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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Contents

1. Intended Use

LINE Immunoblot Testkit for the qualitative detection of Borrelia (*B.) burgdorferi* senu 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 neuroborelliosis 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 bit 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-lymphom of the skin, Lupus erythematodes), 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 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 clinicial 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 presense 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 seronegativitity 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 immuncomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis 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:

Borrelia EU IgG LINE Ctrl-Set	WN224K60
Borrelia EU IgM LINE Ctrl-Set	WN224K80
Borrelia EU + TpN17 IgG LINE Ctrl-Set	WN225K60

IgG or IgM	ready-to-use controls	Abbreviation
1,0 ml IgG, or 1,0 ml IgM	neg. ctrl. / negative control, human serum/plasma with protein stabilisers and preservative, ready for use	**NEG**
1,0 ml IgG, or 1,0 ml IgM	Cut off Ctrl. / Cut off control, human serum/plasma with protein stabilisers and preservative, ready for use	**CO**
1,0 ml IgG, or 1,0 ml IgM	pos. Ctrl. / positive control, human serum/plasma with protein stabilisers and preservative, ready-to-use	**POS**

The positive bands > cut off band can be taken from the supplied certificate.
The negative control shows no bands or no bands relevant for the evaluation > Cut off band.

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 foreceps 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 foreceps
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 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-**V**iral **C**apsid **A**ntigen 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	**O**uter **s**urface **p**rotein **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). Biological significance: *B. burgdorferi* 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).	Specific (3, 8, 22, 28, 30, 31, 32)	*B. afzelii* PKo (originally isolated from human erythema migrans lesion in Germany) / purified via preparative SDS-Page
VlsE recombinant	**V**ariable major protein **l**ike **s**equence **E**. *In vivo*-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). Biological significance: *B.burgdorferi* 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).	Specific	*B. burgdorferi* B31 (originally isolated from an infected tick on Shelter Island, N. Y.*), B. garinii* IP90 (originally isolated from an infected tick in Russia) / Purified from *E. coli* via Ni-NTA affinity chromatography
p39 (BmpA) recombinant	**B**orrelial **m**embrane **p**rotein **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).	Highly specific (4, 5, 6, 8, 14, 15, 18, 31, 32)	*B. afzelii* PKo (originally isolated from human erythema migrans lesion in Germany) / purified from *E. coli* via Ni-NTA affinity chromatography
DbpA highly purified (DbpA Pko)/ recombinant (DbpA PBi, PBr, A14 S)	**D**ecorin **b**inding **p**rotein **A** (also outer surface protein 17 or p17). Plasmid-encoded lipoprotein. The DbpAs from various isolates of the human pathogen species, *B. burgdorferi*, *B. afzelii*, *B. garinii, B. bavariensis* and *B. spielmanii,* were described as sensitive and specific antigens which	Highly specific	*B. bavariensis* PBi and *B. garinii* PBr (originally isolated from the cerebrospinal fluid of a Neuro Borreliosis patient in Germany), *B. spielmanii* A14S (originally isolated

	complement each other in terms of their reactivity (47, 60, 61, 69). They are markers in IgM and IgG serology, particularly for Neuro Borrelioses and Lyme Arthritides (50, 52, 57, 58, 59, 60). Biological significance: Microbial adhesion to the host tissue represents an early, critical step in the pathogenesis of most infectious diseases. The various Borrelia 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 Borrelia 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).		from an erythema migrans lesion in the Netherlands)/ purified from *E. coli* via Ni-NTA affinity chromatography *B. afzelii* PKo (originally isolated from human erythema migrans lesion in Germany) / purified via preparative SDS-Page
p58 (OppA-2) recombinant	**O**ligopeptide **p**ermease **p**rotein **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). Biological significance: OppA is a membrane transporter which possibly plays a role in the adaptation of *B. burgdorferi* s. l. to the host environment (55, 66).	Highly specific	*B. bavariensis* PBi (originally isolated from the cerebrospinal fluid of a Neuro Borreliosis patient in Germany) Purified from *E. coli* via Ni-NTA affinity chromatography
p83/100 recombinant	Chromosomally encoded, protoplasm cylinder-associated antigen (12, 13), preserved within *B. burgdorferi* sensu lato (17). Central marker in IgG serology for advanced Lyme Borrelioses (8, 24, 29).	Highly specific (3, 5, 8, 22, 24, 29, 31)	*B. afzelii* PKo (originally isolated from human erythema migrans lesion in Germany) / purified from *E. coli* via Ni-NTA affinity chromatography
EBV VCA-gp125 affinity purified	Immunodominant Epstein Barr "**V**irus **C**apsid **A**ntigen". IgM antibodies against VCA-gp125 generally disappear again after a few weeks following an EBV infection.	Highly specific marker in IgM serology for an EBV primary infection	gp125 is purified from whole cell lysate (EBV-infected human cells) via affinity chromatography using a monoclonal anti-gp125 antibody
Treponema pallidum TpN17 recombinant (only for WE225G)	Marker for primary, secondary and latent syphilis	highly specific for all infection stages	*Treponema pallidum*/ Purified from *E. coli* via Ni-NTA affinity chromatography

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 ≥ cut-off band are considered.

Recommended IgM evaluation[1]

Band(s) Observed	**Evaluation**
At least 2 of the following bands observed:	**Positive**

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

p39 BmpA, OspC (p23), DbpA-Mix, VlsE-Mix **or** isolated OspC (p23)	
Only one band of: p39 BmpA, DbpA-Mix, VlsE-Mix	**Borderline**
None of the following bands ≥ cut-off band: p39 BmpA, OspC (p23), DbpA-Mix, VlsE-Mix	**Negative**

Recommended IgG evaluation[1]

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 ≥ 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 **V**iral **C**apsid **A**ntigen gp125. If besides gp125, Borrelia antigens also react (with IgM and/or IgG) with an intensity ≥ the IgM cut-off band, the complete EBV status of the 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 ≥ 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 diangosis.
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 occurance 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 occurance 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 spez*.), 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 **Sera group (n=74)**	**Borrelia Europe LINE IgM** **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 Borellia 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 predctions 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	1,515ml	3,03ml	4,545ml	6,06ml	7,575ml	9,09ml	11,11ml	12,12ml	14,14ml	15,15ml

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	17,17ml	18,18ml	20,2ml	21,21ml	23,23ml	24,24ml	26,26ml	27,27ml	29,29ml	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	32,32ml	33,33ml	35,35ml	36,36ml	38,38ml	39,39ml	41,41ml	42,42ml	44,44ml	45,45ml

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	47,47ml	48,48ml	50,5ml	51,51ml	53,53ml	54,54ml	56,56ml	57,57ml	59,59ml	60,6ml