): *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. spielmanii* and *B. bavariensis* (23, 25, 27, 45, 53, 65).

Lyme Borreliosis is a multisystem disease, which takes its course in stages, with a predominantly involvement of skin, joints and nervous system. Due to the wide spectrum of occurring clinical manifestations, the diagnosis of the Lyme-Borreliosis is difficult (25). Differential-diagnostically meaningful is above others the limitation compared with different dermatological (e.g. B-cell-lymphoma of the skin, Lupus erythematoses), neurological (e.g. multiple sclerosis) and internal (e.g. arthritis, carditis) diseases (33).

The serological diagnostic of the Lyme-Borreliosis is, beside others, complicated by the following factors:

- A negative serology does not exclude a Lyme-Borreliosis – especially not in early stages (20).
- The development of IgM-antibodies may fail to appear entirely.
- IgM-antibodies may persist for months (21,36).
- IgG-antibodies may remain detectable even years after a clinical remission (21,36).
- Cross-reactions to other micro-organisms have been observed (34,35). Bacterial caused diseases like Syphilis as well as Herpes-Virus-Infections (especially EBV) are an important factor here (39). False positive antibody responses may occur also at the presence of autoimmune-antibodies (34).

The challenge of the Lyme-Borreliosis-serology is based on the supportive clarification of a clinical reasonable suspicion. Therefore the Lyme-Borreliosis-serology may give important information about the seronegativity or confirm the suspicion of presence of an acute- as well as an advanced infection. However, a positive antibody result must absolutely be assessed in connection with the clinical picture (20).

In accordance with MIQ 12/2000 and DIN 58969-44 July 2005, it is recommended to perform Lyme borelliosis serology in two steps (7, 73). In the first step the samples are tested with a sensitive screening assay (the MiQ 12/2000 recommends to use an ELISA as screening assay). Borderline and positive sera are examined with a confirmatory test (Line Immunoblot/Western Blot) afterwards. The analysis with the Line Immunoblot/Western Blot enables the specific analysis of the antibody response that is aimed against single pathogen antigens.

3. Principle of Test

Proteins of the pathogen-antigen are transferred to the nitrocellulosis membrane by a micro-dispensing method. The nitrocellulosis membrane is then cut into single strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immunocomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alkaline phosphatase conjugated anti-human IgG- respectively IgM-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violet precipitates at each site („antigen bands“) where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interpret the presence of specific IgG- respectively IgM-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1. IgG resp. IgM Nitrocellulose test strips with applied antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	1x	32 strips
2. IgG resp. IgM Cut off Control, human serum, prediluted	1x	1.0ml
3. Dilution-/ washbuffer , pH 7.3 (10x conc.), with Tris and preservative	2x	50 ml
4. IgG- resp. IgM- Conjugate (100x conc.) Anti-human-(goat)-Alcalic Phosphatasis , with preservative	1x	0.7 ml
5. Substrate (BCIP/NBT), ready to use	1x	57 ml
6. Evaluation Record sheet for the notation and deposit of the results	1x	1 pcs.

4.2 Kit for 96 determinations

1. IgG resp. IgM Nitrocellulose test strips with applied antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	3x	32 strips
2. IgG resp. IgM Cut off Control, human serum, prediluted	2x	1.0ml
3. Dilution-/ washbuffer , pH 7.3 (10x conc.), with Tris and preservative	4x	50 ml
4. IgG- resp. IgM- Conjugate (100x conc.) Anti-human-(goat)-Alcalic Phosphatasis , with preservative	3x	0.7 ml
5. Substrate (BCIP/NBT), ready to use	3x	57 ml
6. Evaluation Record sheet for the notation and deposit of the results	3x	1 pcs.

Also available on request:

IgG or IgM- Positive control, human serum, prediluted, 1.0 ml.

The positive bands > For the cut-off band, refer to the certificate supplied with the kit.

(Order No.: IgG: WE224P60 / WE225P60 or IgM: WE224P80)

IgG/IgM- Negative control, human serum, prediluted, 1.0 ml.

The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.

