VIROTECH Borrelia in vivo IgG LINE Immunoblot

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

Order No.: WE222G32; WE222G96

VIROTECH Borrelia in vivo IgM LINE Immunoblot

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

Order No.: WE222M32; WE222M96

VIROTECH Borrelia in vivo + TpN17 IgG LINE Immunoblot

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

Order No.: WE223G32; WE223G96

FOR IN VITRO DIAGNOSIS ONLY

Gold Standard Diagnostics Frankfurt GmbH
Waldstrasse 23 A6
63128 Dietzenbach, Germany
Tel.: +49 6074 23698-0
Fax: +49 6074 23698-900
Email: info.frankfurt@eu.goldstandarddiagnostics.com
Website: clinical.goldstandarddiagnostics.com

CE

Freigabedatum**18.09.2024 11:22**
REV **33 VIROTECH Borrelia in vivo IgG**/IgM LINE Immunoblot EN

Contents

1. Intended Use 3
2. Diagnostic Meaning 3
3. Principle of Test 3
4. Package Contents 4
4.1 Kit for 32 determinations 4
4.2 Kit for 96 determinations 4
5. Storage and Stability 4
6. Precautions and Warnings 5
7. Additionally required material (not supplied) 5
8. Examination Material 5
9. Test Procedure 5
9.1 Preparation of Samples 5
9.2 Preparation of Reagents 5
9.3 Immunoblot Test Procedure 6
9.4 Use of Immunoblot-processors 7
10. Interpretation of Results 7
10.1 Interpretation of the patient samples 7
10.2 Usage of the Cut-Off Control 7
10.3 Meaning of the Antigens 7
10.4 Interpretation criteria 9
10.5 Limits of the Test 11
11. Performance Data 11
11.1 Sensitivity 11
11.2 Specificity 11
11.3 Diagnostical Sensitivity 11
11.4 Diagnostical Specificity 12
11.5 Cross-Reactivity 12
11.6 Prevalence (Expected Values) 12
11.7 Intra-Assay-Precision (Repeatability) 12
11.8 Inter-Assay-Precision (Reproducibility) 12
12. Additional Performance Data for the TpN17 Band of the WE223G32/G96 13
12.1 Diagnostic Sensitivity 13
12.2 Diagnostic Specificity 13
12.3 Crossreactivity 13
13. References 13
14. Symbols 15
15. Test Procedure Scheme 16

1. Intended Use

LINE Immunoblot Testkit for the qualitative detection of *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 *Borrelia 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 species are related to *Borrelia burgdorferi* and are known to be human pathogens in Europe: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii, Borrelia spielmanii* and *Borrelia bavariensis* (23, 25, 27, 42, 43, 44). They are referred to collectively as *Borrelia burgdorferi* sensu lato (s.l.).

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.
- 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 and 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 that Lyme borreliosis serology should be performed in two steps (7, 45). 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

Pathogen antigens are transferred to a nitrocellulose membrane using a special spraying procedure. 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 alkaline phosphatases conjugated anti-human IgG- or 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**	1,0 ml
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**	1,0 ml
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, 0.5 ml.
The positive bands ≥ For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE222P60 / WE223P60 or IgM: WE222P80)

IgG/IgM- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. ≥ Cut-off band.
(Order No.: IgG/IgM: WE222N10 or WE223N60)

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 safely 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 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

Precise adherence to the 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 diluted 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 in vivo LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

For a secure Borrelia diagnostic the 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 and one EBV band for exclusion diagnostic
IgG bands: VlsE-Mix, p39, p83/100, (iv1), (iv2), (iv3), (iv4) and TpN17 band for exclusion diagnostic testing (only with WE 223G)

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 (OspC) and recombinant (VlsE, p83/100, p39, BBA36, BBO323, Crasp3 and pG) *Borrelia burgdorferi* antigens, the EBV **V**iral **C**apsid **A**ntigen gp125 and the TpN17 antigen. The VlsE-Mix consists of two recombinant antigens of the genospecies *Borrelia burgdorferi* s. s. and Borrelia garinii.

