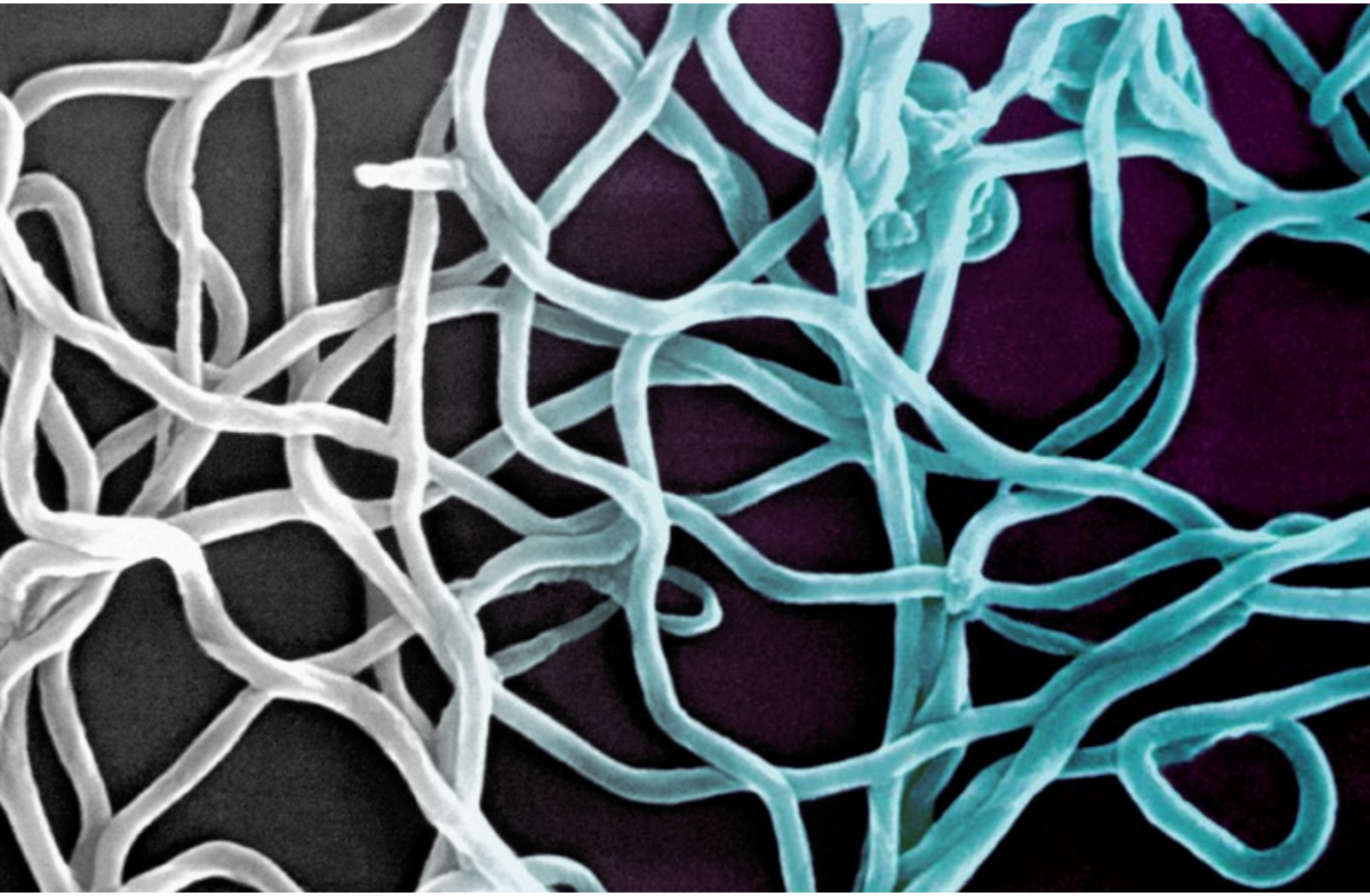
Borrelia burgdorferi sensu lato



Enzyme immunoassays for the diagnosis of **Lyme borreliosis**

ELISA and **IMMUNOBLOT** and **MICROBLOT-ARRAY** kits are optimized and validated for detection of IgG and IgM antibodies in human serum, plasma, cerebrospinal or synovial fluid.

TestLine Clinical Diagnostics

INFECTIOUS SEROLOGY ▸ BACTERIOLOGY ▸ ***BORRELIA***

INTRODUCTION

Lyme borreliosis is a multisystem infectious disease caused by spirochete *Borrelia burgdorferi*. The infection is transmitted by ticks of the genus *Ixodes*.