(Order No.: IgG/IgM: WE224N10 / WE225N10)

5. Storage and Stability

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage or incubation.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. **Nitrocellulose test strips** : Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips and templates are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelflife
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h

Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	or room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic forceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additionally required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic forceps
9. Aqua dest. or deionised water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant. For CSF samples, please refer to the separate instructions for the CSF LINE.

9. Test Procedure

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

9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample. For CSF/serum processing, use only the separate individually calculated CSF / serum dilution for each IgG class (see instructions for the CSF LINE).
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipaemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs and EcoBlots can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality Aqua dest./deionised and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well. Both the concentrated and the dilute dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.
5. **IgG resp. IgM conjugate**
Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**
The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: The antigenstrips must only be tested in the released Ig-class.
(pls. refer to the label on the blot booklet and the marking on each single test strip).

For the correct performance and evaluation of the Borrelia Europe LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

For a secure Borrelia Europe diagnostic the Borrelia Europe LINE shall be proceeded in IgG and IgM.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moistured completely within one minute and can be incubated in supine, lateral position or face-down position.
5. **15 µl patient serum or plasma or 100 µl of the cut-off or positive / negative control** added by pipetting, if at all possible at the upper marked end of the strip. Incubate patient serum and control for **30 minutes** on the rocking platform. Take care that during pipetting and following pour away no cross-contamination of the single patient samples occur.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulosis paper.
7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for **30 minutes** on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with **Aqua dest./deionised**.
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1,5 ml ready to use **substrate solution** each into the channels and allow to develop **10 ± 3 minutes** on the rocking platform.

14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **Aqua dest./deionised** each.
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the included calculation protocol for the interpretation. The inscription of the high-specific band on the protocol sheet make the interpretation of the patient samples easier for you.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot-processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE is fitted out with two controls:

1. **Serum control:**

Only after the incubation with patient serum the serum incubation band appears below the markline.

2. **Conjugate control:**

The LINE strip is fitted out with a conjugate control band which appears after incubation with the respective conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed antigen strip.

Please refer to the protocol sheet for the information of the exact position of the serum- and the conjugate control.

10.1 Interpretation of the patient samples

Please refer to the protocol sheet for position and denotation of reactive bands.

IgM bands: OspC, VlsE-Mix, p39 BmpA, DbpA-Mix and a EBV band for diagnosis by exclusion.

IgG bands: OspC, VlsE-Mix, p39 BmpA, DbpA-Mix or DbpA-PKo, p58, p83 and TpN17 band for exclusion diagnosis (only for WE 225G)

10.2 Usage of the Cut-Off Control

Bands with an intensity weaker than the cut-off band of the cut-off control are not considered for the interpretation.

IgM cut-off band: OspC

IgG cut-off band: VlsE-mix

10.3 Meaning of the Antigens

List of the highly purified and recombinant *Borrelia burgdorferi* antigens, the EBV-Viral Capsid Antigen gp125 and the TpN 17 antigen. VlsE-Mix consists of two recombinant antigens of the genospecies *Borrelia burgdorferi* sensu stricto and *Borrelia garinii*. The DbpA mix consists of the recombinant genospecies *Borrelia garinii*, *Borrelia bavariensis*, *Borrelia spielmanii* and the highly purified *Borrelia afzelii*.

Antigen/ Description	Significance of antigens	Specificity of antibodies in LINE	Original strains/purification
OspC (p23) highly purified	Outer surface protein C. plasmid encoded lipoprotein (6, 22, 26, 28). Important marker for early Lyme Borreliosis manifestations, especially in IgM serology (1, 4, 8, 9, 15, 22, 28, 29, 31, 32). <u>Biological significance:</u>	Specific (3, 8, 22, 28, 30, 31, 32)	<i>B. afzelii</i> PKo (originally isolated from human erythema migrans lesion in Germany) / purified via preparative SDS-Page

