MIKROGEN
DIAGNOSTIK

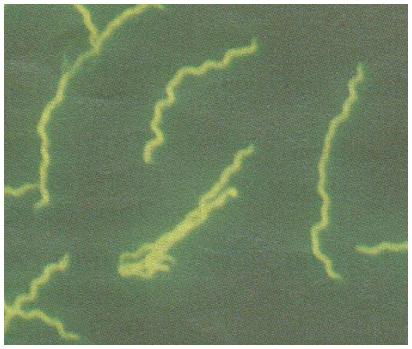
Diagnosis of Lyme disease

2014-03

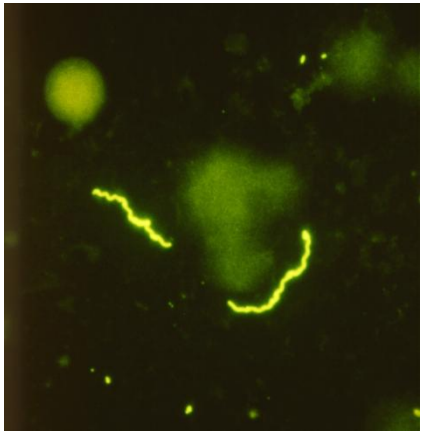
History

- Lyme borreliosis (LB) is the most commonly reported tick-borne infection in Europe and North America
- The disease is a multi-system disorder which can affect a complex range of tissues including the skin, heart, nervous system, and to a lesser extent the eyes, kidneys and liver.
- The term Lyme disease was first used following investigation into a geographical cluster of juvenile rheumatoid arthritis in the town of Old Lyme, Connecticut, USA, in the mid 1970's.
- The disease has, however, been known in Europe under a variety of names (including erythema migrans, acrodermatitis chronica atrophicans, Bannwarth's syndrome) since the 1880's.

The Pathogen

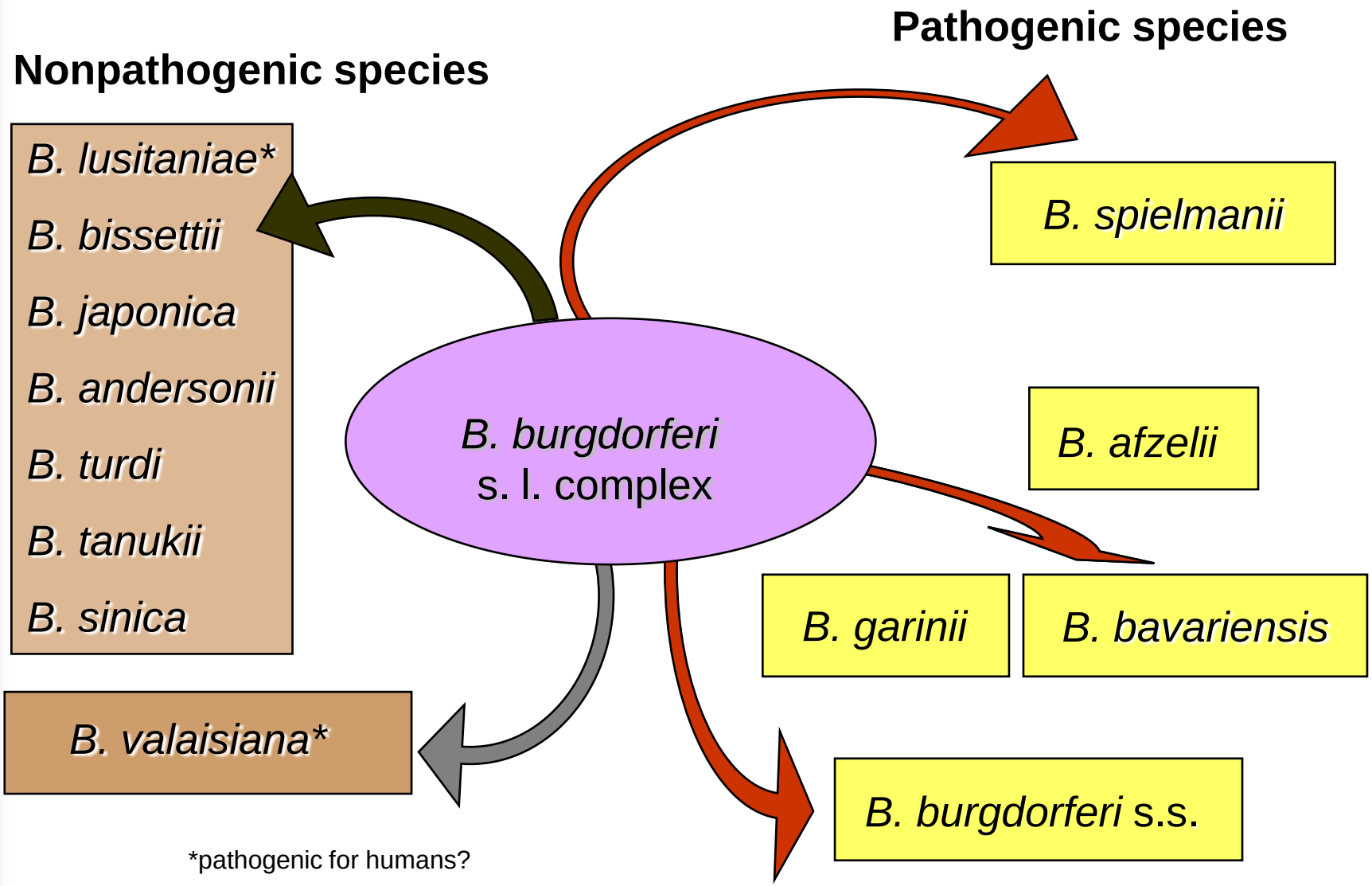


- Family: Spirochaetaceae
- Genera
  - Treponema: Species *T. pallidum* (Lues), …
  - Borrelia
    - Relapsing fever: Species *Borrelia* (*recurrentis*, *hermsii*, *duttonii*, …)
    - **Lyme borreliosis: Species *Borrelia burgdorferi* s.l.**
      - *B. burgdorferi* s.s.
      - *B. garinii*
      - *B. afzelii*
      - *B. spielmanii*
      - *B. bavariensis*



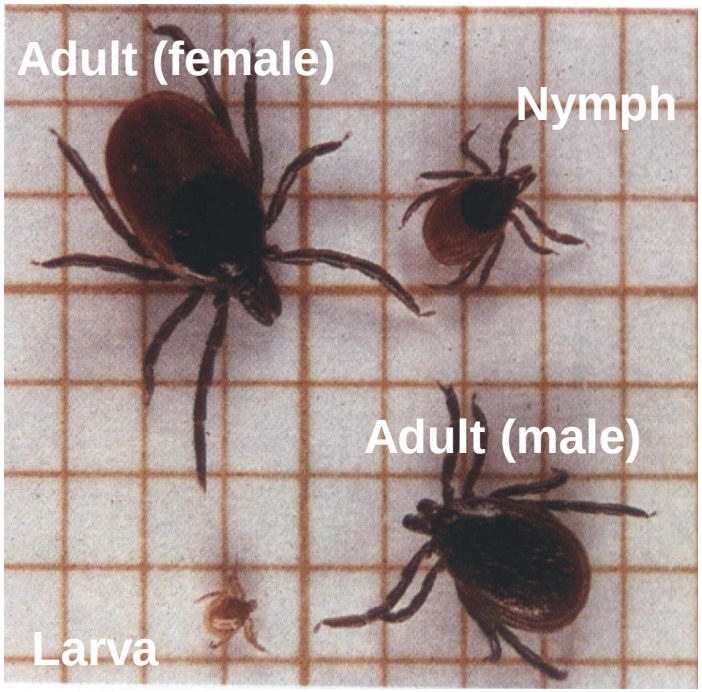
Borreliae in tick midgut

B. burgdorferi s.l. complex



*pathogenic for humans?

The Vector



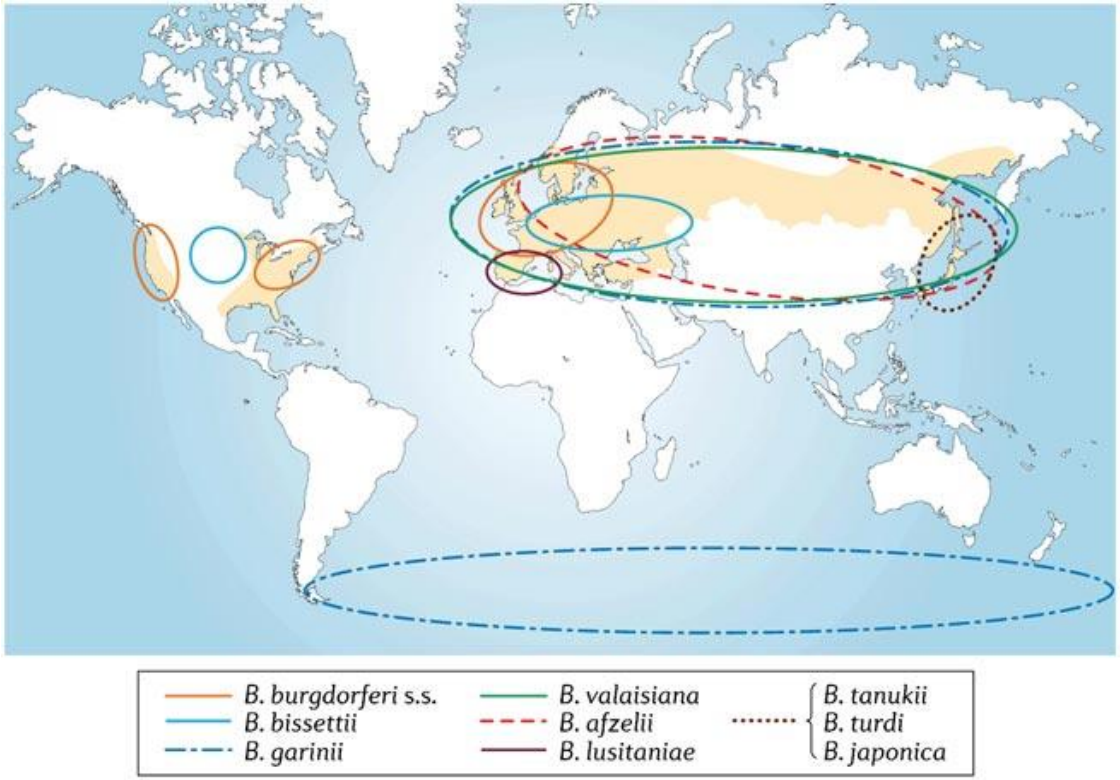
Ixodes ricinus (Europe)
I. persulcatus (Asia, eastern Europe)
I. scapularus (North America east)
I. pacificus (North America west)

Tick during blood meal

Habitat and Seasonality

- The non-parasitic (off-host) phases require
  - a high humidity at the base of the vegetation and
  - ideal conditions are to be found in temperate deciduous woodland with patches of dense vegetation and little air movement coupled with high humidity.
  - The need for questing ticks to maintain a stable water balance is an important factor in determining the location and duration of activity.
- In general, activity will begin in spring and early summer, with ticks being found on vegetation and animals from late March.
- In habitats where desiccation is high, such as open areas, periods of activity will be shortened to only a few weeks - as opposed to several months in dense woodlands. In some areas a second, less intense, phase of questing activity occurs in the autumn.

Geographical Distribution of Borrelia burgdorferi sensu lato



The beige-shaded background indicates the geographical distribution of recorded clinical cases of Lyme borreliosis. The highest species richness is recorded for Eurasia. In the northeastern United States, Borrelia burgdorferi sensu stricto (s.s.) is expanding in population size and geographical range, causing epidemics of Lyme disease in humans. Borrelia afzelii seems to be much less abundant in the British Isles compared with continental Eurasia. The prevalence of B. burgdorferi s.s. phases out towards eastern Europe. Borrelia garinii and Borrelia valaisiana are found across much of terrestrial Eurasia. B. garinii is also maintained by seabird species and Ixodes uriae ticks in pelagic transmission cycles in both hemispheres. Borrelia bissettii and Borrelia lusitaniae have occasionally been found in locations beyond their core range. Borrelia andersonii in the United States, Borrelia sinica in Asia and Borrelia spielmanii sp. nov. in Europe are omitted from the figure, because there is little information on their distribution.