Lyme borreliosis is characterized by early and late clinical symptoms.

PHASES OF LYME BORRELIOSIS

Early localised infection
– lasts days or weeks. It is characterized by erythema migrans (EM), which appears in only 50% of patients. Early symptoms of the disease may include "flu-like" symptoms, headache and lymphadenitis.

Early disseminated infection
– lasts weeks or months. Borrelia are disseminated by blood vessels and the lymphatic system (CNS, joints, heart, eye, skin – secondary EM). At this stage, the most frequently diagnosed symptoms are: neuroborreliosis, paresis neurofacialis, borrelial lymphocytoma (swollen earlobes, knucklebones, etc.) and Bannwarth syndrome.

Late disseminated infection
– lasts months or years. The most typically diagnosed immunopathological changes include Acrodermatitis chronica atrophicans (chronic skin lesions – ACA), chronic neuroborreliosis, and borrelial arthritis.

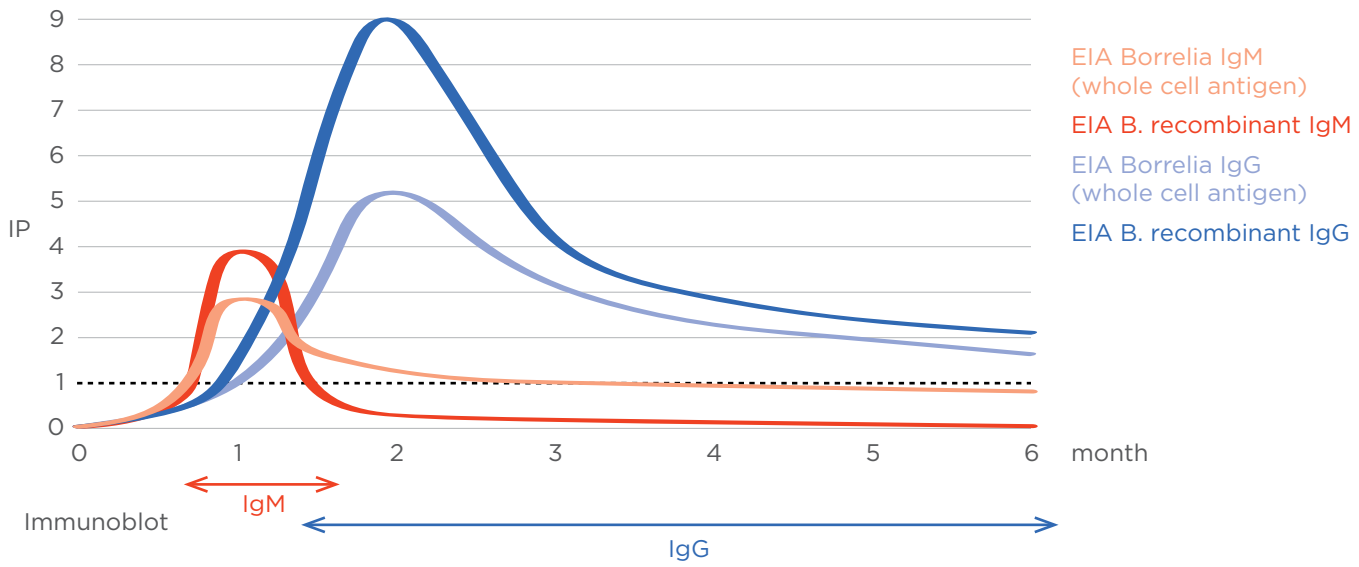
The results of extensive studies have demonstrated that all the genospecies may not only cause the development of erythema migrans (EM), but also have many other clinical manifestations. *Borrelia (B.) garinii* is associated with neurological symptoms, *B. afzelii* with chronic skin disorders (especially ACA), and *B. burgdorferi* sensu stricto is mainly related to joint injuries.

DIAGNOSIS OF THE DISEASE

The diagnosis of the disease is based on anamnesis, clinical picture, and results of laboratory tests. At present, the diagnostic methods of choice are screening of specific IgG and IgM class antibodies by means of ELISA, and subsequent confirmation of the antibodies to specific antigens by means of immunoblot. Direct cultivation or electron microscopy is not applicable in a routine use.

