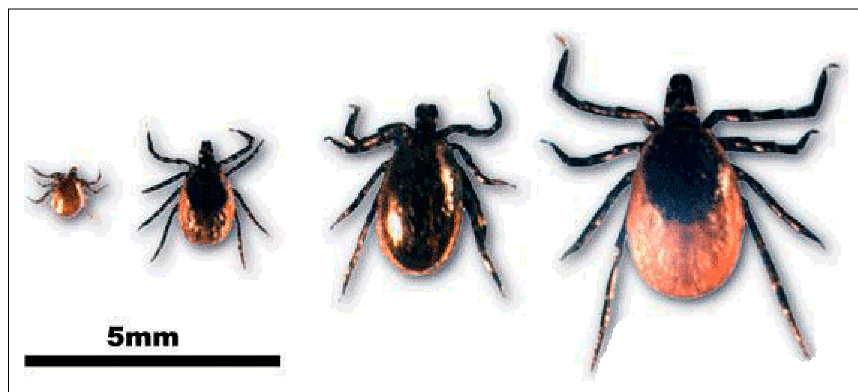
BORRELIA

1. Overview

1.1. History

Lyme borreliosis (Lyme disease) 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 illness is caused by a spirochaete (spiral-shaped bacteria), which is transmitted during the blood feeding of ticks of the genus Ixodes.



Larva, nymph, male and female tick

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. Subsequent studies led to the isolation from the deer tick, Ixodes scapularis (syn, dammini) of a gram-negative spirochaete, which was named Borrelia burgdorferi. The disease has, however, been known in Europe under a variety of names (including erythema migrans, neuroborreliosis, acrodermatitis chronica atrophicans, Bannwarth's syndrome) since the 1880's.
In 1909, Afzelius had associated a red rash (erythema migrans) with the tick, Ixodes ricinus.

In 1948, spirochaetes were observed in EM biopsies and in 1951 a Swedish clinician, Hollstrom, successfully treated EM infected patients with penicillin. Also in 1951, it was suggested that EM, with associated meningitis, was probably the result of an infection by a tick- or other insect-borne bacterium. A transmittable bacterial aetiology for EM had also been indicated by the EM biopsy transplant experiments carried out by Binder et al. in 1955, and Asbrink et al. in 1978. However, EM was considered a relatively harmless condition with no connection made between the lesion and subsequent symptoms caused by the same bacterium.

1.2. Clinical Features

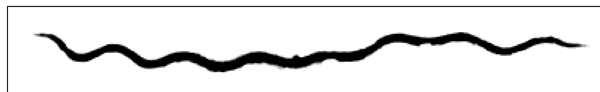
Clinical presentations can generally be divided into three stages but progress from an early to later stage is not inevitable, even if the infection is untreated:

- Stage 1: early dermatitis, appearing days or weeks after the infection.
  Clinical: erythema migrans.
- Stage 2: early disseminated infection, appears weeks or months after infection.
  Clinical: lymphocytic meningoradiculitis (Bannwarth's syndrome), neuroborreliosis.
- Stage 3: late disseminated infection, occuring up to years after infection.
  Clinical: Chronic progressive encephalomyelitis, acrodermatitis chronica atrophicans, chronic arthritis.

Many features of later infection are not specific to Lyme Borreliosis and occur in other conditions.

1.3. The Bacterium

Borrelia burgdorferi, the causative agent of LB, is a gram-negative, microaerophilic bacterium which belongs to the family Spirochaetaceae.

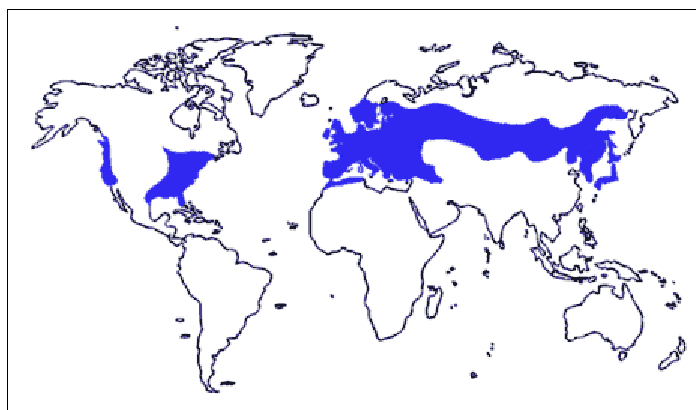


Borrelia burgdorferi

There is evidence that the phenotypic diversity of B. burgdorferi isolates has significant clinical relevance, with different species being associated with particular symptoms, and this could account for the geographical variations in the clinical picture of the disease.