	<p><i>B. burgdorferi</i> s. l. presumably requires OspC for a successful initial infection of the mammal host (48, 63, 70, 71). The spirochaetes express OspC during the blood meal in the tick and the early stage of infection of the mammal host (48). After transmission of the spirochaetes to the mammal, the OspC expression is down-regulated again. The lipoprotein does not appear necessary for a persistent infection (48, 63). Tilly et al. presume that OspC prevents phagocytosis of the spirochaetes during the early phase of the infection of the mammal host (64).</p>		
VlsE recombinant	<p>Variable major protein like sequence E. <i>In vivo</i>-expressed lipoprotein, which demonstrates preserved – cross-genospecies – highly immunogenic epitopes. In IgM serology, reactivity to VlsE is observed particularly in the sera of patients with early Lyme Borreliosis. In IgG serology, reactivity to VlsE is observed in the sera of patients with early and advanced Lyme Borreliosis. In IgG serology, VlsE acts as cross-disease stage Lyme Borreliosis marker. VlsE is a 35 kDa antigen encoded on lp28-1 (2). <u>Biological significance:</u> <i>B. burgdorferi</i> s.l. can persist in infected mammals despite their active immune response. It is presumed that the combinational antigen variation of the VlsE surface protein contributes to this persistence – as "immune escape" mechanism (42, 44, 56).</p>	Specific	<p><i>B. burgdorferi</i> B31 (originally isolated from an infected tick on Shelter Island, N. Y.), <i>B. garinii</i> IP90 (originally isolated from an infected tick in Russia) /</p> <p>Purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>
p39 (BmpA) recombinant	<p>Borrelial membrane protein A. Chromosomally encoded (6, 19), central marker in IgG serology for disseminated Lyme Borreliosis infections (4, 8, 18). The Bmp proteins are lipoproteins with unknown function (43, 57, 62).</p>	Highly specific (4, 5, 6, 8, 14, 15, 18, 31, 32)	<p><i>B. afzelii</i> PKo (originally isolated from human erythema migrans lesion in Germany) / purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>
DbpA highly purified (DbpA Pko)/ recombinant (DbpA PBi, PBr, A14 S)	<p>Decorin binding protein A (also outer surface protein 17 or p17). Plasmid-encoded lipoprotein. The DbpAs from various isolates of the human pathogen species, <i>B. burgdorferi</i>, <i>B. afzelii</i>, <i>B. garinii</i>, <i>B. bavariensis</i> and <i>B. spielmanii</i>, were described as sensitive and specific antigens which complement each other in terms of their reactivity (47, 60, 61, 69). They are markers in IgM and IgG serology, particularly for Neuro</p>	Highly specific	<p><i>B. bavariensis</i> PBi and <i>B. garinii</i> PBr (originally isolated from the cerebrospinal fluid of a Neuro Borreliosis patient in Germany), <i>B. spielmanii</i> A14S (originally isolated from an erythema migrans lesion in the Netherlands)/ purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>

	<p>Borrelioses and Lyme Arthritides (50, 52, 57, 58, 59, 60).</p> <p><u>Biological significance:</u></p> <p>Microbial adhesion to the host tissue represents an early, critical step in the pathogenesis of most infectious diseases. The various <i>Borrelia</i> species express two surface-expressed decorin-binding adhesins, DbpA and B, which mediate bonding of the spirochaetes to the extracellular matrix of the host. The <i>Borrelia</i> reach the dermis with the tick's saliva where they bond to collagen fibres, or via the decorin-binding adhesins A and B to the collagen-associated proteoglycan decorin (46, 49, 72).</p>		<p><i>B. afzelii</i> PKo (originally isolated from human erythema migrans lesion in Germany) / purified via preparative SDS-Page</p>
p58 (OppA-2) recombinant	<p>Oligopeptide permease protein A-2 (OppA-2). Chromosomally encoded lipoprotein preserved between the species (54). Important marker in IgG serology for advanced Lyme Borrelioses (47, 51, 57, 67, 68).</p> <p><u>Biological significance:</u></p> <p>OppA is a membrane transporter which possibly plays a role in the adaptation of <i>B. burgdorferi</i> s. l. to the host environment (55, 66).</p>	Highly specific	<p><i>B. bavariensis</i> PBi (originally isolated from the cerebrospinal fluid of a Neuro Borreliosis patient in Germany)</p> <p>Purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>
p83/100 recombinant	<p>Chromosomally encoded, protoplasm cylinder-associated antigen (12, 13), preserved within <i>B. burgdorferi</i> sensu lato (17). Central marker in IgG serology for advanced Lyme Borrelioses (8, 24, 29).</p>	Highly specific (3, 5, 8, 22, 24, 29, 31)	<p><i>B. afzelii</i> PKo (originally isolated from human erythema migrans lesion in Germany) / purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>
EBV VCA-gp125 affinity purified	<p>Immunodominant Epstein Barr "Virus Capsid Antigen". IgM antibodies against VCA-gp125 generally disappear again after a few weeks following an EBV infection.</p>	Highly specific marker in IgM serology for an EBV primary infection	<p>gp125 is purified from whole cell lysate (EBV-infected human cells) via affinity chromatography using a monoclonal anti-gp125 antibody</p>
<i>Treponema pallidum</i> TpN17 recombinant (only for WE225G)	<p>Marker for primary, secondary and latent syphilis</p>	highly specific for all infection stages	<p><i>Treponema pallidum</i> / Purified from <i>E. coli</i> via Ni-NTA affinity chromatography</p>