Kurtenbach *et al. Nature Reviews Microbiology* advance online publication;
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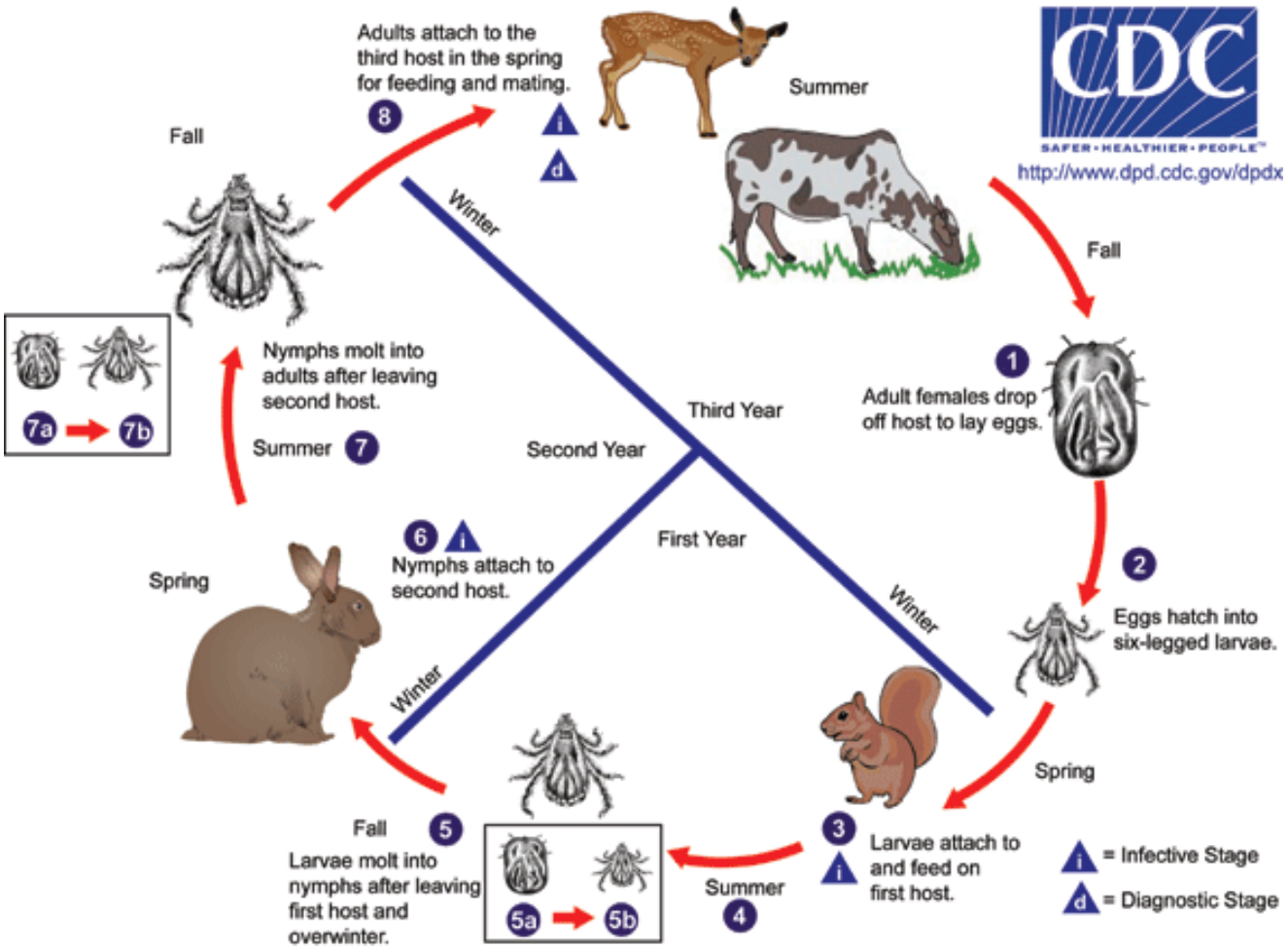
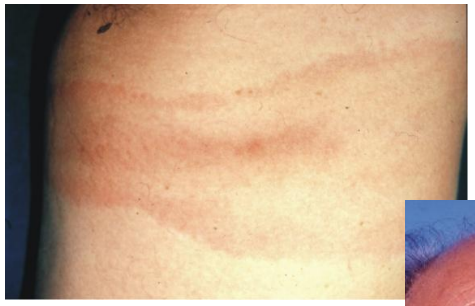
Transmission of Borrelia to different hosts

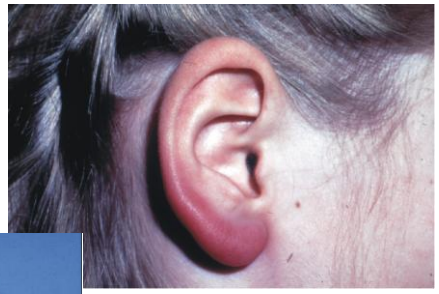


Clinical Symptoms

Early localised Lyme borreliosis



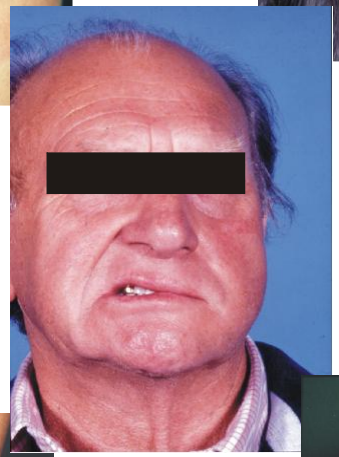
Erythema migrans



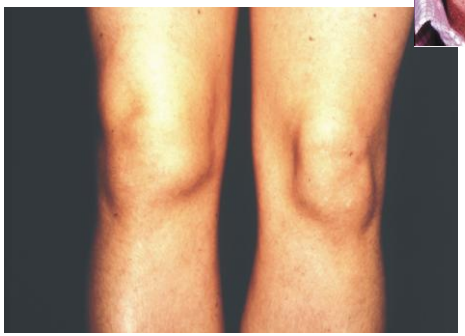
Borrelial lymphocytoma

Early disseminated Lyme borreliosis

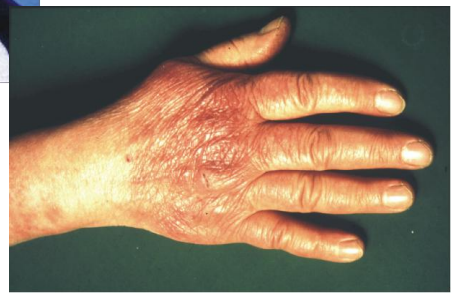
Neuroborreliosis (facial palsy)



Late Lyme borreliosis



Arthritis



Acrodermatitis chronica atrophicans (ACA)

Treatment, Prophylaxis

- Most infections with B. burgdorferi are asymptomatic and self-limiting, so that individuals carrying antibodies but without clinical symptoms do not require treatment.
- Patients showing symptoms with adequate supporting laboratory evidence for diagnosis should be treated to prevent possible progression of the disease. A range of antibiotics are available (tetracyclines, penicillin, cephalosporins, etc.) but their selection and use vary in different countries.
- The value of the **prophylactic use of antibiotics** following a tick-bite has been assessed in a number of studies, with mixed results. Since a minority of ticks in endemic areas are infected and infection can be prevented by prompt removal of the attached tick this approach is not generally recommended.
- A **vaccine** for use in humans based on outer surface proteins (OspA) was available in the USA (LYMErix, Smithkline Beecham) until recently, but has now been withdrawn. For an effective vaccine in Europe it will probably be necessary to produce a "cocktail" of such proteins, since European B. burgdorferi are more heterogeneic.

Two step approach in serodiagnosis is recommended

1. step

Screening assay IgG-ELISA and IgM-ELISA

In case of suspected neuroborreliosis: examination of paired serum/CSF samples drawn on the same day for intrathecally produced antibodies (CSF/serum index)

positive or borderline

negative

negative serological result

no further tests from the present serum
in case of short disease duration serological follow up

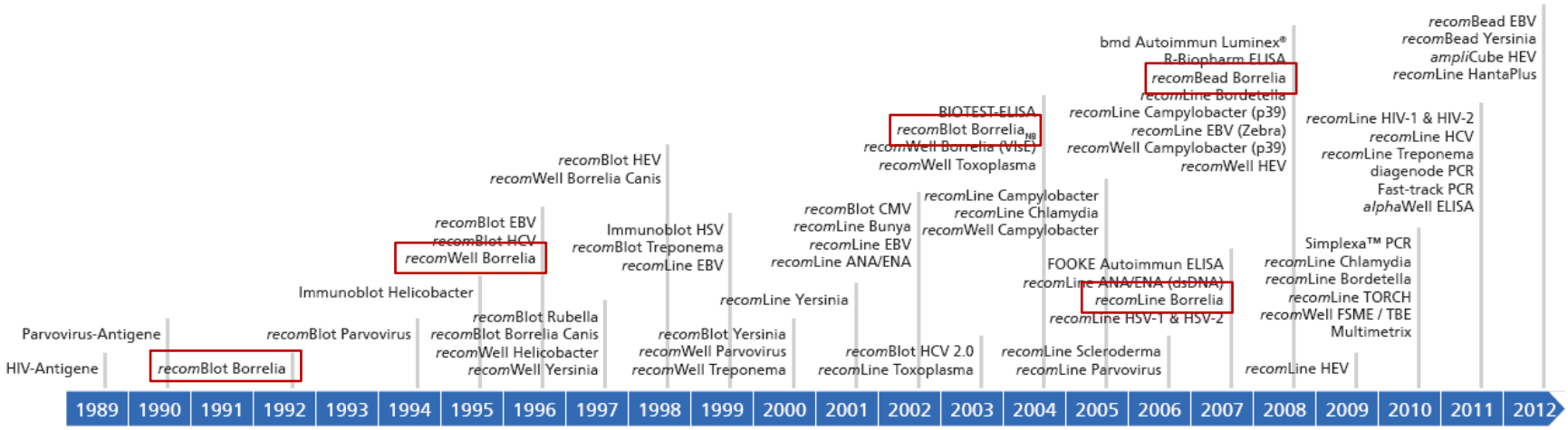
2. step

Confirmatory assay IgG-immunoblot and IgM-immunoblot

Important Borrelia proteins

Antigen	Biological Importance	Diagnostic Relevance	Mikrogen Feature
VlsE	• Variable membrane protein containing highly conserved regions • Circumvention of the host immune system	• Highly specific and sensitive • Key antigen for IgG detection • Early marker	• Recombinant • Fusion protein from several genospecies
OspC	• Surface protein • Important for the passage from tick to human • Binds plasminogen	• Highly specific and sensitive • Key antigen for IgM detection • Early marker	• Recombinant • 4 genospecies
p18 (DbpA, Osp17)	• Decorin-binding protein • Essential for spreading of Borrelia around the body	• Most heterogenous antigen between species • Highly specific and, when suitably combined, highly sensitive • Key antigen for IgG detection	• Recombinant • 5 genospecies
p100	• Membrane protein • Function unknown	• Highly specific • IgG marker	• Recombinant
OspA	• Surface protein • Binds to the intestinal tract of the tick	• (Detection of immunised people (USA))	• Recombinant
p39 (BmpA)	• Borrelial membrane protein	• Very specific and sensitive • IgG marker	• Recombinant
p58 (OppA-2)	• Oligopeptide-binding protein	• Very specific and sensitive • IgG marker • Scientifically evaluated	• Recombinant
p41 (Fla)	• Component of flagellin	• Relatively unspecific	• Recombinant

24 Years of Competence in Diagnosis of Lyme Disease



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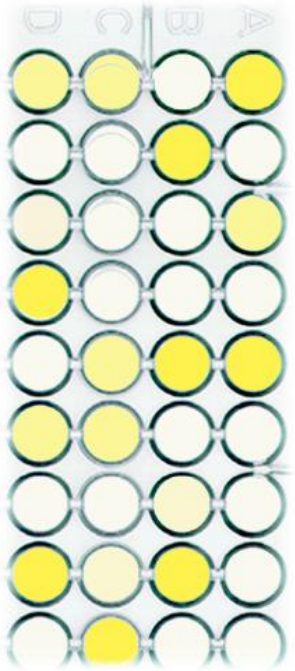
Your partner for diagnosis of Lyme disease

- **Screening** — ***recom*Well Borrelia IgG**, ***recom*Well Borrelia IgM**
- **Confirmation** — ***recom*Line Borrelia IgG**, ***recom*Line Borrelia IgM**, ***recom*Bead Borrelia IgG**, ***recom*Bead Borrelia IgM**

*recom*Well Borrelia IgG, IgM

A reliable screening system



Test	Antigen / Species	
IgM	**OspC**	*B. afzelii*, *B. garinii*
	p41/internal	*B. bavariensis*
	VlsE	Fusion protein
IgG	**p100**	*B. afzelii*
	OspC	*B. burgd.* sensu stricto, *B. garinii*
	VlsE	Fusion protein
	p18 (DbpA)	*B. afzelii*

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*recom*Well Borrelia - Performance

Sensitivity

Clinically defined sera	Number	IgG	IgM	IgG/IgM
Erythema migrans	64	34 (53 %)	51 (80 %)	55 (86 %)
Neuroborreliosis	81	80 (99 %)	52 (64 %)	81 (100 %)
Arthritis	46	46 (100 %)	23 (50 %)	46 (100 %)
Acrodermatitis	17	17 (100 %)	10 (59 %)	17 (100 %)

Specificity and Seroprevalence

Blood donors, year 2003 (no clinical markers, n = 200)	IgG	IgM
negative	180	185
positive or equivocal	20	15
confirmed by western blot	19	9
Prevalence	9,5 %	4,5 %
Specificty	99 %	97 %