1.4. Geographical Incidence

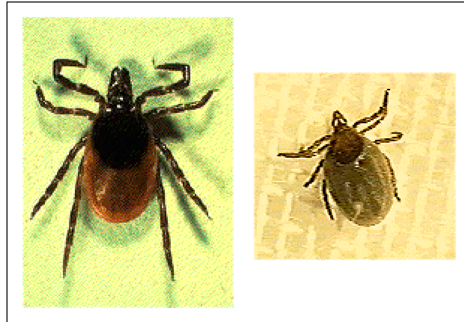
Following the discovery of B. burgdorferi as the causative agent of LB in 1982, the disease emerged as the most prevalent arthropod-borne infection in temperate climate zones around the world. Clinically confirmed cases of LB have been reported all over
Eurasia, as well in the USA and Canada:



Lyme disease regions worldwide

1.5. Transmission Vector

A large number of ticks have been reported as carriers of B. burgdorferi, however the presence of the spirochaete in questing ticks does not necessarily mean that the ticks are effective in transmitting the disease. In Europe the main vector is Ixodes ricinus, while I. persulcatus is primarily responsible for transmission in Asia. In the Northeastern and the upper Midwestern United States I. scapularis (syn. dammini) is the main vector, and I. pacificus is the vector in the West of the USA.



Adult female and engorged nymph of Ixodes scapularis

The process of transmission occurs through salivation during the feeding process on an animal host. The ticks have a three-stage life cycle; larva, nymph, and adult, with the ticks feeding only once during each stage. An undisturbed feeding adult tick can remain attached for more than seven days, however disease transmission usually takes place from 24 hours onwards. After feeding, the tick will drop off the host and locate on or near the soil surface while they transform to the next instar or, in the case of adult females, lay up to 2000 eggs.

1.6. Diagnosis

The diagnosis of Lyme borreliosis (LB) should be made only after careful evaluation of the patient's clinical history, physical findings, laboratory evidence and exposure risk evaluation. Exposure to ticks prior to disease manifestations is essential for the diagnosis of LB. Since an awareness or recollection of a tick-bite is not always present, however, this should not exclude the diagnosis of LB.

Laboratory evidence of infection, by demonstration of specific antibodies is not essential for the diagnosis of erythema migrans (EM) since some of the EM patients, especially treated cases, have no antibody response. For all other clinical manifestations of LB, laboratory evidence of infection is essential for the diagnosis. The diagnosis of early and late neuroborreliosis requires demonstration of intrathecal antibody production.

2. Tests in use

2.1. Immunofluorescence Assay (IFA)

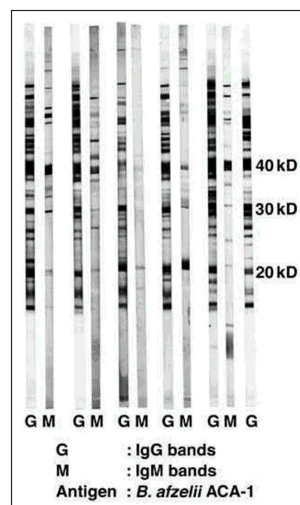
This technique was one of the first serodiagnostic tests developed for Lyme borreliosis and is still a testing method which is used in some countries. The main advantage of immunofluorescence, the ease of antigen preparation, is to an extent countered by the disadvantage of requiring a trained microscopist and problems associated with large-scale testing. IFA cannot be effectively automated.