Antigen/ Description	**Significance of antigens**	**Specificity of antibodies in LINE**	**Original strains/purification**
OspC (p23), purified native antigen	**O**uter **s**urface **p**rotein **C**. plasmid encoded lipoprotein (6, 22, 26, 28). Important marker for early Lyme Borreliosis manifestations 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 (46, 47, 48, 49). The spirochaetes express OspC during the blood meal in the tick and the early stage of infection of the mammal host (46). 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 (47, 47). Tilly et al. presume that OspC prevents phagocytosis of the spirochaetes during the early phase of the infection of the mammal host (50).	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 *B. burgdorferi* 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 (51, 52, 53).	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.	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
p83/100, recombinant	Chromosomally encoded, protoplasm cylinder-associated antigen (12, 13), preserved within *B. burgdorferi* s. l. (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
BBA36 (iv1)*, recombinant	*In vivo expressed* 22 kDa *B. burgdorferi* antigen encoded on lp54. BBA36 demonstrates preserved – cross-genospecies – highly immunogenic epitopes. BBA36 is an important marker for advanced Lyme Borrelioses (disseminated infections) in IgG serology (10).	Highly specific	*B. afzelii* MMS (originally isolated from an infected tick from Germany) /purified from *E. coli* via Ni-NTA affinity chromatography
BBO323 (iv2)*, recombinant	*In vivo expressed 4*2 kDa *B. burgdorferi* antigen encoded chromosomally. BBO323 demonstrates preserved – cross-genospecies – highly immunogenic epitopes. BB=323 is an important marker for advanced Lyme Borrelioses (disseminated infections) in IgG serology. (54)	Specific	*B. burgdorferi* ZS7 (originally isolated from an infected tick from Germany) /purified from *E. coli* via Ni-NTA affinity chromatography
Crasp3 (iv3)*, recombinant	**C**omplement **r**egulator-**a**cquiring **s**urface **p**rotein3. *In vivo expressed* 21 kDa *B. burgdorferi* surface antigen encoded on cp32-8. Member of the Erp family. Important marker for advanced Lyme Borrelioses (disseminated infections) in IgG serology. Crasp3 supports complement resistance (11, 54).	Highly specific	*B. burgdorferi* ZS7 (originally isolated from an infected tick from Germany) /purified from *E. coli* via Ni-NTA affinity chromatography

pG (iv4)*, recombinant	*In vivo expressed* 22 kDa *B. burgdorferi* antigen encoded on cp32-3. Member of the Erp family. Important marker for advanced Lyme Borrelioses (disseminated infections) in IgG serology (16).	Highly specific	*B. burgdorferi* ZS7/ *B. afzelii* MMS (originally isolated from an infected tick from Germany) /purified from *E. coli* via Ni-NTA affinity chromatography
EBV VCA-gp125	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 WE223G)	Marker for primary, secondary and latent syphilis	highly specific for all infection stages	*Treponema pallidum/* Purified from *E. coli* via Ni-NTA affinity chromatography

*(iv1-4) = antigens expressed in vivo (iv)

10.4 Interpretation criteria

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

Recommended overall assessment (IgG and IgM) of Borrelia antigens
For a secure Borrelia diagnostic the LINE shall be proceeded and interpreted in IgG and IgM.

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

Occured band(s) in IgM		**Occured band(s) in IgG**	**Interpretation**
No bands respectively bands < cut off	**or**	No bands respectively bands < cut off or 1 IgG-band (except VlsE)	**negative**
1 IgM-band (except OspC)	**or**	VlsE IgG-band	**borderline**
OspC IgM-band **or** ≥ 2 IgM-bands	**or**	≥ 2 IgG-bands	**positive**
1 IgM-band	**and**	1 IgG-band	**positive (*)**

(*) The constellation of the bands in the last grey row shows the combination of only one band in IgM plus one band in IgG. The total result (IgG and IgM) has to be considered positive.