Accuracy

	IgG/IgM
Intra assay	VC < 4 %
Inter assay	VC < 12 %

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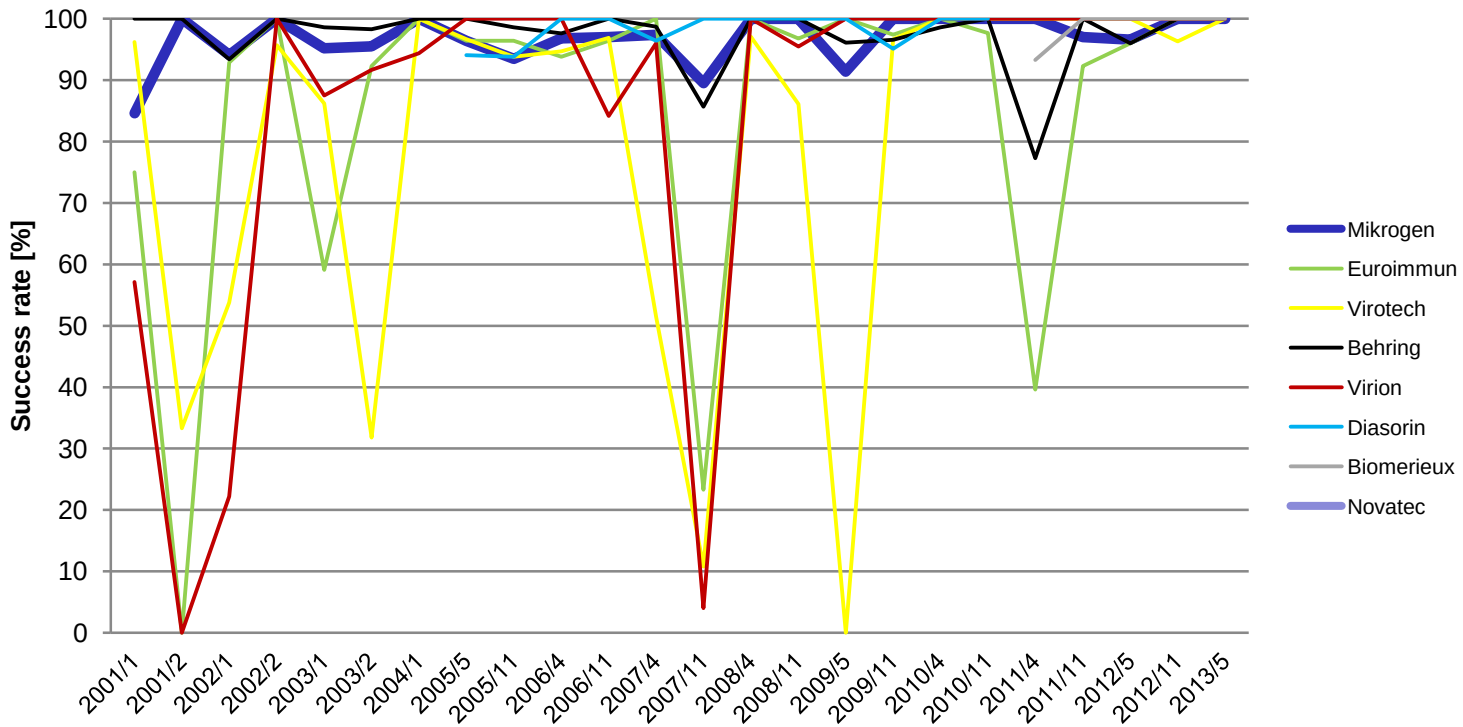
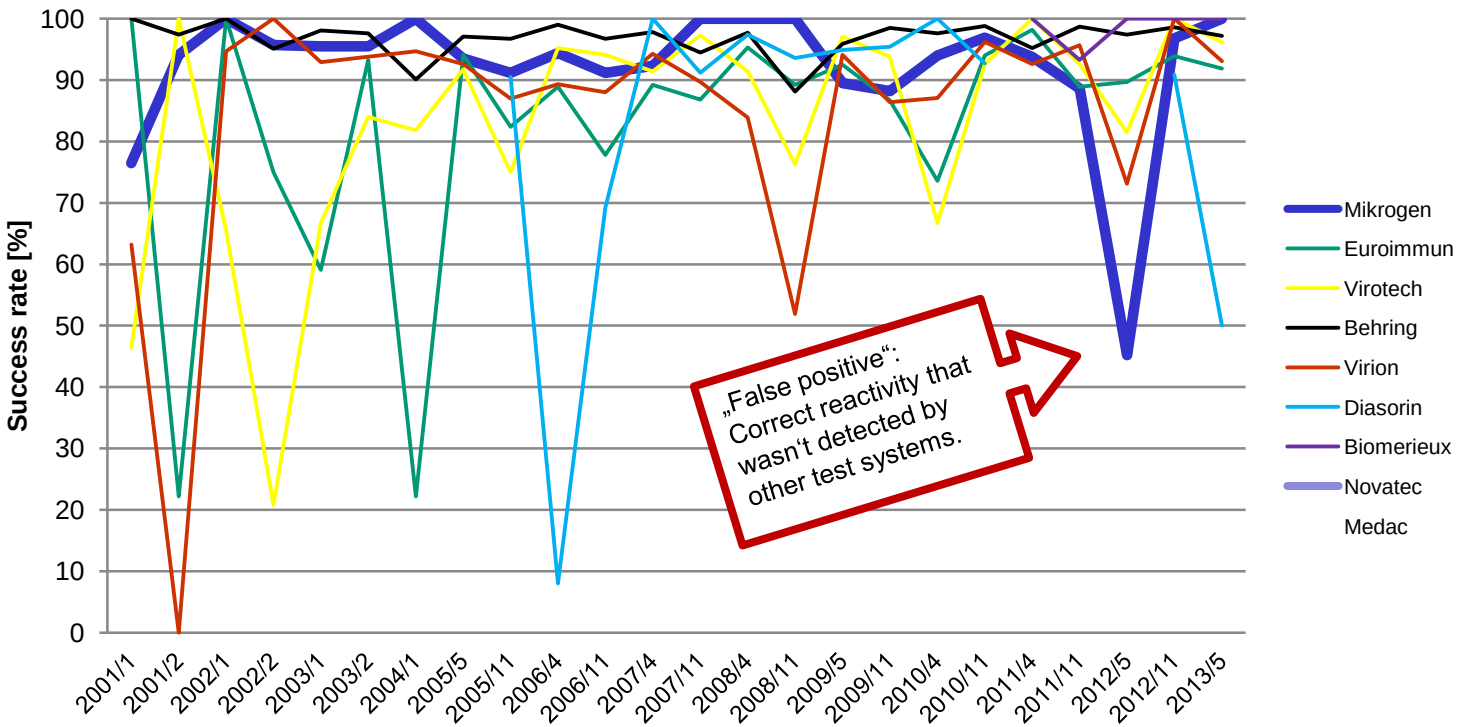
External Quality Assessment Instand e.V.
*recom*Well Borrelia IgG
Success rates



MIKROGEN DIAGNOSTIK

External Quality Assessment Instand e.V.
*recom*Well Borrelia IgM
Success rates



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Product Advantages *recom*Well Borrelia IgG, IgM

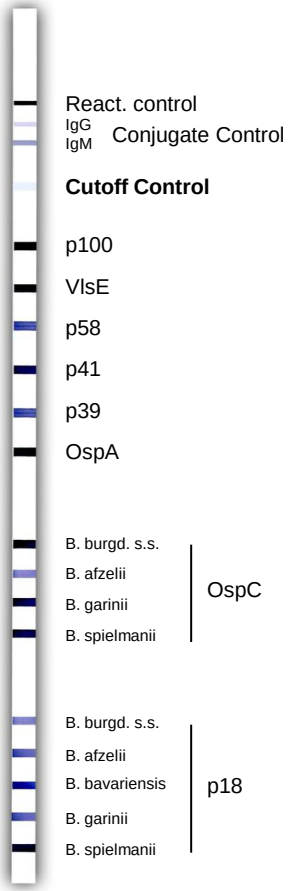
- Only ELISA relying exclusively on recombinant antigens
- IgM ELISA without RF absorption, saving time and money
- Optimal combination of immunodominant antigens leading to verification of early and late stage of disease
- CE-certified instruction for CSF/serum ratio
- Possible quantification of specific IgG and IgM

MIKROGEN

Your partner for diagnosis of Lyme disease

- **Screening**
  - *recom*Well Borrelia IgG
  - *recom*Well Borrelia IgM
- **Confirmation**
  - ***recom*Line Borrelia IgG**
  - ***recom*Line Borrelia IgM**
  - *recom*Bead Borrelia IgG
  - *recom*Bead Borrelia IgM

*recom*Line Borrelia IgG, IgM



→ **Conjugate control line**

→ **Integrated cutoff control**

→ **15 recombinant antigens covering 5 pathogenic genospecies**

→ **5 different p18 (DbpA) for the safe detection of acute Neuroborreliosis**

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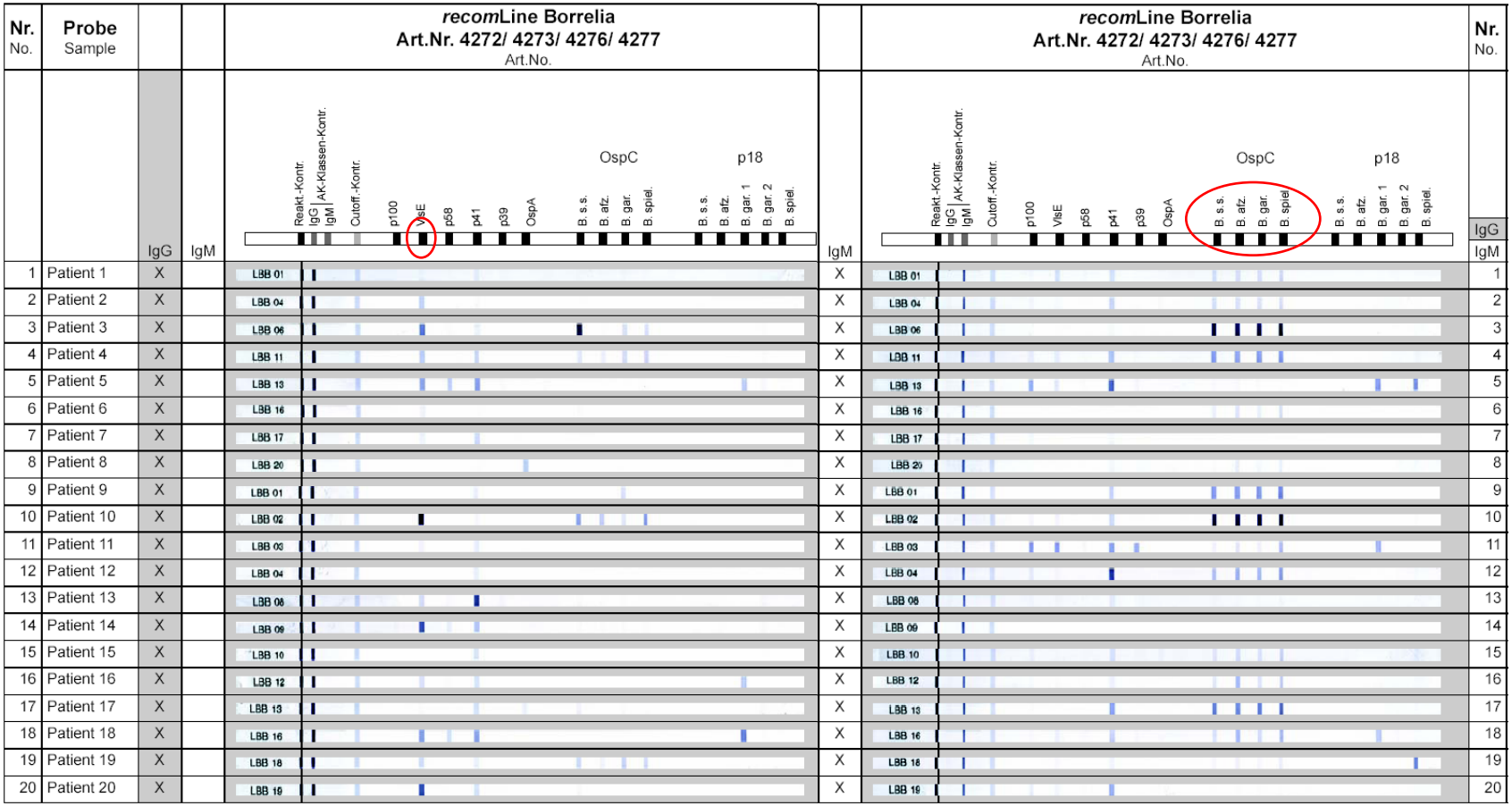
*recom*Line Borrelia IgG, IgM