2.2. Enzyme Linked Immunosorbent Assay (ELISA)

This procedure, which has a number of important advantages over IFA, is now the most commonly used serodiagnostic screening method for LB. ELISA can be used alone or in combination with Immunoblot. The major advantages of this method is the easy automatisation for large-scale testing, coupled with the avoidance of subjective interpretation. The majority of commercial kits use either semi-purified Bacterial lysate antigens, one or several purified Bacterial antigens, or recombinant antigens. The usefulness of ELISA is dependent on the choice of cut-off levels, which influence the sensitivity and specificity of the ELISA.

2.3. Immunoblot (Western Blot)

This assay, also known as Western blot (WB), is important in the specification of immune responses to Borrelia proteins. It should be used for diagnostic purposes, as part of a two step process. The first step is a sensitive assay like an ELISA or IFA for screening, the second step is to confirm reactive samples by WB. Currently, use of the criteria of Engstrom et al (1995, J. Clin. Microbiol. 33; 419-422) is recommended for interpretation of IgM immunoblots and those of Dressler et al (1993, J. Infect. Dis. 167; 392-400) for IgG. The standardisation of criteria for the interpretation of WB in Europe is the subject of a current study by EUCALB.



Borrelia Western blot. Three commonly seen bands are indicated as a guide to molecular weights.

3. Borrelia antigene used in Biomedica ELISAs

To improve the diagnostic specificity, the Biomedica Borrelia assays use a combination of three to five recombinant antigens.
These recombinant antigens are produced by genetically modified microorganisms, and are highly purified during downstream processing.

Most other assays on the market use antigens produced from Borrelia whole cell lysates, which could consist of up to 25 different Borrelia antigen fragments, as reported in the literature[1].

The Biomedica microwell strips are coated with the following recombinant antigens:

Antigen	Used in Biomedica Borrelia IgG	Used in Biomedica Borrelia IgM	Name	Specifity	Lyme disease phase
18 kDa	+ (B. afzelli)	-	P18	high	late
22 kDa	+ (B. sensu stricto and B. garinii)	+ (B. afzelli and B. garinii)	OspC [2]	high	very early
41 kDa	+ (p41i used: = inner part of flagellin of B. garinii = 14 kDa)	+ (p41i used: = inner part of flagellin of B. garinii = 14 kDa)	Flagellin	low	early
100 kDa	+ (B. afzelli)	-	Protein of the membrane vesicles on the surface	high	late

The nomenclature of the antigens takes place according to their molecular weights, which was estimated by Western blot.

The advantages of using recombinant antigens instead of bacteria lysates are:

	Assays with recombinant antigens	Assays with bacteria lysates
Antigen quality	proteins well defined	proteins not well defined
Balance of antigens	well adjustable	None or bad adjustable
Lot to lot constance	high	low
Cross reactivities	low	high

[1] F. Tewald, R. Braun, Clinical Laboratory (1998) 11:899
[2] The OspC antigen is patent protected worldwide

4. Serology

4.1. Minimum Standards

Serological diagnosis is a balance between sensitivity and specificity of the assays, which is depending on the choice of the cut-off level. Irrespective of methods used, a high level of specificity, i.e. a limited number of false positives, is always more important than a high level of sensitivity, since a lack of sensitivity can usually be compensated by rising antibodies in later samples.

A minimum specificity of at least 98% in assays is recommended. The cut-off level giving such a specificity should be established with serum samples from healthy donors.

A complete evaluation of an assay, e.g. a commercial kit, should include validation of the recommended cut-off level in the population where the assay is to be used. The performance of the assay should also be investigated with samples of patients with diseases known to cross-react with Lyme borreliosis, e.g. syphilis, or disorders known to cause false positive reactions, e.g. rheumatoid factor in IgM assays. The sensitivity of the assay should be established in samples from clinically confirmed cases of Lyme borreliosis at different stages.

These recommendations of EUCALB (European Union Concerted Action on Lyme Borreliosis) were discussed with experts in non-EU countries, US and Asia and were adopted by World Health Organisation (WHO) as general recommendations.

4.2. Limitations

Sensitivity:

Antibody response in early Lyme borreliosis may be weak or absent, especially in erythema migrans. Antibody response may be absent in serum but present in cerebrospinal fluid (CSF) in early cases of neuroborreliosis. There is no good evidence for the existence of seronegative late LB.