Serological diagnosis of borreliosis is difficult regarding to the large genetic diversity of the species *Borrelia burgdorferi* s.l., possible cross reactivity with unrelated antigens of other microorganisms, and borrelia richness to heat shock proteins. Diagnosis is also complicated as well by different individual serological reactivity. The production of antibodies can be extremely slow in the early phase of the disease. On the other hand, the IgG and IgM antibodies can persists for more than ten years.

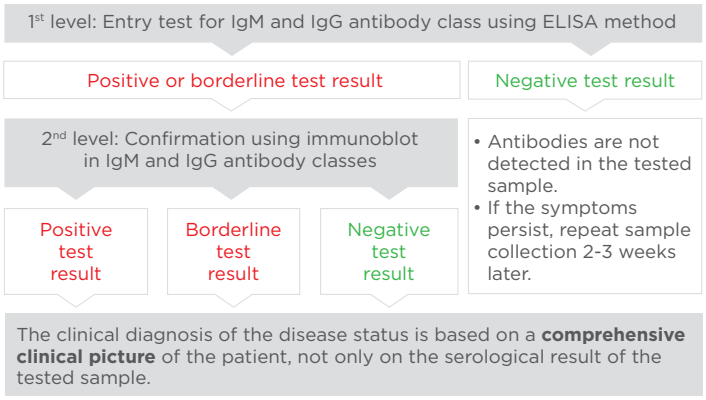
ANTIBODY RESPONSE



TWO-LEVEL ANTIBODY DETECTION

The IgM and IgG class antibodies are detected in two levels with two types of tests. First, the samples are divided by ELISA method into two groups according to their positive or negative test results. Provided that the test result is negative and the symptoms of infection persist, the second (control) collection is performed in 2-3 weeks. The positive and borderline results are recommended to be confirmed by immunoblot. The result of the test does not indicate the diagnosis, but it may support it.

The number of disagreements between immunoblot (second level testing) and ELISA results (1st level) is reduced when the ELISA method is based on recombinant antigens as are the TestLine assays.

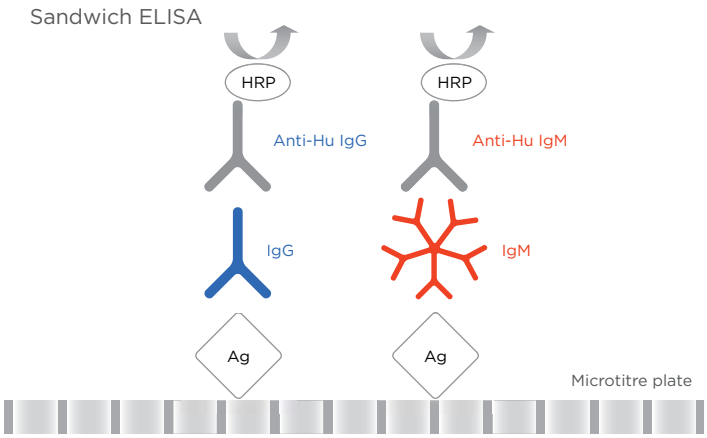


Two-level antibody detection (adapted from MiQ 12 2000 Lyme borreliosis, B. Wilske et al.)

ELISA

TEST PRINCIPLE

The assays are based on a sandwich type of ELISA method.



USER COMFORT

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF included
- Semiquantitative evaluation of results (Index of Positivity)