Erythema migrans



- IgG: VlsE
- IgM: OspC

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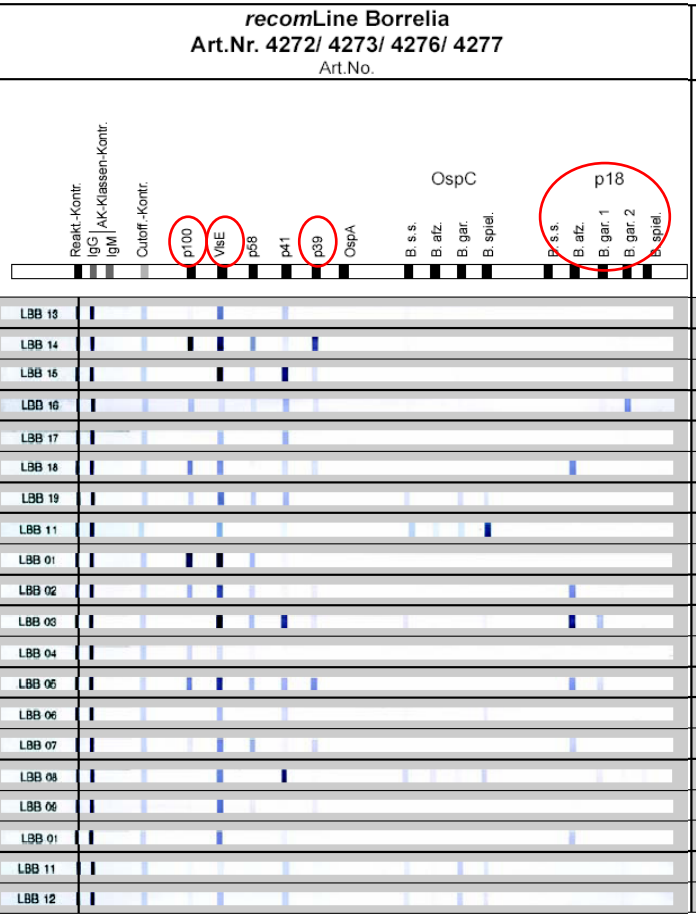
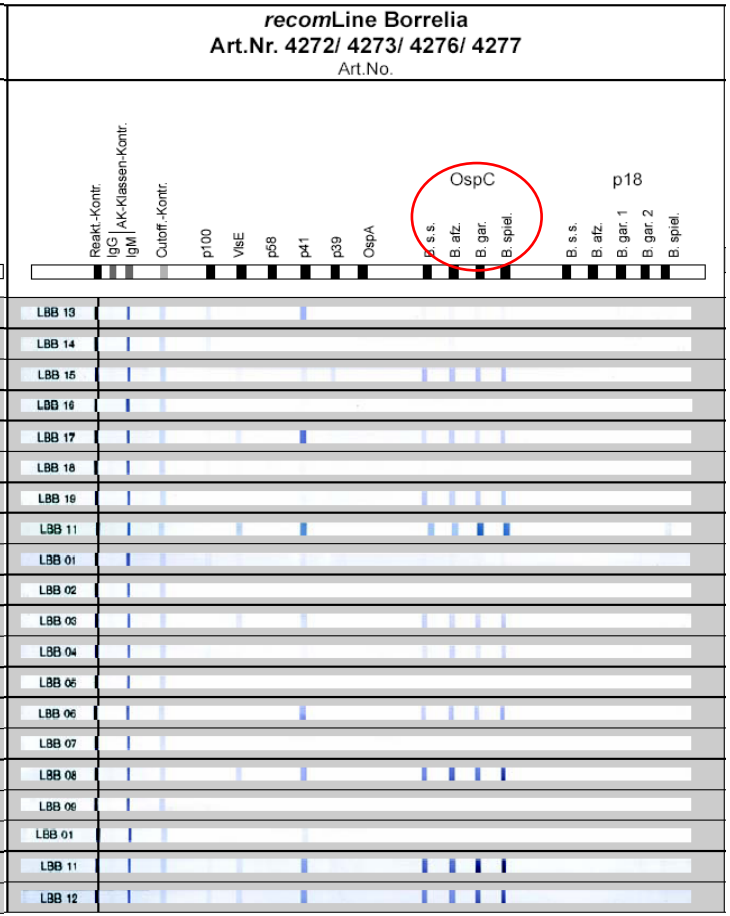
*recom*Line Borrelia IgG, IgM

Neuroborreliosis

Nr. No.	Probe Sample	IgG	IgM
1	Patient 1	X	
2	Patient 2	X	
3	Patient 3	X	
4	Patient 4	X	
5	Patient 5	X	
6	Patient 6	X	
7	Patient 7	X	
8	Patient 8	X	
9	Patient 9	X	
10	Patient 10	X	
11	Patient 11	X	
12	Patient 12	X	
13	Patient 13	X	
14	Patient 14	X	
15	Patient 15	X	
16	Patient 16	X	
17	Patient 17	X	
18	Patient 18	X	
19	Patient 19	X	
20	Patient 20	X	





- **IgG: p100, VlsE, p39 and p18**
- **IgM: OspC**

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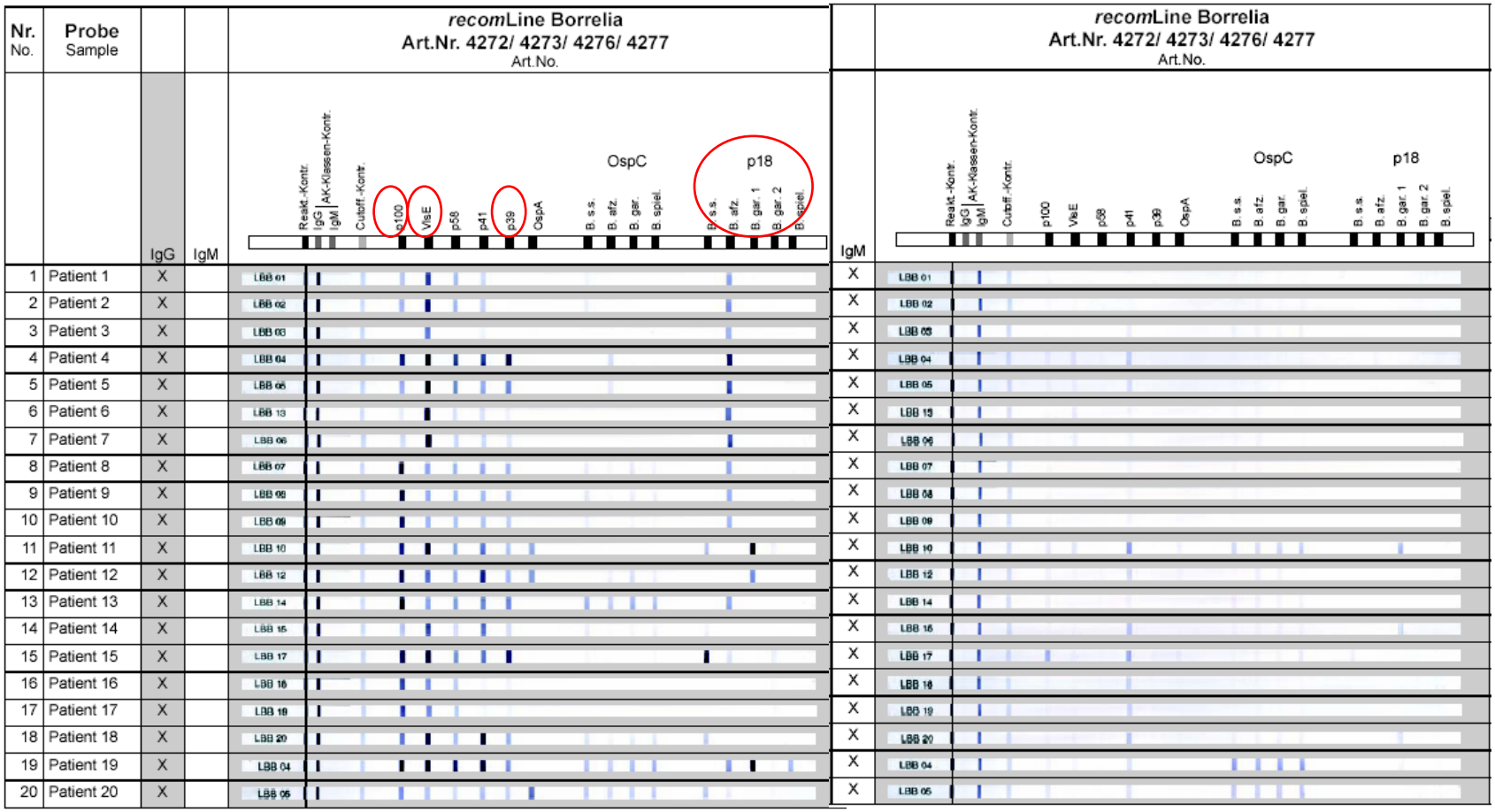
*recom*Line Borrelia IgG, IgM

Arthritis



- IgG: p100, VlsE, p39 and p18
- IgM: no relevance

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*recom*Line Borrelia - Performance

Diagnostic Specificity

*recom*Line Borrelia	Two comparison tests negative	
	IgG	IgM
Negative	171	169
Borderline	0	0
Positive	0	0
Specificity	100 %	100 %

Detection Rate

	IgG positive	IgM positive	IgG/IgM positive
Blood donor sera* (n = 200)	21 (10.5 %)	5 (2.5 %)	25 (12.5 %)

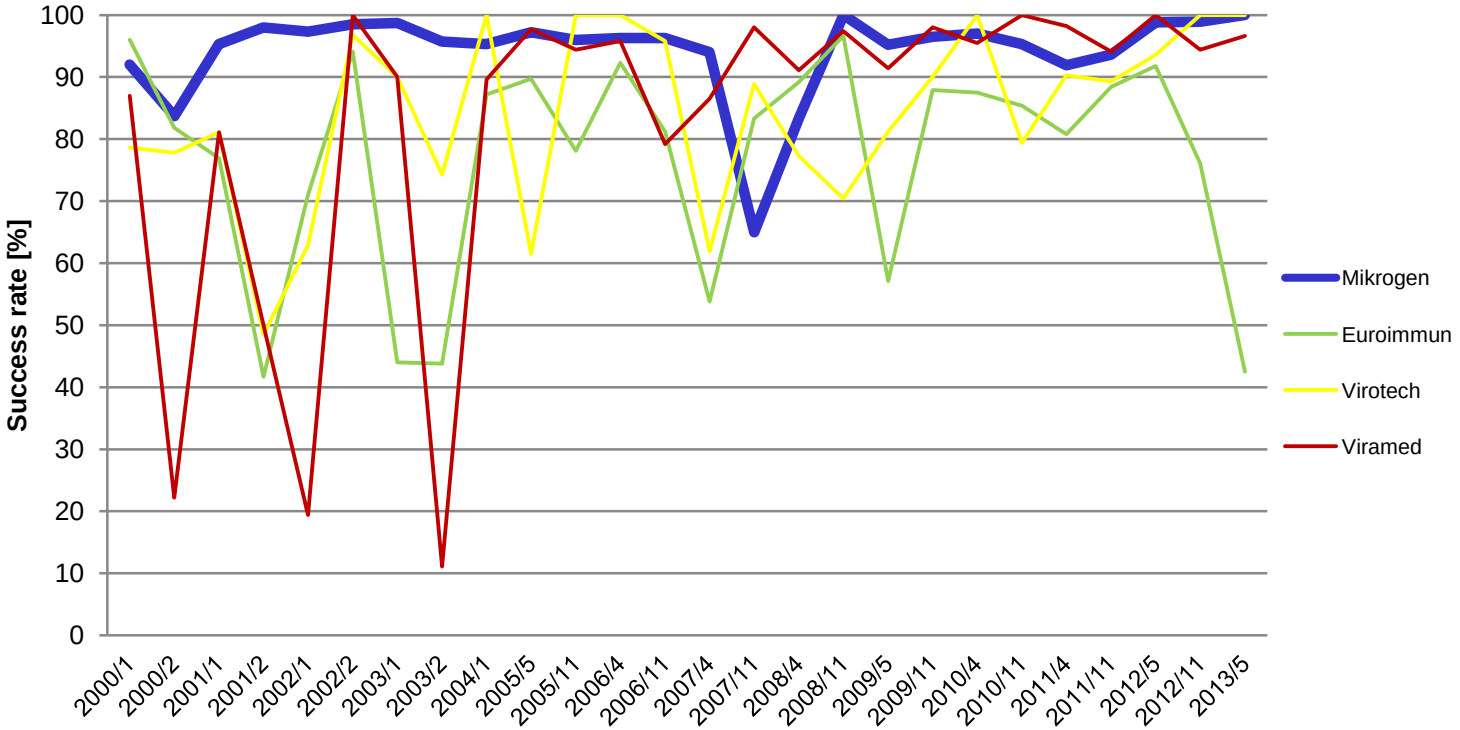
*from the south German region

Diagnostic Sensitivity

	IgG positive	IgM positive	IgG/IgM positive
Arthritis (n = 28)	27 (96 %)	6 (21 %)	27 (96 %)
Acrodermatitis (n = 11)	11 (100 %)	1 (9 %)	11 (100 %)
Neuroborreliosis (n = 35)	29 (83 %)	18 (51 %)	33 (94 %)
Erythema migrans (n = 42)	18 (43 %)	30 (71 %)	33 (79 %)