Specificity:

Examples of problems caused by the serological assays:

1. too low cut-off levels.
2. known cross-reacting antibodies, e.g. syphilis.
3. false positive IgM due to rheumatoid factor in indirect assays.

Example of problems caused by the other diseases:

1. finding of CSF antibodies, not due to intrathecal antibody production, but to leakage of serum antibodies caused by a damaged serum/CSF barrier.
2. oligoclonal stimulation, seen in some infections, e.g. EBV, CMV and Mycoplasma pneumoniae, can result in an increase of antibodies to a large number of antigens.
3. false positive reactions, predominantly IgM and mainly in sonicate antigen ELISA, caused by some autoimmune diseases or disease of unknown origin, e.g. Multiple Sclerosis, SLE.

Problems caused by high antibody prevalence in the general population:

High seroprevalence of specific antibodies in the general population in high-endemic areas will cause the problem of relevance to clinical disease. Clinicians must take local seroprevalence into account when interpreting the clinical relevance of positive serology in patients.

5. Diagnostic Guidelines

Erythema migrans (EM):

Specific IgG and/or IgM can be found in 40-60% of untreated cases, particularly in patients with signs of haematogenous spread, e.g. multiple EM and extensive general symptoms. A significant rise of specific IgG and/or IgM between paired blood samples, one taken at the first visit and a second 2-3 weeks later gives the highest diagnostic specificity. Significant titre decreases after treatment can sometimes be seen between a sample taken at the start of treatment and another at least three months later. Early treatment and a superficial lesion can result in absence of specific antibody response. The role of serology in EM diagnosis is supporting but not essential.

Borrelia lymphocytoma or lymphadenosis benigna cutis (BL):

A significant rise of specific IgG and/or IgM between paired blood samples, one taken at the first visit and a second 3-6 weeks later can be demonstrated and is essential for the diagnosis. Significant titre decreases after treatment can sometimes be seen between a sample taken at the start of treatment and another at least three months later.

Acrodermatitis chronica atrophicans (ACA):

For the diagnosis of acrodermatitis chronica atrophicans, it is essential to demonstrate high levels of specific IgG antibodies.

Early neuroborreliosis:

A significant rise of specific IgG and/or IgM between paired blood samples, one taken at the first visit and a second 2-4 weeks are supportive for the diagnosis, but serum antibodies can be absent in early cases. Specific antibodies in CSF are found earlier than in serum. For the diagnosis of early neuroborreliosis, it is essential to demonstrate intrathecal antibody production, which requires simultaneously drawn blood and CSF samples.

Chronic neuroborreliosis:

For the diagnosis of chronic neuroborreliosis, it is essential to demonstrate intrathecal antibody production, in simultaneously drawn blood and CSF samples, and to demonstrate specific IgG antibodies in serum.

Lyme arthritis:

For the diagnosis in early cases of Lyme arthritis, it is essential to demonstrate a significant rise of specific IgG and/or IgM between paired blood samples or high levels of specific IgG. For chronic Lyme arthritis cases, it is essential to demonstrate high levels of specific IgG antibodies.

Lyme carditis:

For the diagnosis of Lyme carditis, it is essential to demonstrate a significant rise or a significant decrease of specific IgG antibodies in paired serum samples.