Recommended interpretation with positive EBV-gp125 in IgM-serology

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 (55). This can give rise to a false-positive result for Lyme borreliosis. To minimise faulty diagnoses of this sort, VIROTECH Borrelia in vivo IgM LINE Immunoblot contains the Epstein Barr **V**iral **C**apsid **A**ntigen gp125. If in IgM and/or IgG serology not only Borrelia antigens, but also gp125 reacts with an intensity of at least the value of the IgM cut-off band, the complete EBV status of the serum should be checked as a precaution (e.g. with VIROTECH EBV IgG LINE Immunoblot; Order No.: WE102G32/96 and VIROTECH EBV IgM LINE Immunoblot; Order No.: WE102M32/96).
The **EBV-gp125** band has not been validated for CSF diagnostic testing.

Recommended Evaluation of the TpN17 Band

The *Treponema pallidum* **TpN17 Antigen Band (only with WE223G)**

In Lyme borreliosis serum diagnostic testing, crossreactions are observed with other microorganisms. Herpes virus infections (particularly EBV) and bacterial infections, such as syphilis, play an important role. Lyme borreliosis MiQ12/2000 makes the following recommendation: “If the screening test is borderline or positive (in Lyme borreliosis serology), a syphilis test (e.g. TPHA) should be performed, to exclude false positive findings due to crossreacting antibodies aganst Treponema.”
The TpN17 band serves to recognise false borderline or positive results in Lyme borreliosis serum diagnostic testing due to crossreacting antibodies from *Treponema pallidum* infection (syphilis).

If in the VIROTECH Borrelia in vivo + TpN17 IgG LINE Immunoblot the TpN17 band reacts ≥ the IgG cut-off band and at the same time there are Borrelia antigens in the IgM and/or in the IgG, the complete syphilis status of the serum should be tested as a precaution (e.g. with the VIROTECH Treponema pallidum IgG LINE Immunoblot Order No.: WE150G16/32 and VIROTECH Treponema pallidum IgM LINE Immunoblot Order No.: WE150M16/32).

It is essential to note the following points:

a. The TpN-17 band cannot replace complete syphilis differential diagnosis with respect to sensitivity and specificity.
b. A negative TpN17 antigen band does not in principle exclude the possibility that antibodies against *Treponema pallidum* may be present.
c. A positive result with the TpN17 antigen band must be confirmed with an appropriate *Treponema pallidum* confirmatory test (e.g. VIROTECH WE150).
d. The TpN-17 band has not been validated for use in CSF diagnostic testing.

Typical constellations of findings

The sequence of the antigens on the Borrelia in vivo LINE strip was chosen in a way that the antigens that react preferably with antibodies of patients with early Lyme-Borreliosis (e.g.: OspC, VlsE-IgM, VlsE-IgG) are located in the upper part of the strip (near the markline). Antigens which react preferably with antibodies of patients with advanced Lyme-Borreliosis (e.g.: p83, BBA36, BBO323, Crasp3, pG) are in the lower part of the strip (away from the markline). By this already the visual impression of band allocation gives notice of the stage of infection (from early to advanced Lyme Borreliosis).

Examples of band constellations in following stages of infection:

Borreliosis-stage	IgM-Serology	IgG-Serology
Early Lyme-Borreliosis	OspC	VlsE
	VlsE	VlsE
	p39	VlsE
	more than 2 bands	no bands or VlsE
	OspC	no bands
disseminated Lyme-Borreliosis	No up to all IgM-bands can occur.	VlsE and p39
		2 bands

progressed Lyme-Borreliosis	IgM-bands step increasingly into the background.	With the progress of the infection usually more and more IgG-bands are arising in different combinations. p39, p83, VlsE, iv1-4 (BBA36, BBO323, Crasp3 und pG)