10.4 Interpretation criteria

The interpretation of the serological result shall always include the clinical picture, epidemiological data and further diagnostical parameter.

Only bands with an intensity \geq cut-off band are considered.

Recommended IgM evaluation¹

Band(s) Observed	Evaluation
At least 2 of the following bands observed: p39 BmpA, OspC (p23), DbpA-Mix, VlsE-Mix or isolated OspC (p23)	Positive
Only one band of: p39 BmpA, DbpA-Mix, VlsE-Mix	Borderline
None of the following bands \geq cut-off band: p39 BmpA, OspC (p23), DbpA-Mix, VlsE-Mix	Negative

Recommended IgG evaluation¹

Band(s) Observed	Evaluation
At least 2 of the following bands observed: p83/100, p58 (OppA-2), p39 BmpA, OspC (p23), DbpA-Mix and/or DbpA-PKo, VlsE-Mix	Positive
Only one band of: p83/100, p58 (OppA-2), p39 BmpA, OspC (p23), DbpA-Mix and/or DbpA-PKo, VlsE-Mix	Borderline
None of the following bands \geq cut-off band: p83/100, p58 (OppA-2), p39 BmpA, OspC (p23), DbpA-Mix and/or DbpA-PKo, VlsE-Mix	Negative

* Both DbpA bands (Mix and PKo) are evaluated together in the IgG as a single band.

Recommended evaluation with positive VCA-gp125 in IgM serology

In the course of primary EBV infections, polyclonal B-cell stimulation can lead to antibody reactivity against *Borrelia burgdorferi* sensu lato antigens. This can lead to false positive Lyme borreliosis findings. To minimise false diagnoses of this sort, the VIROTECH Borrelia Europe IgM LINE Immunoblot contains the Epstein Barr Viral Capsid Antigen gp125. If besides gp125, Borrelia antigens also react (with IgM and/or IgG) with an intensity \geq the IgM cut-off band, the complete EBV status of the

¹ according to MIQ 12/2000 and DIN 58969-44 July 2005 [7,73]

serum should be checked as a precaution (e.g. with the VIROTECH EBV IgG LINE Immunoblot; Order No.: WE102G32/96 and VIROTECH EBV IgM LINE Immunoblot; Order No.: WE102M32/96).

Recommended evaluation of the TpN17 band

The *Treponema pallidum* TpN17 antigen band (only for WE225G)

Crossreactions with other microorganisms are observed in Lyme borreliosis serodiagnosis. Herpes virus infections (particularly EBV) and some bacterial infections, such as syphilis, are important here. Lyme borreliosis MiQ12/2000 recommends the following: "If the screening test for Lyme borreliosis serology is borderline or positive, a syphilis screening test (e.g. TPHA) should be performed, to exclude false positives from crossreacting antibodies against *Treponema*."

The TpN17 band serves to recognise false borderline or positive results in Lyme borreliosis serodiagnosis due to crossreacting antibodies from a *Treponema pallidum* infection (syphilis).