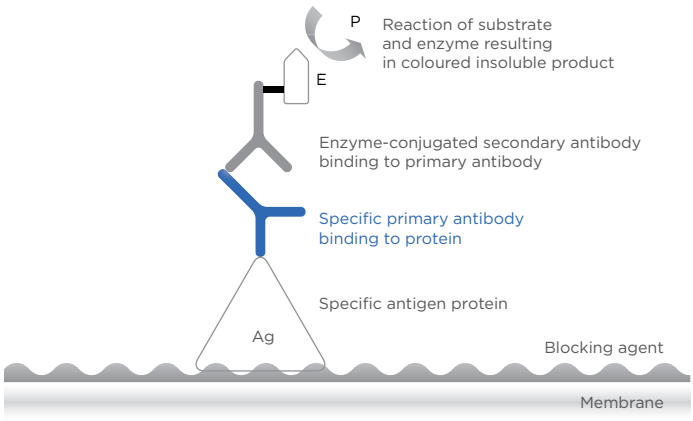
PROTOCOL SUMMARY

Step No.	Test steps
1	Dilute samples • serum/plasma 1:101 (10 µl + 1 ml) • cerebrospinal fluids 1:2 (110 µl + 110 µl) • synovial fluids 1:21 (20 µl + 400 µl), 1:41 (10 µl + 400 µl)
2	Pipette Controls and diluted samples 100 µl • blank = empty well
3	Incubate 30 minutes at 37°C
4	Aspirate and wash the wells 4 times
5	Add 100 µl Conjugate • blank = empty well
6	Incubate 30 minutes at 37°C
7	Aspirate and wash the wells 5 times
8	Add 100 µl Substrate (TMB-Complete) • Including blank
9	Incubate 15 minutes at 37°C
10	Add 100 µl Stopping solution • Including blank
11	Read colour intensity at 450 nm evaluation of strips

IMMUNOBLOT

TEST PRINCIPLE

Antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



USER COMFORT

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Positive and Negative controls
- Control line on the strip
- Easy assay procedure

PROTOCOL SUMMARY

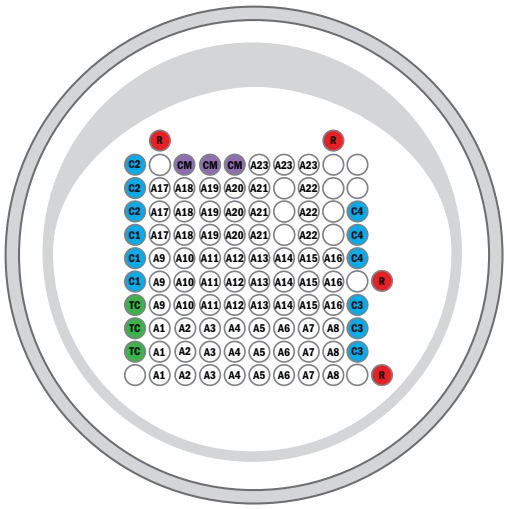
Step No.	Test steps
1	Pipette Universal solution 2 ml
2	Strips soaking 10 min. at room temperature • Shaker
3	Aspirate
4	Dilute samples • serum/plasma 1:51 (30 µl + 1,5 ml) • cerebrospinal fluids 1:2 (0,75 ml + 0,75 ml) • synovial fluids 1:17,5 (90 µl + 1,5 ml)
5	Pipette Controls and diluted samples 1.5 ml
6	Incubate 30 min. at room temperature • Shaker
7	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. • Shaker
8	Pipette Conjugate 1.5 ml
9	Incubate 30 min. at room temperature • Shaker
10	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. • Shaker
11	Pipette Substrate solution (BCIP/NBT) 1.5 ml
12	Incubate 15 min. at room temperature • Shaker
13	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. • Shaker
14	Sticking and evaluation of strips

RESULTS INTERPRETATION

MODEL SITUATIONS IN EVALUATION OF BORRELIA TEST

IgM		IgG		Interpretation
ELISA	BLOT	ELISA	BLOT	
–	–	–	–	Specific antibodies were not detected.
+	+	–	–	In most cases an early stage of the disease.
+	+	+	+	In most cases acute infection.
–	–	+	+	In most cases a late stage of the disease.
+	–	–	–	Probably a non-specific response of EIA test - results should be evaluated as negative. If symptoms persist, retest the sample after 2-3 weeks.
–	–	+	–	
+	–	+	–	
+	+	+	–	Probably an early stage of the disease, earlier IgG capture by EIA and/or by IMMUNOBLOT.
+	+	–	+	
+	–	+	+	Persisting or residual IgM antibodies after treatment detected by using EIA and/or by IMMUNOBLOT, sample IgG positive in EIA as well as IMMUNOBLOT.
–	+	+	+	
–	–	–	+	Recessing residual IgG antibodies after treatment detectable only by using IMMUNOBLOT.
–	+	+	–	A unique capture of antibodies passing between IgM and IgG. IMMUNOBLOT can still detect residual IgM and EIA can already detect IgG.
–	+	–	–	Probably an early stage of the disease or a heat-shock protein response or persistence of antibodies after treatment in IgM.