10.5 Limits of the Test

1. A negative blot result does not completely exclude the possibility of a B. *burgdorferi s.l.* infection. The sample may be taken before the occurence 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). Likewise, cross-reactions may occur at Herpes-virus (EBV, CMV, Parvovirus) (34, 39). If the VIROTECH Borrelia in vivo + TpN17 IgG LINE Immunoblot (WE223G) not only shows reactivity to Lyme borreliosis antigens, but also reactivity to the TpN17 antigen, please refer to the comments under 9.4 (Recommended Evaluation of the TpN17 band.
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 in vivo IgM LINE Immunoblot not only exhibits IgM and/or IgG reactivity against Borrelia antigens, but also reactivity against EBV-gp125, the differential diagnosis of mononucleosis must be excluded.
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

When calculating the sensitivity the diagnosis group Erythema migrans (EM), Erythema chronica migrans (ECM) and Multiple Erythema migrans (MEM), Neuroborreliosis as well as the diagnosis group Arthritis and Acrodermatitis chronica atrophicans (ACA) have been dealt separately.

11.1 Sensitivity

For the detection of the sensitivity, clinically characterised sera collectives, that have been pre-determined with a Western Blot as reference method (finding), have been tested.
Sera Collectives: ACA n = 18, Neuroborreliosis n = 8, MEM, EM, ECM n = 72.
Borderline results have not been considered for the calculation of the sensitivity.

Referring to the finding, a sensitivity of 97,0 % has been calculated.

11.2 Specificity

For the detection of the specificity, 85 blood donor sera, that have been pre-determined with an ELISA as reference method (finding), have been tested.
Borderline results have not been considered for the calculation of the specificity.

Referring to the finding, a specificity of 97,2% has been calculated.

11.3 Diagnostical Sensitivity

For obtaining the diagnostical sensitivity, clinical characterised sera of patients with EM, ECM, MEM (n=122), Neuroborreliosis (n=53) as well with ACA and Arthritis (n=65), have been tested (source of sera: Prof. Dr. Hofmann, Munich; Dr. Talaska, Brieskow-Finkenheerd).

Sera Collective (n=122)		LINE		
		negative	**borderline**	**positive**
Diagnostical Finding/Clinic	**EM, ECM, MEM**	17	10	95

Sera Collective (n=53)		LINE		
		negative	**borderline**	**positive**
Diagnostical Finding/Clinic	**Neuroborreliosis**	4	2	47

Sera Collective (n=65)		LINE		
		negative	**borderline**	**positive**
Diagnostical Finding/Clinic	**ACA, Arthritis**	0	0	65

Borderline results have not been considered for the calculation of the diagnostical sensitivity.

For the sera collective EM, ECM and MEM a diagnostical sensitivity of 84,8%, for the sera collective Neuroborreliosis a diagnostical sensitivity of 92,2% and for the sera collective ACA and Arthritis a diagnostical sensitivity of 100% have been calculated

11.4 Diagnostical Specificity

28 human sera from persons without clinically suspected Lyme-Borreliosis were tested. From these 28 sera only one serum was positive and one serum was borderline.

The thus calculated diagnostical specificity is 96,3%.

11.5 Cross-Reactivity

Autoimmune diseases

10 autoimmune sera were tested. All sera have been negative in the Borrelia in vivo LINE.

Treponema pallidum

From 15 Treponema pallidum positive sera tested, four sera were positive and one serum was borderline in the Borrelia in vivo LINE. The reference tests used (ELISA, Western Blot) showed significantly more false positive results.

EBV

Without the exclusion diagnostic marker from 12 sera with primary EBV infection, two sera were positive and one serum was borderline (comperative tests showed significantly more unspecific Borrelia positive results than the Borrelia in vivo LINE). As all 12 sera reacted with the gp125-band, these sera could be recognized as false positive sera.

11.6 Prevalence (Expected Values)

87 blooddonors have been tested. Thereof 6% were positive in the overall assessment.