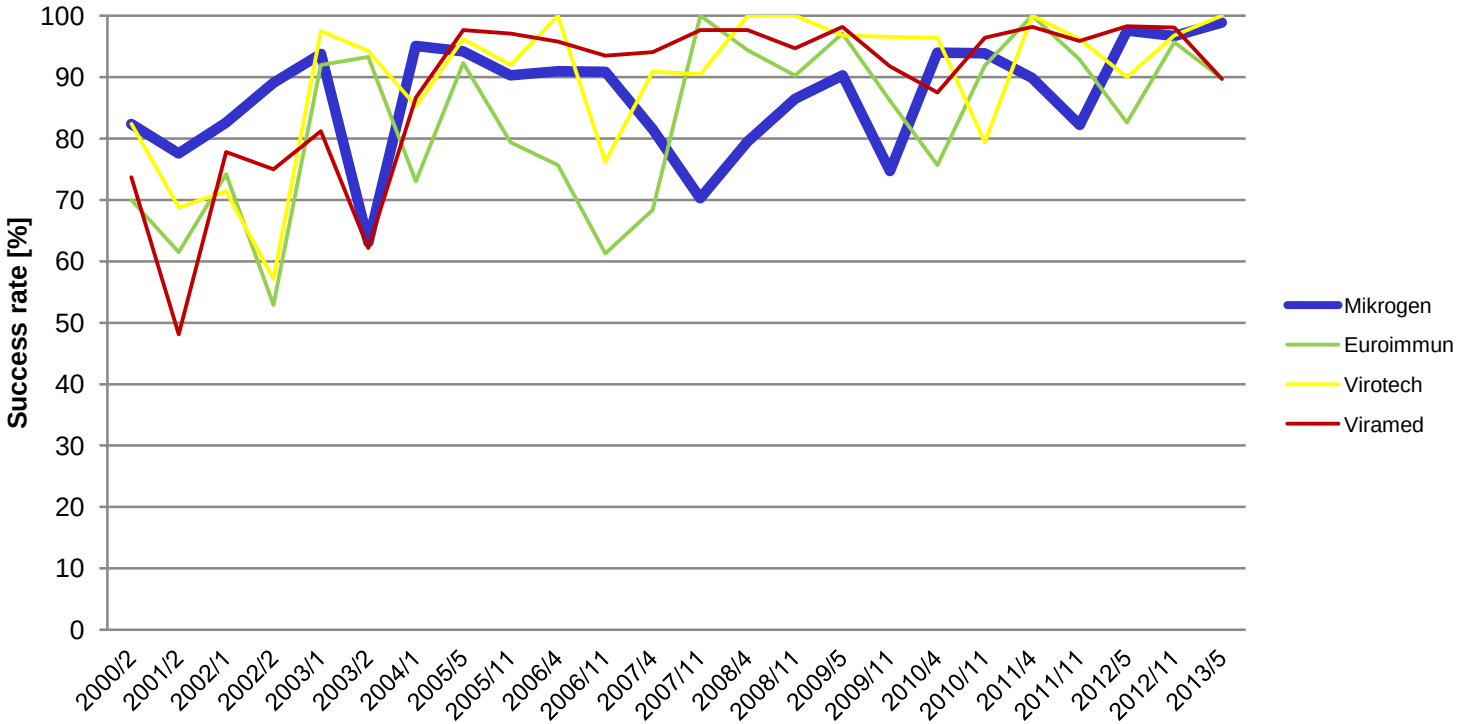
External Quality Assessment Instand e.V. *recom*Line Borrelia / *recom*Blot Borrelia IgG

Success rates

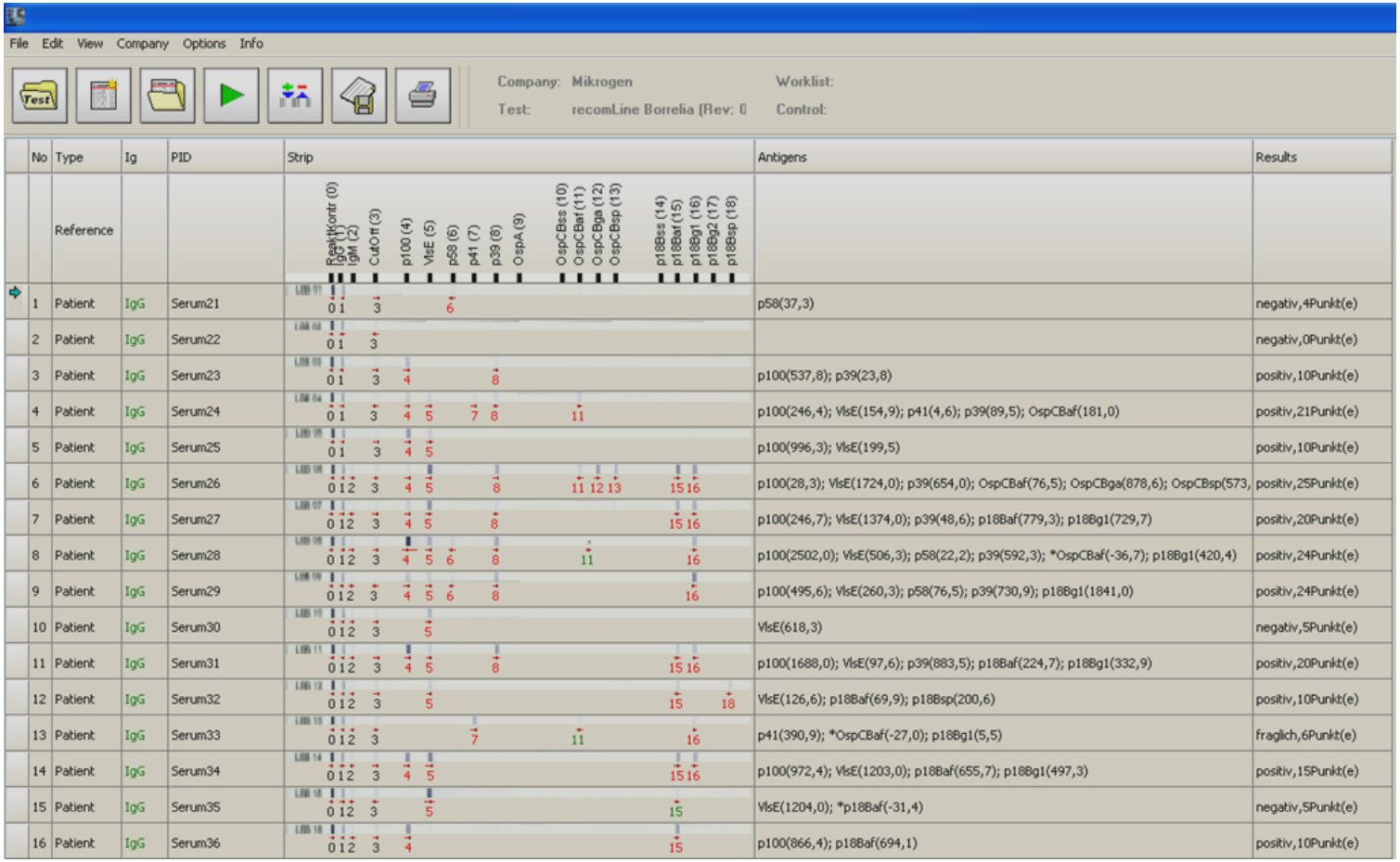


MIKROGEN DIAGNOSTIK

External Quality Assessment Instand e.V.
*recom*Line Borrelia / *recom*Blot Borrelia IgM
Success rates



Digital Evaluation with *recom*Scan



No	Type	Ig	PID	Strip	Antigens	Results
	Reference			Reaktivkontr (0) IgG (1) IgM (2) CutOff (3) p100 (4) VlsE (5) p58 (6) p41 (7) p39 (8) OspA (9) OspCBss (10) OspCBar (11) OspCBga (12) OspCBsp (13) p18Bss (14) p18Baf (15) p18Bg1 (16) p18Bg2 (17) p18Bsp (18)		
1	Patient	IgG	Serum21	0 1 3 6	p58(37,3)	negativ,4Punkt(e)
2	Patient	IgG	Serum22	0 1 3		negativ,0Punkt(e)
3	Patient	IgG	Serum23	0 1 3 4 8	p100(537,8); p39(23,8)	positiv,10Punkt(e)
4	Patient	IgG	Serum24	0 1 3 4 5 7 8 11	p100(246,4); VlsE(154,9); p41(4,6); p39(89,5); OspCBaf(181,0)	positiv,21Punkt(e)
5	Patient	IgG	Serum25	0 1 3 4 5	p100(996,3); VlsE(199,5)	positiv,10Punkt(e)
6	Patient	IgG	Serum26	0 1 2 3 4 5 8 11 12 13 15 16	p100(28,3); VlsE(1724,0); p39(654,0); OspCBaf(76,5); OspCBga(878,6); OspCBsp(573,	positiv,25Punkt(e)
7	Patient	IgG	Serum27	0 1 2 3 4 5 8 15 16	p100(246,7); VlsE(1374,0); p39(48,6); p18Baf(779,3); p18Bg1(729,7)	positiv,20Punkt(e)
8	Patient	IgG	Serum28	0 1 2 3 4 5 6 8 11 16	p100(2502,0); VlsE(506,3); p58(22,2); p39(592,3); *OspCBaf(-36,7); p18Bg1(420,4)	positiv,24Punkt(e)
9	Patient	IgG	Serum29	0 1 2 3 4 5 6 8 16	p100(495,6); VlsE(260,3); p58(76,5); p39(730,9); p18Bg1(1841,0)	positiv,24Punkt(e)
10	Patient	IgG	Serum30	0 1 2 3 5	VlsE(618,3)	negativ,5Punkt(e)
11	Patient	IgG	Serum31	0 1 2 3 4 5 8 15 16	p100(1688,0); VlsE(97,6); p39(883,5); p18Baf(224,7); p18Bg1(332,9)	positiv,20Punkt(e)
12	Patient	IgG	Serum32	0 1 2 3 5 15 18	VlsE(126,6); p18Baf(69,9); p18Bsp(200,6)	positiv,10Punkt(e)
13	Patient	IgG	Serum33	0 1 2 3 7 11 16	p41(390,9); *OspCBaf(-27,0); p18Bg1(5,5)	fraglich,6Punkt(e)
14	Patient	IgG	Serum34	0 1 2 3 4 5 15 16	p100(972,4); VlsE(1203,0); p18Baf(655,7); p18Bg1(497,3)	positiv,15Punkt(e)
15	Patient	IgG	Serum35	0 1 2 3 5 15	VlsE(1204,0); *p18Baf(-31,4)	negativ,5Punkt(e)
16	Patient	IgG	Serum36	0 1 2 3 4 15	p100(866,4); p18Baf(694,1)	positiv,10Punkt(e)

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Product Advantages *recom*Line Borrelia IgG, IgM

- Unique scope of immunodominant antigens leading to verification of early and late stages of Lyme disease
- Maximum sensitivity due to distinct antigen bands for p18 (DbpA) and OspC
- Reliable detection of acute Neuroborreliosis due to 5 different p18 (DbpA) antigens
- High reliability due to scientifically evaluated antigens
- Mikrogen holds the patents of important antigens

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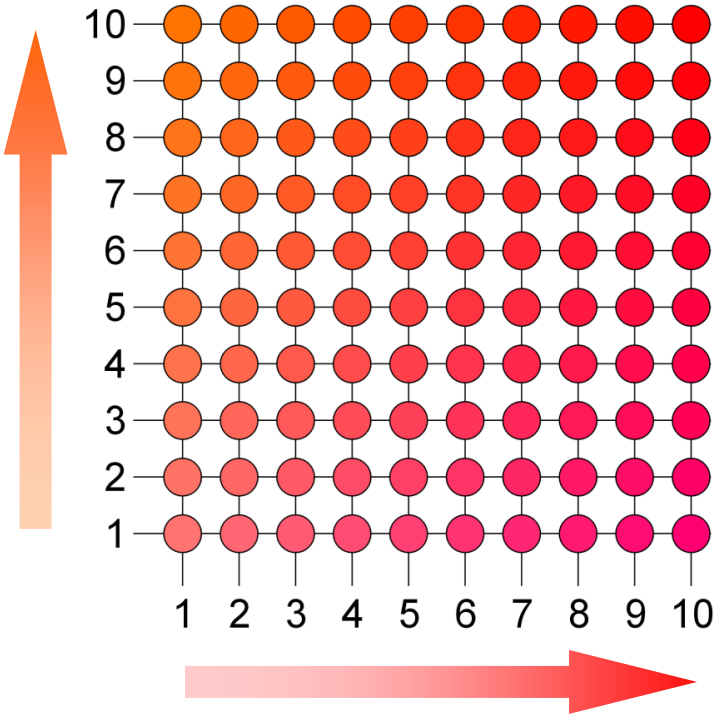
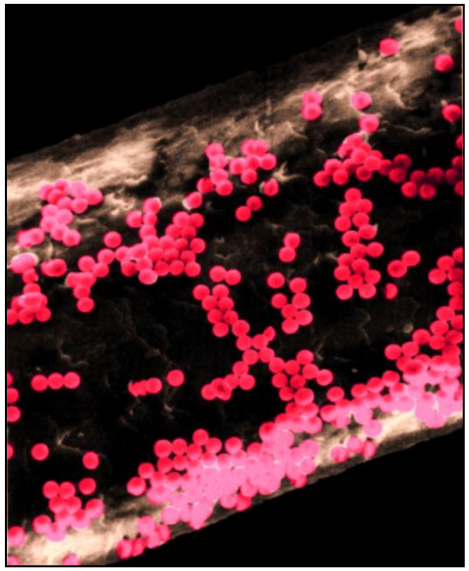
Your partner for diagnosis of Lyme disease