If in the VIROTECH Borrelia Europe + TpN17 IgG LINE Immunoblot, the TpN17 band reacts \geq the IgG cut-off band and, at the same time, Borrelia antigens react in IgM and/or in IgG, the complete syphilis status of the serum should be checked as a precaution (e.g. with VIROTECH *Treponema pallidum* IgG LINE Immunoblot and VIROTECH *Treponema pallidum* IgM LINE Immunoblot WE150).

It is essential to note the following:

- a. Because of limitations in sensitivity and specificity, the TpN-17 band cannot replace complete syphilis differential diagnosis.
- b. A negative TpN17 antigen band does not in principle exclude the possible presence of antibodies to *Treponema pallidum*.
- c. A positive result with the TpN17 antigen band must be confirmed with suitable *Treponema pallidum*-confirmatory tests (e.g.: VIROTECH WE150).
- d. The TpN-17 band is not validated for use in CSF diagnosis.

10.5 Limits of the Test

1. A negative Blot result does not completely exclude the possibility of a Borrelia-infection. The sample may be taken before the occurrence of antibodies, or the antibody titre exists below the detection limit of the test.
2. The treatment of the patients with antibiotics during the early stage of the disease (35, 37) may lead to a suppression of the immune response, so that no anti-*B. burgdorferi*-specific antibodies may be detected.
3. The cross-reaction between *Borrelia* and other spirochaetes may lead to an occurrence of Borrelia-associated bands in the Western Blot, what may lead to a false positive result. Sera of patients with e.g. the following infections may cross-react: Syphilis (*Treponema pallidum*), Framboesie (*Treponema pertenue*), relapsing fever (*Borrelia spei*), Leptospirosis (*Leptospira spec.*) (38). There can also be cross-reactions with herpes viruses (HSV, CMV) and with parvovirus (34, 39). If VIROTECH Borrelia Europe + TpN17 IgG LINE Immunoblot (WE225G) not only exhibits reactivity to Lyme borreliosis antigens, but also to the TpN17 antigen, the comments under 9.4 (recommended evaluation of the TpN17 band) must be considered.
4. Within the context of EBV primary infections it may come to antibody reactivities against *Borrelia burgdorferi* sensu lato antigens due to polyclonal b-cell stimulation (34, 39). If the VIROTECH Borrelia Europe IgM LINE Immunoblot is not only reactive to Borrelia antigens (IgM and/or IgG), but also against EBV-gp125, mononucleosis must be excluded by differential diagnosis.
5. In rare cases patients may show „inverse“-bands (dark background, white bands), these are not to be considered, means the Immunoblot can not be assessed in such cases. The serum should be checked using other serological methods.
6. Please refer also to the listed limits of the test in chapter 2.: "Diagnostic Relevance".

11. Performance Data

11.1 Sensitivity

To determine the sensitivity, clinically characterised groups of sera were tested for IgG and IgM, after prior testing with another LINE as reference method (findings). The following groups of sera were studied: MEM, EM, ECM (n=26), neuroborreliosis (n=14), ACA sera (n=10), Lyme arthritis (n=24).

Sera group (n=74)		Borrelia Europe LINE IgG + IgM		
		negative	borderline	positive
Findings	negative	0	0	2
	borderline	0	0	1
	positive	0	4	67

Relative to the findings, this corresponds to sensitivity of > 99.9%.

11.2 Specificity

To determine the specificity, 60 blood donor sera were tested for IgG and IgM, after prior testing with another Borrelia LINE as reference method (findings).

Sera group (n=60)		Borrelia Europe LINE IgG + IgM		
		negative	borderline	positive
Findings	negative	48	8	1
	borderline	0	1	0
	positive	1	0	1

Relative to the findings, this corresponds to specificity of 98%.

11.3 Diagnostic Sensitivity

To determine the diagnostic sensitivity, clinically characterised sera were tested for IgG and IgM. The following groups of sera were studied: MEM, EM, ECM (n=26), neuroborreliosis (n=14), ACA sera (n=10), Lyme arthritis (n=24).