11.7 Intra-Assay-Precision (Repeatability)

For the determination of the repeatability, 30 blot strips of a nitrocellulose-membrane have been incubated in an IgG- and an IgM-examination testrun with a serum, that shows weak to strong antigenband-reactions.
The bands show an uniform intensity on the whole nitrocellulose-sheet.

11.8 Inter-Assay-Precision (Reproducibility)

For the determination of the reproducibility 3 sera each have been tested in IgM and IgG (one negative serum, a cut-off control, a positive control). The determination has been effected in 10 different test runs proceeded by 3 different test persons.

In all testings, the serological allegations have been exactly reached.

12. Additional Performance Data for the TpN17 Band of the WE223G32/G96

12.1 Diagnostic Sensitivity

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

Sera Collective (n=64)	Borrelia in vivo LINE Immuno Assay
negative	4
borderline	1
positive	59

12.2 Diagnostic Specificity

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

12.3 Crossreactivity

79 potentially cross-reactive sera (EBV primary infection, autoimmune sera) and 43 pregnancy sera were tested in the IgG. The TpN17 band gives a specificity of >99.9%.

13. References

1. Aguero-Rosenfeld et al., 1993 Serodiagnosis in early Lyme disease. J. Clin. Microbiol. 31:3090-3095
2. Zhang, J-R. et al.; Antigenic variation in Lyme disease Borrelia by promiscuous recombination of VMP-like sequence cassettes; Cell 1997. 89:275-285
3. Bruckbauer et al., 1992 Cross reactive Proteins of *Borrelia burgdorferi*. Eur. J. Clin Microbiol. Infect. Dis. 11:224-232.
4. Dressler et al., 1993 Western blotting in the seradiagnosis of Lyme disease J. infect Dis. 176:392-400
5. Engstroem et al., 1995 Immunoblot Interpretation criteria for Serodiagnosis of Early Lyme Disease. J. Clin. Microbiol. 33:419-427
6. Fawcett et al., 1993 Detection of antibodys to the recombinant P39 protein of *Borrelia burgdorferi* using enzyme immunoassay and immunoblotting. J. Rheumatol. 20:734-738
7. Wilske et al., 12/2000, Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik für Lyme-Borreliose, pp. 38ff., Urban&Fischer Verlag
8. Hauser et al., 1997 Interpretation criteria for Standardized Western Blots for three European species of *Borrelia burgdorferi* sensu lato. J. Clin. Microbiol. 35:1433-1444
9. Marianne J. Mathiesen et al., 1996 Analysis of the human antibody response to outer surface protein C (OspC) of *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Med. Microbiol. Immunol. 185:121-129
10. Wallich, R. et al.; Artificial-infection protocols allow immunodetection of novel Borrelia burgdorferi antigens suitable as vaccine candidates against Lyme disease; Eur. J. Immunol. 2003. 33:708-719
11. Kraiczy, P. et al.; Immune evasion of Borrelia burgdorferi: mapping of a complement inhibitor factor H-binding site of BbCRASP-3, a novel member of the Erp protein family; Eur. J. Immunol. 2003. 33:697-707
12. LeFebvre et al., 1990 The 83-kilodalton antigen of *Borrelia burgdorferi* which stimulates immunoglobulin M (IgM) and IgG responses in infected hosts is expressed by a chromosomal gene. Clin. Microbiol. 28:1673-1676
13. Luft et al., 1992 The 93-kilodalton protein of *Borrelia burgdorferi*: an immundominant protoplasma cylinder antigen. Infect. Immun. 60:4309-4321
14. Ma et al., 1992 Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. J. Clin. Microbiol. 30:370-376
15. Moskophidis et al., 1995 Wertigkeit des Immunoblots in der Serodiagnostik der Lyme Borreliose. lab. Med. 19:231-237