- **Screening** — *recom*Well Borrelia IgG; *recom*Well Borrelia IgM
- **Confirmation** — *recom*Line Borrelia IgG; *recom*Line Borrelia IgM

_recom_Bead Borrelia IgG 2.0
_recom_Bead Borrelia IgM 2.0

Beads with individual Colour Code allow multiplex Detection in a Sample

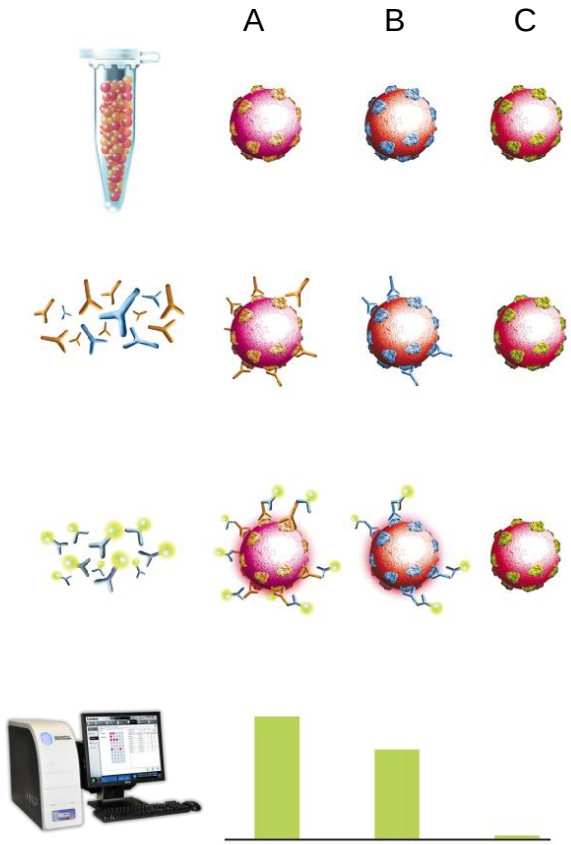




Human hair with
Luminex particles

- Polystyrene beads, 100 different bead populations exist. The populations can be distinguished from each other by their specific colour code.
- Each bead population can be coated with a specific antigen or antigen mixture, thus allowing analysis of up to 100 parameters in one single sample simultaneously.

Antibody Detection by Bead Technology



Example

Mixture of 3 bead populations (A, B and C), individually coated with specific recombinant antigens.

Sample (contains antibodies against antigens on bead population A and B) is incubated with a mixture of 3 bead populations.

Specifically bound antibodies in from the sample are labelled with fluorescence coupled (R-Phycoerythrin) conjugate antibodies.

Fluorescence intensity of the individual bead populations is measured.

LX 100/200 versus MAGPIX

*recom*Bead 2.0 test systems are compatible with both systems

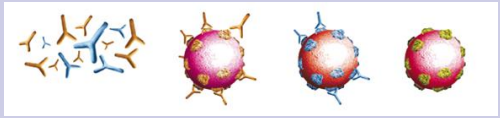
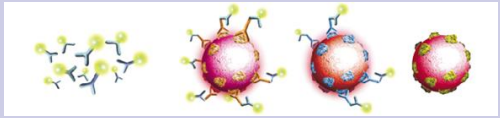
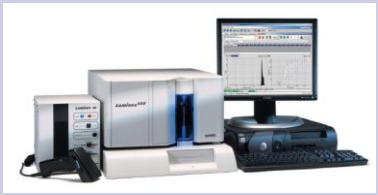
	LX 100/200	MAGPIX
Optics	Laser/APD/PMT	LED/CCD camera
Hardware	Flow cytometry based	Fluorescence imager
Bead compatibility	MicroPlex, MagPlex	MagPlex
Dynamic range	3,5 Log	
Microtiter plate	96 Well	
Footprint (incl. PC)	80 cm	64,8 cm
Weight	49 kg	17,5 kg
Software	xPonent	

MIKROGEN DIAGNOSTIK

The *recom*Bead 2.0 Test Format offers:

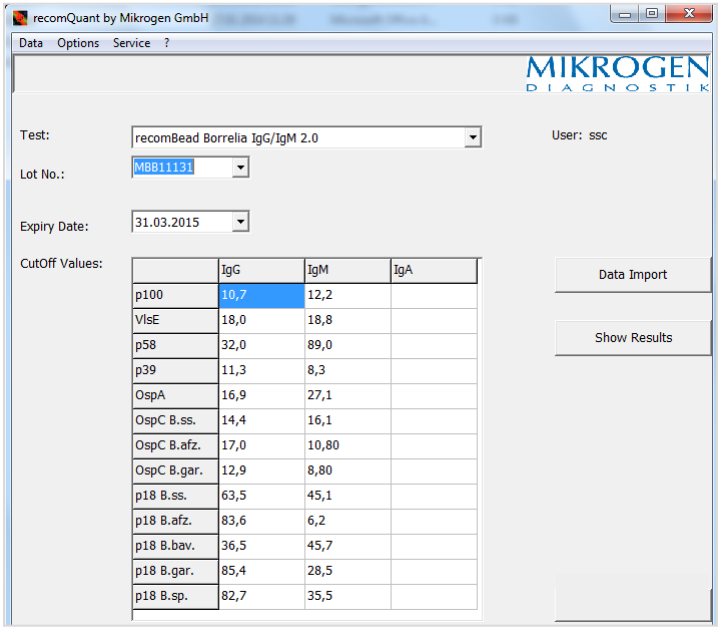
Paramagnetic Beads	• Easy washing on magnetic plate, fully automatable
Different Analysis systems	• New, affodable analysis system MAGPIX® • Compatible with LX100®/LX200®
High Throughput	• Simultaneous and separate detection of individual antigen specific antibodies • Confirmatory assay ideally suited for high sample throughput
Automation	• Fully automated processing and analysis possible • Integration into an existing laboratory information system possible
Speed	• 20 minutes sample incubation • 20 minutes conjugate incubation • Analysis result in less than 3 hours
High Flexibility	• Flexible combination of different test systems and conjugate classes on one plate possible - unique protocol and procedure for all Mikrogen *recom*Bead 2.0 test systems • Conjugate and buffer from different *recom*Bead 2.0 tests can be used for different parameters and batches. • Use of single microtiter bars possible
Precision	• Very high accuracy and reproducibility of test results
Safety	• Integration of all controls necessary for validation in each sample run • Incubation control, conjugate control, negative control
Low Sample Volume	• 10 µl sample volume

Test Procedure

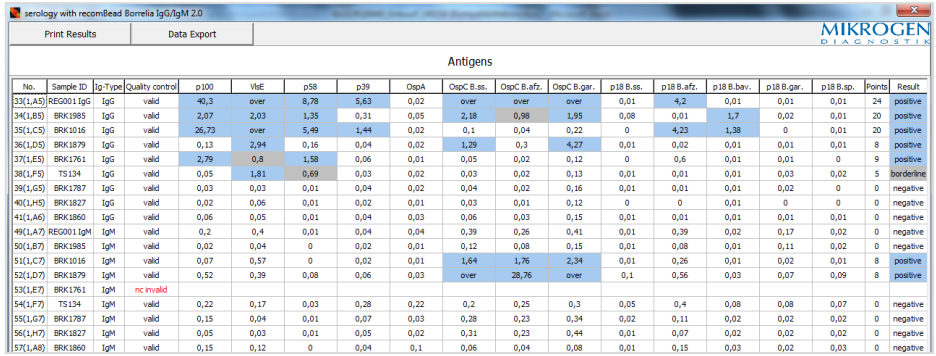
50 µl Beadmix + 50 µl Diluted sample IgG/IgM	Incubation: 20‘, 37°C 
	Wash 3x on magnetic plate The microtiter plate **must not** be knocked out!
50 µl Conjugate	Incubation: 20‘, 37°C 
	Wash 3x on magnetic plate The microtiter plate **must not** be knocked out!
100 µl System fluid	Measuring: MAGPIX or LX 100/200  
	Analysis: *recom*Quant

*recom*Quant – Software for Mikrogen Bead-Assays

- Data export to LIS
- Automatic Ig classification
- Input of lot data via barcode or download
- Automatic lot management



	IgG	IgM	IgA
p100	10,7	12,2	
VlsE	18,0	18,8	
p58	32,0	89,0	
p39	11,3	8,3	
OspA	16,9	27,1	
OspC B.ss.	14,4	16,1	
OspC B.afz.	17,0	10,80	
OspC B.gar.	12,9	8,80	
p18 B.ss.	63,5	45,1	
p18 B.afz.	83,6	6,2	
p18 B.bav.	36,5	45,7	
p18 B.gar.	85,4	28,5	
p18 B.sp.	82,7	35,5	



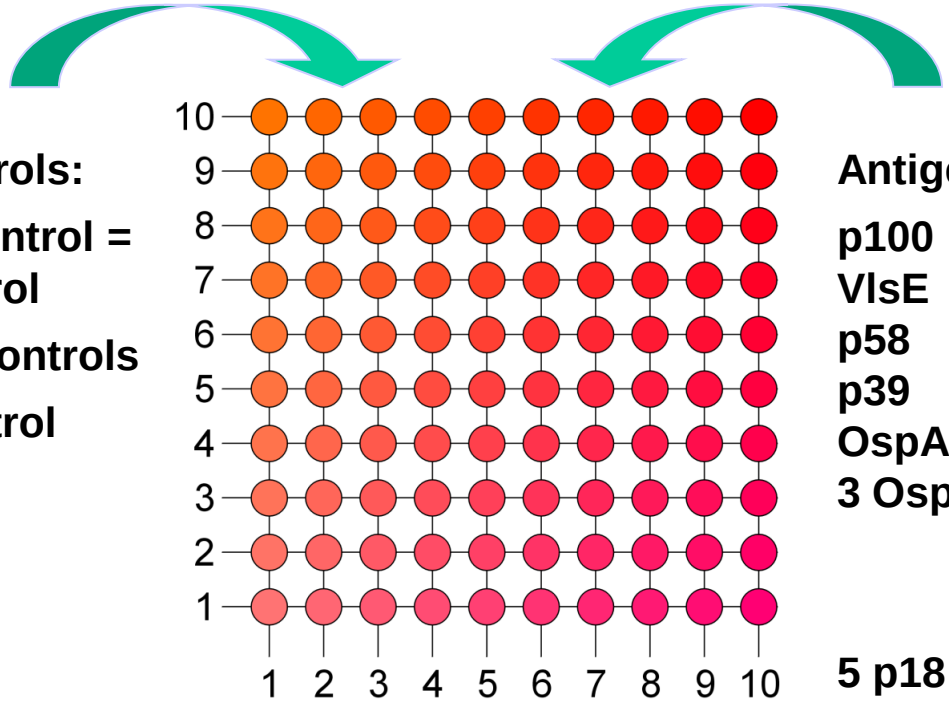
No.	Sample ID	Ig-Type	Quality control	p100	VlsE	p58	p39	OspA	OspC B.ss.	OspC B.afz.	OspC B.gar.	p18 B.ss.	p18 B.afz.	p18 B.bav.	p18 B.gar.	p18 B.sp.	Points	Result
33(1,A5)	REG001 IgG	IgG	valid	40,3	over	8,78	5,63	0,02	over	over	over	0,01	4,2	0,01	0,01	0,01	24	positive
34(1,B5)	BRK1985	IgG	valid	2,07	2,03	1,35	0,31	0,05	2,18	0,98	1,95	0,08	0,01	1,7	0,02	0,01	20	positive
35(1,C5)	BRK1016	IgG	valid	26,73	over	5,49	1,44	0,02	0,1	0,04	0,22	0	4,23	1,38	0	0,01	20	positive
36(1,D5)	BRK1879	IgG	valid	0,13	2,94	0,16	0,04	0,02	1,29	0,3	4,27	0,01	0,02	0,01	0,01	0,01	8	positive
37(1,E5)	BRK1761	IgG	valid	2,79	0,8	1,58	0,06	0,01	0,05	0,02	0,12	0	0,6	0,01	0,01	0	9	positive
38(1,F5)	TS134	IgG	valid	0,05	1,81	0,69	0,03	0,02	0,03	0,02	0,13	0,01	0,01	0,01	0,03	0,02	5	borderline
39(1,G5)	BRK1787	IgG	valid	0,03	0,03	0,01	0,04	0,02	0,04	0,02	0,16	0,01	0,01	0,01	0,02	0	0	negative
40(1,H5)	BRK1827	IgG	valid	0,02	0,06	0,01	0,02	0,01	0,03	0,01	0,12	0	0	0,01	0	0	0	negative
41(1,A6)	BRK1860	IgG	valid	0,06	0,05	0,01	0,04	0,03	0,06	0,03	0,15	0,01	0,01	0,01	0,01	0,01	0	negative
49(1,A7)	REG001 IgM	IgM	valid	0,2	0,4	0,01	0,04	0,04	0,39	0,26	0,41	0,01	0,39	0,02	0,17	0,02	0	negative
50(1,B7)	BRK1985	IgM	valid	0,02	0,04	0	0,02	0,01	0,12	0,08	0,15	0,01	0,08	0,01	0,11	0,02	0	negative
51(1,C7)	BRK1016	IgM	valid	0,07	0,57	0	0,02	0,01	1,64	1,76	2,34	0,01	0,26	0,01	0,02	0,01	8	positive
52(1,D7)	BRK1879	IgM	valid	0,52	0,39	0,08	0,06	0,03	over	28,76	over	0,1	0,56	0,03	0,07	0,09	8	positive
53(1,E7)	BRK1761	IgM	nc invalid															
54(1,F7)	TS134	IgM	valid	0,22	0,17	0,03	0,28	0,22	0,2	0,25	0,3	0,05	0,4	0,08	0,08	0,07	0	negative
55(1,G7)	BRK1787	IgM	valid	0,15	0,04	0,01	0,07	0,03	0,28	0,23	0,34	0,02	0,11	0,02	0,02	0,02	0	negative
56(1,H7)	BRK1827	IgM	valid	0,05	0,03	0,01	0,05	0,02	0,31	0,23	0,44	0,01	0,07	0,02	0,02	0,02	0	negative
57(1,A8)	BRK1860	IgM	valid	0,15	0,12	0	0,04	0,1	0,06	0,04	0,08	0,01	0,15	0,03	0,02	0,03	0	negative