6. Comparison of available Borrelia ELISAs

Parameter	Biomedica	Biomedica	Dako	Behring	Biomed	Invitro Diag.
Cat. no.	BI-21032	BI-21042	K6029	Enzygnost	108 105	EI 02.01
Antibodies measured	IgG against recombinant p21 (= OspC) p41i p18 p100	IgM against recombinant p21 (= OspC) p41i	IgG + IgM against B. burgdorferi flagellum p41	IgG + IgM against Borrelia detergent extract	IgM against recombinant OspC p41 p41i	IgG + IgM against bacteria lysate
Assay principle	sandwich ELISA HRPO / TMB	sandwich ELISA HRPO / TMB	sandwich ELISA HRPO / OPD	sandwich ELISA HRPO / OPD	sandwich ELISA AP / PNPP	sandwich ELISA HRPO / TMB
Solid phase	wells	wells	wells	wells	wells	wells
Number of tests	96	96	192	192	96	96
Storage	4 °C	4 °C	2 - 8 °C	2 - 8 °C	2 - 8 °C	4 - 8 °C
Shelf life	12 months	12 months	6 months	6 months	6 months	6 months
Positive controls	1	1	1	2	1	1
Negative controls	1	1	0	1	1	1
Cutoff controls	1	1	1	0	1	0
Sample type	plasma, serum, cerebrospinal fluid	plasma, serum, cerebrospinal fluid	serum	plasma, serum	serum	serum
Sample size	10 - 50 µl	10 - 50 µl	10 µl	10 µl	10 µl	10 µl
Sample pretreatment	none	none	none	none	Rheuma factor absorption	none
Assay procedure	3 steps	3 steps	3 steps	3 steps	3 steps	3 steps
Incubation time	1 h / 30 min / 15 min	1 h / 30 min / 15 min	1 h / 1 h / 10 min	30 min / 30 min / 30 min	1 h / 30 min / 30 min	1 h / 30 min / 15 min
Incubation temperature	37 °C / 18 - 26 °C	37 °C / 18 - 26 °C	20 - 25 °C	37 °C / 18 - 26 °C	37 °C	37 °C / 18 - 26 °C
Specificity	possible crossreaction with syphilis	possible crossreaction with syphilis	crossreaction with syphilis, rheumatoid factor	crossreaction with endemic recurring fever, autoimmune pos. samples	crossreaction with rheumatoid factor, IgG	crossreaction with syphilis, rheumatoid factor
Interassay prec.	7.6 - 9.1 %	8.1 - 8.3 %	4.5 - 14.9 %	-	-	-
Intraassay prec.	6.6 - 6.8 %	6.7 - 6.8 %	3.9 - 6.7 %	-	-	-
Comments	High specifity, due to a mix of 6 recombinant antigens used	High specifity, due to a mix of 3 recombinant antigens used	p41 antigene used is very unspecific. False-positives by other spirochetal diseases			

Parameter	Viroimmun	Viroimmun	Virotech	BioMérieux
Cat. no.	EG 111	EM 111	E 122.00	30 298
Antibodies measured	IgG against bacteria lysate	IgM against bacteria lysate	IgG + IgM against bacteria lysate	IgG + IgM against bacteria lysate
Assay principle	sandwich ELISA HRPO / TMB	sandwich ELISA HRPO / TMB	sandwich ELISA HRPO / OPD	sandwich ELISA
Solid phase	wells	wells	wells	tubes
Number of tests	96	96	96	60
Storage	2 - 8 °C	2 - 8 °C	2 - 8 °C	4 °C
Shelf life	6 months	6 months	6 months	6 months
Positive controls	1	1	1	1
Negative controls	1	1	1	1
Cutoff controls	1	1	1	0
Sample type	serum	serum	serum	serum
Sample size	10 - 50 µl	10 - 50 µl	10 - 50 µl	100 µl
Sample pretreatment	Rheuma factor absorption	Rheuma factor absorption	Rheuma factor absorption	none
Assay procedure	3 steps	3 steps	3 steps	automated
Incubation time	30 min / 30 min / 10 min	30 min / 30 min / 10 min	1 h / 30 min / 15 min	40 min
Incubation temperature	18 - 26 °C	18 - 26 °C	37 °C	18 - 26 °C
Specificity	-	-	-	-
Interassay prec.	-	-	-	-
Intraassay prec.	-	-	-	-
Comments				

7. Implications

Impact of strain heterogeneity on Lyme disease serology in Europe: Comparison of enzyme-linked immunosorbent assays using different species of Borrelia burgdorferi sensu lato

(1) *Hauser et al., Journal of Clinical Microbiology (1998) 36: 427-436*

In this study, four commercially available IgM and IgG ELISAs were compared using different Borrelia extracts.