16. Wallich, R. et al.; Molecular cloning and immunological characterization of a novel linear-plasmid-encoded gene, pG, of Borrelia burgdorferi expressed only in vivo; Infection and Immunity 1995 Sept:3327-3335
17. Roessler et al., 1995 Molecular and immunological characterization of the p83/100 protein of various *Borrelia burgdorferi* sensu lato isolates Med. Microbiol. Immunol. 184:23-32
18. Roessler et al., 1997 Heterogeneity of BmpA (P39) among European isolates of *Borrelia burgdorferi* sensu lato and Influence of Interspecies varability on Serodiagnosis. J. Clin. Microbiol. 35:2725-2758
19. Simpson et. al., 1990 reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to *Borrelia burgdorferi*. J. Clin. Microbiol. 28:1329-1337
20. RKI (1999), Ratgeber Infektionskrankheiten, Lyme-Borreliose, Epidemiologisches Bulletin, überarbeitete Auflage
21. Craft, J.E., Grodzicki, R.L. and Steere, A.C. (1984), Antibody response in Lyme disease: evaluation of diagnostic tests, J. Inf. Dis. 149:789-95
22. Wilske et al., 1986 Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriol. Mikrobiol. Hyg. A 263:92-102
23. Dressler, F. (1994) Lyme borreliosis in European children and adolescents, Clinical and Experimental Rheumatology 12 (Suppl. 10) :49-54
24. Wilske et al., 1988 Immunochemische Analyse der Immunantwort bei Spätmanifestationen der Lyme Borreliose. Zbl. Bakt. Hyg. A 267:549-558
25. Pfister,H-W., Wilske, B. (1994) Lyme borreliosis: basic science and clinical aspects, The Lancet Vol. 343: 1013-1015.
26. Wilske & Preac-Mursic 1993 Microbiological diagnosis of Lyme Borreliose in: Aspects of Lyme borreliosis: Weber, Burgdorferi eds. Springer, Berlin 267-300
27. Dressler, F., Ackermann, R. and Steere, A.C. (1994), Antibody responses to the three genomic groups of Borrelia burgdorferi in European Lyme Borreliosis, J. Infect. Dis. 169: 313-318
28. Wilske et al., 1993 Immunological and Molecular Polymorphism of OspC, an Immunodominant Major Outer Surface Protein of *Borrelia burgdorferi*. J. Clin. Microbiol. 61:2182-2191
29. Wilske et al., 1994 Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. 183:43-59
30. Wilske, 1995 Diagnostik der *Borrelia burgdorferi*-Infektion. Internist 36:114-119
31. Wilske et al., 1997 *Borrelien*. Diagnostische Bibliothek 48:1-12, Blackwell Verlag
32. Zöller et al., 1991, Validity of Western immunoblot band patterns in the serodiagnosis of Lyme borreliosis, J. Clin. Microbiol. 29:174-182
33. Oschmann und Kraiczy, (1998), Lyme-Borreliose und Frühsommer-Meningoenzephalitis“, UNI-MED-Verlag
34. Horst, H. (1997), Einheimische Zeckenborreliose (Lyme-Krankeit) bei Mensch und Tier, 3., überarbeitete Auflage, Spitta Verlag: 128-130
35. Tewald, F. Braun, R. (1998), Durchführung und Interpretation serologischer Tests bei Verdacht auf Borrelieninfektion, Clin. Lab. 44: 897-902
36. Craft, J.E., Fischer, D.K., Shimamoto, G.T. and Steere, A.C. (1986), Antigens of *Borrelia burgdorferi* recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G late in the illness., J. Clin. Invest. 78: 934-39
37. Shrestha M., R.L. Grodzicki, A.C. Steere (1985) Diagnosing early Lyme disease. Am. J. Med. 78: 235-40
38. Magnarelli, L.A., J.F. Anderson and R.C. Johnson (1987), Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J. Infect. Dis. 156: 183-88
39. Goosens, H.A.T., Bogaard, van den A.E., Nohlmans, M.K.E., (1999), Epstein-Barr Virus and Cytomegalovirus Infections cause false.positive results in IgM two-test protocol for early Lyme-Borreliosis, Infection 27 No.3: 231
40. Burgdorfer, W., Barbour, A.G., Hayes S.F. et al. (1982), Lyme disease - a tick -borne spirochetosis?, Science 216:1317-19.
41. Steere, A.C. (1989), Lyme Disease, N. Engl. J. Med. 321:586-96.
42. Fingerle, V., Schulte-Spechtel, U.C., Ruzic-Sabljic, E., Leonhard, S., Hofmann, H., Weber, K., Pfister, K., Strle, F., Wilske, B. (2007) Epidemiological aspects and molecular characterizartion of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species Borrelia spielmanii sp. Nov. Int J Med Microbiol. 2008; 298(3-4): 279-90
43. Herzberger, P., Siegel, C., Skerka, C., Fingerle, V., Schulte-Spechtel, U., van Dam, A., Wilske, B., Brade, V., Zipfel, P.F., Wallich, R., Kraiczy, P. (2007) Human pathogenic *Borrelia spielmanii* sp. nov. resist complement-mediated killing by direct binding of immune regulators factor H and FHL-1. Infect Immun. 75(10): 4817-25