*recom*Bead Borrelia 2.0 - Antigens

*recom*Bead Borrelia 2.0 is a qualitative test for the detection of IgG or IgM antibodies against *Borrelia burgdorferi sensu lato* in human serum, plasma or CSF

Integrated controls:

- **Incubation control = positive control**
- **2 conjugate controls**
- **Negative control**



Antigens (IgG + IgM):

p100
VlsE
p58
p39
OspA
3 OspC
- **B.b. sensu stricto**
- **B. afzelii**
- **B. garinii**

5 p18
- **B.b. sensu stricto**
- **B. afzelii**
- **B. bavariensis**
- **B. garinii**
- **B. spielmanii**

*recom*Bead Borrelia 2.0 – Performance

Seroprevalence

	IgG positive	IgM positive	IgG/IgM positive
Blood donors (n = 200)	27 (13,5 %)	11 (5,5 %)	35 (17,5 %)*

* Within the 35 (17,5%) IgG/IgM positive samples 30 (15,0%) showed the typical antibody pattern of an infection with *Borrelia burgdorferi*. The remaining 5 samples (2,5%) exhibit weak unspecific reactivities (background).

Sensitivity

	IgG positive	IgM positive	IgG/IgM positive
Arthritis (n = 27)	27 (100 %)	6 (22 %)	27 (100 %)
Acrodermatitis (n = 11)	11 (100 %)	2 (18 %)	11 (100 %)
Neuroborreliosis (n = 30)	26 (87 %)	16 (53 %)	28 (93 %)
Erythema migrans (n = 38)	21 (55 %)	25 (66 %)	30 (79 %)

*recom*Bead Borrelia - Courses

Serological scar, course over two years

				Antigene														
No.	Sample	IgG/IgM	Status	p100	VlsE	p58	p39	OspA	OspC B.ss.	OspC B.a.	OspC B.g.	p18 B.ss	p18 B.a.	p18 B.g.1	p18 B.g.2	p18 B.sp.	Punkte	Result
2 (B1)	2304322	IgG	gültig	0,3	**11,5**	**0,8**	0,1	0,0	0,1	0,2	0,3	0,0	**3,1**	0,0	0,2	0,0	9	positiv
2 (B1)		IgM	gültig	0,1	0,2	0,0	0,1	0,0	**1,3**	**1,1**	**1,8**	0,0	0,5	0,0	0,0	0,2	8	positiv
3 (C1)	2441065	IgG	gültig	0,3	**15,0**	**1,0**	0,1	0,0	0,1	0,3	0,3	0,0	**3,6**	0,0	0,2	0,0	9	positiv
3 (C1)		IgM	gültig	0,1	0,2	0,0	0,1	0,0	**1,6**	**1,5**	**2,3**	0,0	0,5	0,1	0,0	0,3	8	positiv
4 (D1)	2969118	IgG	gültig	0,4	**16,9**	**1,4**	0,1	0,0	0,2	0,4	0,3	0,0	**3,8**	0,0	0,2	0,0	12	positiv
4 (D1)		IgM	gültig	0,2	0,2	0,0	0,1	0,0	**1,8**	**1,5**	**2,7**	0,0	**0,7**	0,1	0,0	0,4	9	positiv
5 (E1)	3278918	IgG	gültig	0,2	**11,2**	**0,8**	0,1	0,0	0,1	0,2	0,3	0,0	**3,2**	0,0	0,2	0,0	9	positiv
5 (E1)		IgM	gültig	0,1	0,2	0,0	0,1	0,0	**1,1**	**1,0**	**1,6**	0,0	0,4	0,1	0,0	0,3	8	positiv
6 (F1)	3386864	IgG	gültig	0,2	**9,9**	**0,8**	0,1	0,0	0,1	0,2	0,2	0,0	**3,1**	0,0	0,2	0,0	9	positiv
6 (F1)		IgM	gültig	0,1	0,1	0,0	0,1	0,0	**1,1**	**1,0**	**1,8**	0,0	0,5	0,1	0,0	0,3	8	positiv

Serological scar (child, 22 months), reinfection with booster effect in IgG

				Antigene														
No.	Sample	IgG/IgM	Status	p100	VlsE	p58	p39	OspA	OspC B.ss.	OspC B.a.	OspC B.g.	p18 B.ss	p18 B.a.	p18 B.g.1	p18 B.g.2	p18 B.sp.	Punkte	Result
13 (E2)	3315491	IgG	gültig	0,2	**13,6**	**1,4**	0,1	0,0	0,1	0,1	0,2	0,1	**1,2**	0,1	0,2	0,0	12	positiv
13 (E2)		IgM	gültig	0,1	0,3	0,0	0,1	0,0	0,6	0,6	**0,8**	0,0	0,3	0,0	0,0	0,5	1	negativ
11 (C2)	3341132	IgG	gültig	0,6	**35,9**	**3,3**	0,3	0,2	0,1	0,2	0,3	0,1	**3,0**	0,2	0,2	0,0	12	positiv
11 (C2)		IgM	gültig	0,1	0,6	0,0	0,1	0,0	**1,0**	**0,9**	**1,3**	0,0	0,3	0,1	0,1	**0,9**	9	positiv
10 (B2)	3407848	IgG	gültig	0,2	**27,2**	**1,9**	0,2	0,1	0,1	0,2	0,2	0,1	**2,6**	0,1	0,2	0,0	12	positiv
10 (B2)		IgM	gültig	0,1	0,4	0,0	0,1	0,0	**0,8**	**0,8**	**1,2**	0,0	0,3	0,0	0,1	0,5	8	positiv

Serological scar after therapy, reinfection with booster effect in IgG, persisting IgM

				Antigene														
No.	Sample	IgG/IgM	Status	p100	VlsE	p58	p39	OspA	OspC B.ss.	OspC B.a.	OspC B.g.	p18 B.ss	p18 B.a.	p18 B.g.1	p18 B.g.2	p18 B.sp.	Punkte	Result
19 (C3)	3203716	IgG	gültig	0,1	**6,5**	**0,8**	0,0	0,0	**0,7**	0,3	**0,9**	0,0	0,0	0,0	0,3	0,0	6	fraglich
19 (C3)		IgM	gültig	0,1	0,3	0,0	0,2	0,0	**3,3**	**3,4**	**5,3**	0,0	0,1	0,1	0,0	0,1	8	positiv
18 (B3)	3290899	IgG	gültig	0,4	**31,9**	**5,8**	0,6	0,0	0,4	0,2	**0,7**	0,0	**3,0**	0,2	0,3	0,0	13	positiv
18 (B3)		IgM	gültig	**1,1**	0,4	0,0	0,2	0,0	**3,3**	**3,4**	**5,1**	0,0	0,4	0,1	0,0	0,0	12	positiv
20 (D3)	3408101	IgG	gültig	0,1	**10,2**	**1,8**	0,3	0,0	0,4	0,2	**0,9**	0,0	**1,7**	0,1	0,3	0,0	13	positiv
20 (D3)		IgM	gültig	0,5	0,2	0,0	0,2	0,0	**3,4**	**3,5**	**5,4**	0,0	0,1	0,1	0,0	0,1	8	positiv

MIKROGEN DIAGNOSTIK

Summary – *recom*Bead Borrelia 2.0

- Immuno-dominant, recombinant antigens of the five pathogenic Borrelia genospecies
  - Broadest Borrelia genospecies antigen spectrum
  - Highest sensitivity and specificity
- Short and easy processing – system solutions
- Integrated controls (as bead regions)
- Wash assay
  - Avoids drift of reactivity (over the plate)
  - No sensitivity problem

Summary - *recom*Bead 2.0 Product Line

- Paramagnetic beads
  - Easy washing on magnetic plate, fully automatable
- New, affordable analysis system MAGPIX®
- Compatible with analysis systems LX100®/LX200®
- Short incubation times: ∑ 40 min (formerly 90 min)
- High flexibility
  - Combination of different test systems and conjugate classes on one plate
  - Unique protocol and procedure for all Mikrogen *recom*Bead 2.0 test systems
  - Conjugate and buffer can be used for different parameters and batches.
  - Use of single microtiter bars possible
- One-step sample dilution and low sample volume
- Integration of all controls necessary for validation in each sample run:
  - Incubation control = Positive control
  - Conjugate control
  - Negative control for the detection of unspecific reactivities

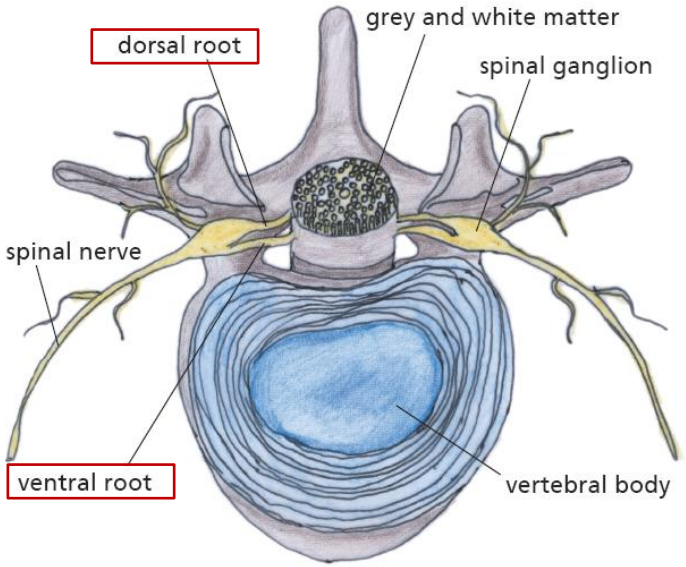
Lyme Neuroborreliosis

- Lyme neuroborreliosis (LNB) is an infectious disorder of the nervous system caused by tick-borne spirochetes of the Borrelia burgdorferi (Bb) sensu lato complex.
- Clinical features of LNB are diverse and differ in European and American patients – most probably because of different bacteria species.
- The diagnosis of defined LNB is supported by i) neurological symptoms, ii) a lymphocytic pleocytosis in the cerebrospinal fluid (CSF) and iii) intrathecally produced Borrelia burgdorferi (Bb) specific antibodies.
- LNB should be treated with antibiotics to achieve rapid resolution of symptoms and theoretically to avoid spreading and persistence of infection. The choice of the best antibiotic, the preferred mode of administration, and the duration of treatment are the still debated issues.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis | Myglanda A et al. | European Journal of Neurology 2010, 17: 8–16

Clinical Appearance

- On average, the symptoms of acute LNB develop 4-6 weeks after a tick bite.
- 90% of LNB patients suffer from a painful meningopolyradiculitis (Bannwarth syndrome).
  - Inflammations of the nerve roots cause severe pain in the corresponding regions. Complaints typically get worse in the night.
- Deficiencies of the brain nerves may occur (60%). This very frequently (80%) affects the nervus fascialis. In this paralysis, the mimic facial musculature of one or both sides of the face fails to work (facial nerve paresis).
- Chronic LNB is described very rarely – in this case, the central nervous system is involved.
- The most frequent manifestation is myelitis
- Isolated meningitis can also occur as a form of LNB, although mainly in children.
- All forms of early LNB generally respond well to antibiotic therapies.