Enzyme-linked immunosorbent assay using recombinant OspC and the Internal 14-kDa flagellin fragment for serodiagnosis of early Lyme disease

(2) *Rauer et al., Journal of Clinical Microbiology (1998) 36: 857-861*

The OspC / 14-kDa antigens ELISA seems to be a suitable test for the detection of an IgM response in early Lyme disease.

Neurocognitive abnormalities in children after classic manifestations of Lyme disease

(3) *Bloom et al., Pediatr. Infect. Dis. J. (1998) 17: 189-196*

Children may develop neurocognitive symptoms along with or after classic manifestations of Lyme disease. This may represent an infectious or postinfectious encephalopathy related to Borrelia burgdorferi infection.

Relationship between the Borrelia burgdorferi specific immune response and different stages and syndromes in neuroborreliosis

(4) *Oschmann et al., Infection (1997) 25: 292-297*

One hundred untreated neuroborreliosis patients were investigated by IgG/IgM immunoblot to find out if different stages and syndromes are characterized by different patterns of their Borrelia burgdorferi specific immune responses in CSF and serum.

The spirochetal etiology of Lyme disease

(5) *Steere et al., The New England Journal of Medicine (1983) 308: 733-740*

A newly recognized spirochete was recovered from the blood, skin lesions and cerebrospinal fluid of patients with Lyme disease and from adult ticks in Connecticut.

Immunodominant proteins of Borrelia burgdorferi, the etiological agent of Lyme borreliosis

(6) *Wilske et al., World J. of Microbiology and Biotechnology (1991) 7: 130-136*

Borrelia burgdorferi proteins have been analysed for cross-reactivity with immune sera from unrelated bacteria, and sera from patients with different stages of the disease.

Diagnostic testing for Lyme disease
(7) *Callister et al., (1990) Feb-March: 11-14*

When Lyme disease is confirmed using accurate diagnostic testing, appropriate antimicrobial therapy may then reverse a potentially debilitating pathologic process.

A new immunodominant Borrelia burgdorferi antigen - p18
(8) *Soutschek et al., (1997) poster presented at the 2nd Frankfurter borrelia workshop*

The sensitivity of the IgG Western blot was improved significantly, while the specifity remains unchanged. For IgM measurement, the diagnostic value of antigen p18 is only of limited value.

Use of recombinant antigens of Borrelia burgdorferi in serologic tests for diagnosis of Lyme borreliosis
(9) *Magnarelli et al., Journal of Clinical Microbiology (1996) 2: 237-240*

With relatively high degrees of specificity, ELISAs with recombinant antigens, particularly OspC and p41-G, can help to confirm Borrelia burgdorferi infections.

Molecular characterization of the p83/100 proteins of various Borrelia burgdorferi sensu lato strains
(10) *Rößler et al., pp 15-18*

To investigate the molecular diversity and the relationship to species and OspA-serotype classification, strains of different Borrelia strains were cloned, sequenced, and studied for alignment with published p83/100 sequences.

Antigenic variability of Borrelia burgdorferi
(11) *Wilske et al., Lyme Disease and Related Disorders (1988) 539: 126-143*

Borrelia burgdorferi isolates were examined for common antigenic components and to differentiate them from relapsing fever borreliae and treponemes, and to characterize the variable proteins of Borrelia burgdorferi in more detail.

Immunological and molecular heterogeneity of the outer surface protein C (OspC) of Borrelia burgdorferi sensu lato
(12) *Jauris-Heipke et al., pp 11-14*

The molecular and immunological heterogeneity of OspC has major implications for diagnosis and vaccine development in Lyme borreliosis.

Identification of a protein in several Borrelia species which is related to OspC of the Lyme disease spirochetes
(13) *Marconi et al., Journal of Clinical Microbiology (1993) 31: 2577-2583*

A protein that is genetically and antigenically related to OspC is expressed in all species of the genus Borrelia tested.

Antigenic variation and variation in expression of immunodominant Borrelia burgdorferi antigens: implications for using recombinant antigens for serodiagnosis

(14) *Wilske et al., pp 179-182*

In general, IgM antibodies recognized conserved epitopes of OspC, IgG antibodies - in contrast - strain-specific epitopes. Heterogeneity of flagellin plays a larger role than expected from the sequence data.