DISTRIBUTION OF ANTIGENS AND CONTROL SPOTS IN THE MICROPLATE WELL (IgM)



Description of antigens

A1 – VlsE Ba	A9 – OspB	A17 – NapA
A2 – VlsE Bg	A10 – OspA Ba	A18 – OspE
A3 – VlsE Bs	A11 – OspA Bg	A19 – p17
A4 – p83	A12 – OspA Bs	A20 – OmpA
A5 – p58	A13 – OspC Ba	A21 – p44
A6 – p41 Ba	A14 – OspC Bg	A22 – Asp62
A7 – p41 Bs	A15 – OspC Bs	A23 – VCA-p18
A8 – p39	A16 – OspC Bsp	

Description of control spots

R – Reference	
TC – Test control	
CM – Conjugate control IgM	
C1 – Calibration 1	
C2 – Calibration 2	
C3 – Calibration 3	
C4 - Calibration 4	

PROTOCOL SUMMARY

Step No.	Test steps
1	Pipette Universal solution 150 µl
2	Strips soaking 10 min. at room temperature
3	Aspirate
4	Dilute samples • serum/plasma 1:51 (10 µl + 500 µl) • cerebrospinal fluids 1:3 (50 µl + 100 µl) • synovial fluids 1:21 (10 µl + 200 µl)
5	Pipette Controls and diluted samples 100 µl
6	Incubate 30 min. at room temperature
7	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
8	Pipette Conjugate 100 µl
9	Incubate 30 min. at room temperature
10	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
11	Pipette Substrate solution (BCIP/NBT) 100 µl
12	Incubate 15 min. at room temperature
13	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
14	Dry and evaluate strips

USER COMFORT

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Fully automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity

ORDERING INFORMATION

ELISA

Cat. No.	Product	No. of Tests
BGV096	EIA Borrelia VlsE IgG	96
BM0096	EIA Borrelia IgM	96
BrG192	EIA Borrelia recombinant IgG (192)	192
BrM192	EIA Borrelia recombinant IgM (192)	192
BaGVD2	EIA Borrelia afzelii VlsE IgG (192)	192
BaM192	EIA Borrelia afzelii IgM (192)	192
BsGV96	EIA Borrelia b. sensu stricto VlsE IgG	96
BsM096	EIA Borrelia b. sensu stricto IgM	96
BgGVD2	EIA Borrelia garinii VlsE IgG (192)	192
BgM192	EIA Borrelia garinii IgM (192)	192
SK-BGV096	SmartEIA Borrelia VlsE IgG	96
SK-BM0096	SmartEIA Borrelia IgM	96
SK-BrG096	SmartEIA Borrelia recombinant IgG	96
SK-BrM096	SmartEIA Borrelia recombinant IgM	96
SK-BaGV96	SmartEIA Borrelia afzelii VlsE IgG	96
SK-BaM192	SmartEIA Borrelia afzelii IgM	96
SK-BsGV96	SmartEIA Borrelia b. sensu stricto VlsE IgG	96
SK-BsM096	SmartEIA Borrelia b. sensu stricto IgM	96
SK-BgGV96	SmartEIA Borrelia garinii VlsE IgG	96
SK-BgM096	SmartEIA Borrelia garinii IgM	96

SmartEIA kits are designed for automated processing using the Agility® analyser

IMMUNOBLOT

Cat. No.	Product	No. of Tests
BGL020	BLOT-LINE Borrelia/HGA IgG	20
BML020	BLOT-LINE Borrelia/HGA IgM	20
BaGL20	BLOT-LINE Borrelia afzelii IgG	20
BaML20	BLOT-LINE Borrelia afzelii IgM	20
BgGL20	BLOT-LINE Borrelia garinii IgG	20
BgML20	BLOT-LINE Borrelia garinii IgM	20
BsGL20	BLOT-LINE Borrelia b. sensu stricto IgG	20
BsML20	BLOT-LINE Borrelia b. sensu stricto IgM	20
BD-BGL024	BlueBLOT-LINE Borrelia IgG	24
BD-BML024	BlueBLOT-LINE Borrelia IgM	24
SwIm03	Immunoblot Software	1 ks

The BlueBLOT-LINE kits are designed for automatic processing using BlueDiver® analyser

MICROBLOT-ARRAY

Cat. No.	Product	No. of Tests
BGMA096	Microblot-Array Borrelia IgG	96
BMMA096	Microblot-Array Borrelia IgM	96

ANTIBODY INDEX STANDARD SERA

Cat. No.	Product	No.	Units
BrGAI2	Borrelia recombinant AI - Standard IgG	0.2	ml
BrMAI2	Borrelia recombinant AI - Standard IgM	0.2	ml
BgGAI2	Borrelia garinii AI - Standard IgG	0.2	ml
BgMAI2	Borrelia garinii AI - Standard IgM	0.2	ml
SwAI01	Antibody Index Software	1	pc

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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