Cross-section of the vertebral body

Early LNB **(> 95 %)**
Neurological symptoms for < 6 months
With manifestations confined to PNS (cranial nerves, spinal roots or peripheral nerves) (Bannwarth syndrome)
With CNS manifestations
Late LNB **(< 5 %)**
Neurological symptoms for more than 6 months
With PNS manifestations
With CNS manifestations

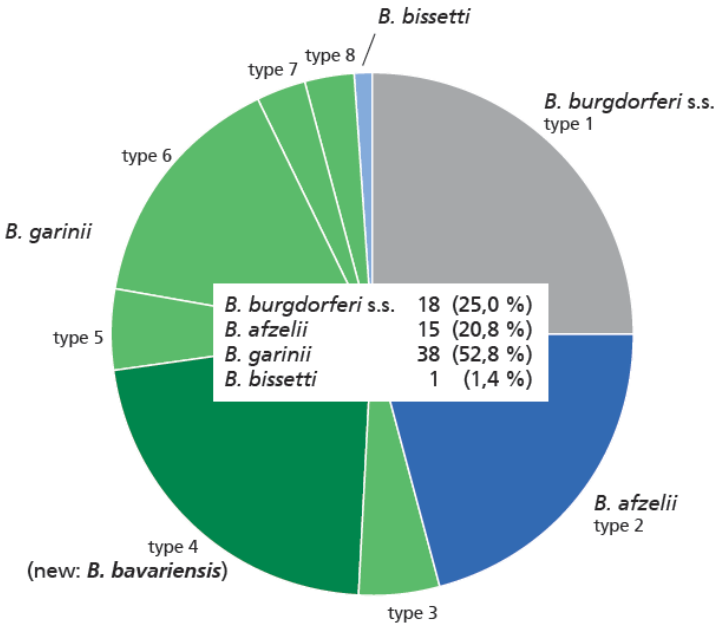
PNS, peripheral nervous system.

Classification LNB

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis | Myglanda A et al. | European Journal of Neurology 2010, 17: 8–16

Pathogenesis

- All pathogenic borrelia species (involvement of B. spielmanii is unclear) can cause LNB in principle. Borrelia garinii, however, is found in more than half of CSF isolates. It is interesting that especially B. garinii type 4 seem to be able to enter the nervous tissue.
- It is not known how borreliae finally pass from the skin to the central nervous system.
  - There are two possibilities in principle – via the bloodstream or along peripheral nerves.
  - Both hypotheses may be correct and, for example, Borrelia burgdorferi sensu stricto, which in the US frequently causes meningitis as LNB, reaches the CNS via blood, and B. garinii, here in Europe, via the peripheral nerves.



Species distribution in CSF. OspA subtypes (type 1 to type 8): Lecture Fr. Prof. Wilske, Mikrogen symposium 15.05.2009

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis 1/2

Myglanda A et al. | European Journal of Neurology 2010, 17: 8–16

Method	Recommendations
PCR	PCR on CSF samples has a low sensitivity, but **may be useful in very early LNB with negative antibody index (AI), or in patients with immunodeficiency**. Because of low sensitivity and unknown specificity, PCR cannot be recommended as a diagnostic method in patients with chronic symptoms or for follow-up of therapy.
Cultivation	Because of its low sensitivity, slow growth and restriction to a few specialized laboratories, **culture of Bb is limited to special indications such as atypical clinical presentation or patients with immune deficiencies**.
Bb-specific antibodies in serum and CSF	Antibody tests for serum and CSF (AI) are **useful in the diagnosis of LNB, but are hampered by a low sensitivity in patients with symptom duration <6 weeks, and by low specificity, if judged without other criteria**. Because of the low specificity, antibody results can only be interpreted together with clinical data and CSF inflammation parameters. Therefore, **antibody testing should only be carried out in patients with symptoms suggestive of LNB.**

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis 2/2

Myglanda A et al. | European Journal of Neurology 2010, 17: 8–16

Method	Recommendations
Microscope-based assays	There is not enough evidence to recommend microscope-based assays as a routine diagnostic tool.
Chemokine CXCL13	There is not enough evidence to recommend CXCL13 test as a routine diagnostic tool or in follow-up after treatment.
Antigen detection	There is not enough evidence to recommend antigen detection assays as a routine diagnostic tool or in follow-up after treatment.
Detection of antibodies against circulating immune complexes	There is not enough evidence to recommend immune complex tests as a routine diagnostic tool.
Lymphocyte trans-formation test (LTT)	There is not enough evidence to recommend LTT as a routine diagnostic tool or in followup after treatment.
Cyst formation	There is not enough evidence to recommend examination for cyst formation ("cysts" spheroplasts or L-forms of Bb can be induced in vitro by stressors such as high temperature or change in pH) as a diagnostic tool.
CD57+/CD3- lympho-cyte subpopulation	There is not enough evidence to recommend examination for lymphocyte subpopulations as a diagnostic tool.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis

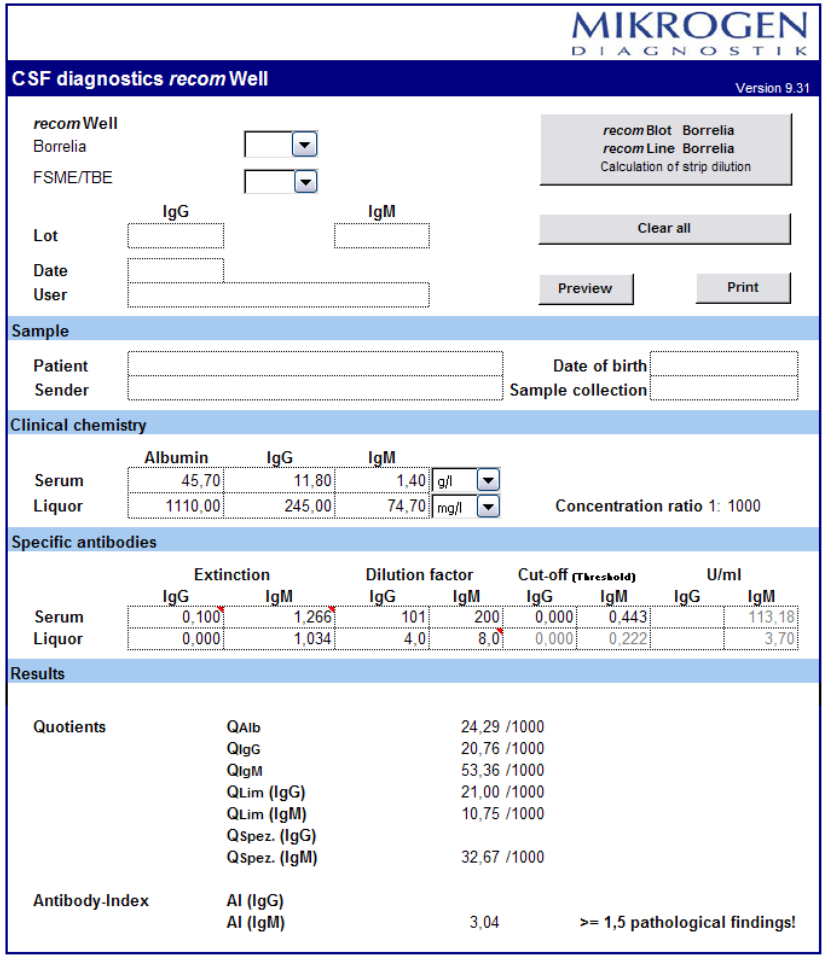
Myglanda A et al. | European Journal of Neurology 2010, 17: 8–16

Recommendations - Choice of laboratory methods

1. Investigation of CSF/serum pair for Bb-specific antibodies, intrathecal antibody production and signs of CSF inflammation is obligatory for laboratory diagnosis of LNB.
2. Culture and PCR may be corroborative in very early LNB.
3. At present, no further methods are recommendable.

CSF Analysis with *recom*Well Borrelia

- Analyse serum and CSF (from the same day) using the usual serological screening ELISA.
- Subsequently determine extinction of serum and CSF with appropriate dilutions (linear portion of the dilution curve) using a single point quantification or standard curve. Now, data from the clinical chemistry are required too.
- (Automatically) calculate the AI using the values from the clinical chemistry and extinctions obtained.


MIKROGEN DIAGNOSTIK

CSF diagnostics *recom*Well Version 9.31

*recom*Well Borrelia

FSME/TBE

*recom*Blot Borrelia
*recom*Line Borrelia
Calculation of strip dilution

Clear all

Preview Print

	IgG	IgM
Lot		
Date		
User		

Sample

Patient		Date of birth	
Sender		Sample collection	

Clinical chemistry

	Albumin	IgG	IgM	
Serum	45,70	11,80	1,40	g/l
Liquor	1110,00	245,00	74,70	mg/l

Concentration ratio 1: 1000

Specific antibodies

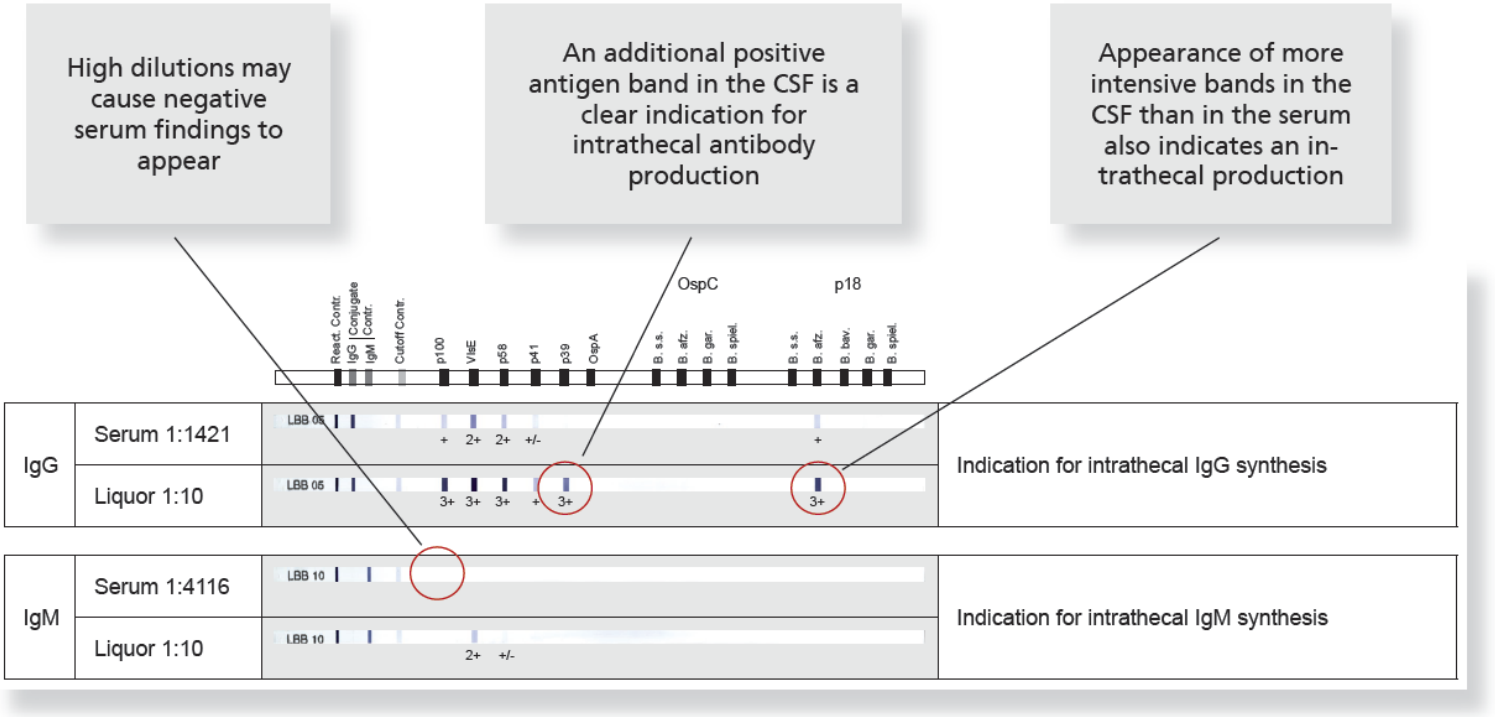
	Extinction IgG	Extinction IgM	Dilution factor IgG	Dilution factor IgM	Cut-off (Threshold) IgG	Cut-off (Threshold) IgM	U/ml IgG	U/ml IgM
Serum	0,100	1,266	101	200	0,000	0,443		113,18
Liquor	0,000	1,034	4,0	8,0	0,000	0,222		3,70

Results

Quotients	QAlb	24,29 /1000	
	QIgG	20,76 /1000	
	QIgM	53,36 /1000	
	QLim (IgG)	21,00 /1000	
	QLim (IgM)	10,75 /1000	
	QSpez. (IgG)		
	QSpez. (IgM)	32,67 /1000	
Antibody-Index	AI (IgG)		
	AI (IgM)	3,04	>= 1,5 pathological findings!

CSF analysis software

Visualization of Specific Intrathecal Antibodies in *recom*Line Borrelia



LNB Testing with *recom*Well Borrelia and *recom*Line Borrelia

Clinically defined CSF/serum pairs, n = 47

n	known clinical data	anti-B.b. antibodies (serum)	IgG AI positive/ borderl.	IgM AI positive borderl.	IgG: confirmation with *recom*Line	IgM: confirmation with *recom*Line
21	Neuroborreliosis[1]	yes	21 (100%)	18 (86%)	19/21 (90%)	18/18 (100%)
7	negative[2]	no	0	0	-	-
19	Multiple Sclerosis	2 yes 17 no	0	0	-	-

[1] Cell count >5/µl and disturbed blood-cerebrospinal fluid barrier (at least in one of possibly several samples of one patient) and Ig synthesis (at least in one of possibly several samples of one patient), anti-*Borrelia burgdorferi* antibodies in the serum.
[2] Cell count <5/µl, intact blood-CSF barrier, no intrathecal Ig synthesis, no anti-B.b. antibodies in the serum.