	Borrelia Europe LINE IgG			Borrelia Europe LINE IgM		
	Sera group (n=74)			Sera group (n=74)		
	negative	borderline	positive	negative	borderline	positive
EM, ECM, MEM	5	12	9	1	4	21
Neuroborreliosis	0	3	11	5	2	7
ACA, Lyme arthritis,	0	0	34	9	3	22

11.4 Diagnostic Sensitivity IgG + IgM Overall

Sera group (n=74)	Borrelia Europe LINE		
	negative	borderline	positive
EM, ECM, MEM	0	4	22
Neuroborreliosis	0	0	14
ACA, Lyme arthritis,	0	0	34

11.5 Cross-reactivity

A total of 52 potentially cross-reactive sera were tested in comparison to a Borrelia Western blot. The table shows the results for the overall evaluation for IgG + IgM.

	Borrelia Western Blot			Borrelia Europe LINE		
	negative	borderline	positive	negative	borderline	positive
Autoimmune (n=22)	15	1	6	15	4	3
Primary EBV infections (n=10)	6	3	1	5	5	0
Syphilis (n=20)	6	3	11	9	2	9

11.6 Prevalence (Expected Values)

To determine the infection rate, 60 blood donor samples were submitted to the IgG and IgM tests. None of these sera was positive in the IgG test. 3.3% were positive in the IgM test.

Serum group (n=60)	Borrelia Europe LINE IgG	Borrelia Europe LINE IgM
negative	56	52
borderline	4	6
positive	0	2

11.7 Intra-Assay Precision (Repeatability)

To determine the repeatability, 30 blood strips of a nitrocellulose were incubated in the IgG test mixture and 30 in the IgM test mixture with a serum that gave weak to strong reactions for the antigen bands.

The bands gave homogenous intensities throughout the nitrocellulose sheet.

11.8 Inter-Assay Precision (Reproducibility)

To determine the reproducibility, 3 sera (one negative and two positive sera) were tested in the IgG and in the IgM tests. The determination was performed by 3 operators in 10 different test mixtures.

The serological predictions were fulfilled in all tests.

12. Additional Performance Data for the TpN17 Band of the WE225G32/G96

12.1 Diagnostic Sensitivity

To determine the diagnostic sensitivity, 64 clinically characterised syphilis sera were tested in IgG. The TpN17 band exhibits sensitivity of 93.7%.

Group of Sera (n=64)	VIROTECH Borrelia Europe + TpN17 IgG LINE Immunoblot
negative	4
borderline	1
positive	59

12.2 Diagnostic Specificity

To determine the diagnostic specificity, 116 clinically characterised Lyme borreliosis sera were tested in IgG. The TpN17 band exhibits specificity of >99.9%.

12.3 Crossreactivity

A total of 79 potentially crossreactive sera (EBV primary infections, autoimmune sera) and 43 pregnancy sera were tested in IgG. The TpN17 band exhibits specificity of >99.9%.

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14. Symbols



=> refer to user manual

15. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	30 minutes	15 µl Patient serum/ plasma/ 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	3 x 5 minutes	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	30 minutes	with 1,5 ml working dilution (1 + 100)
Washing	3 x 5 minutes 1 x 1 minute	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	10 ± 3 minutes	with 1,5 ml ready to use substrate solution each
Stopping	3 x without incubation in between	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	1	2	3	4	5	6	7	8	9	10
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515m	3,03ml	4,545m	6,06ml	7,575m	9,09ml	11,11m	12,12m	14,14m	15,15m

Number of strips	11	12	13	14	15	16	17	18	19	20
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17m	18,18m	20,2ml	21,21m	23,23m	24,24m	26,26m	27,27m	29,29m	30,3ml

Number of strips	21	22	23	24	25	26	27	28	29	30
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	32,32m	33,33m	35,35m	36,36m	38,38m	39,39m	41,41m	42,42m	44,44m	45,45m

Number of strips	31	32	33	34	35	36	37	38	39	40
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47m	48,48m	50,5ml	51,51m	53,53m	54,54m	56,56m	57,57m	59,59m	60,6ml