44. Wang, G., van Dam, A.P., Dankert, J. (1999) Phenotypic and genetic characterization of a novel *Borrelia burgdorferi* sensu lato isolate from a patient with lyme borreliosis. J Clin Microbiol 37: 3025-3028
45. Normenausschuss Medizin (NAMed) im DIN, DIN 58969-44, Medizinische Mikrobiologie-Serologische und molekularbiologische Diagnostik von Infektionskrankheiten –Teil44: Immunoblot (IB); Spezielle Anforderungen für den Nachweis von Antikörpern gegen *Borrelia burgdorferi*, Juli 2005, Beuth Verlag GmbH.
46. Grimm, D., Tilly, K., Byram, R., Stewart, P.E., Krum, J.G., Bueschel, D.M., Schwan, T.G., Policastro, P.F., Elias, A.F., Rosa, P.A. (2004) Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc Natl Acad Sci U S A 101: 3142-3147
47. Tilly, K., Krum, J.G., Bestor, A., Jewett, M.W., Grimm, D., Bueschel, D., Byram, R., Dorward, D., Vanraden, M.J., Stewart, P., Rosa, P. (2006) *Borrelia burgdorferi* OspC protein required exclusively in a crucial early stage of mammalian infection. Infect Immun 74: 3554-3564
48. Xu, Q., Seemanapalli, S.V., McShan, K., Liang, F.T. (2006) Constitutive expression of outer surface protein C diminishes the ability of *Borrelia burgdorferi* to evade specific humoral immunity. Infect Immun 74: 5177-5184
49. Xu, Q., McShan, K., Liang, F.T. (2007a) Identification of an ospC operator critical for immune evasion of *Borrelia burgdorferi*. Mol Microbiol 64: 220-231
50. Tilly, K., Bestor, A., Jewett, M.W., Rosa, P. (2007) Rapid clearance of Lyme disease spirochetes lacking OspC from skin. Infect Immun 75: 1517-1519
51. Bankhead, T., Chaconas, G. (2007) The role of VlsE antigenic variation in the Lyme disease spirochete: persistence through a mechanism that differs from other pathogens. Mol Microbiol. 65(6): 1547-58
52. Bykowski, T., Babb, K., von Lackum, K., Riley, S.P., Norris, S.J., Stevenson, B. (2006) Transcriptional regulation of the *Borrelia burgdorferi* antigenically variable VlsE surface protein. J Bacteriol 188: 4879-4889
53. Norris, S.J. (2006) Antigenic variation with a twist - the Borrelia story. Mol Microbiol 60: 1319-1322
54. Nowalk et al. (2006), Serologic Proteome Analysis of *Borrelia burgdorferi* Membrane-Associated Proteins, Infection and Immunity 74, No.7: 3864-3873
55. Poggensee, G. et al (2008), Lyme.Borreliose: Forschungsbedarf und Forschungsansätze, Bundesgesundheitsblatt 50: 1329-1339

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