

Review

Diagnosis of Lyme Borreliosis in Europe

BETTINA WILSKE

ABSTRACT

In Europe, Lyme borreliosis is caused by at least three species, *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. Thus microbiological diagnosis in European patients must consider the heterogeneity of Lyme disease borreliae for development of diagnostic tools such as PCR primers and diagnostic antigens. According to guidelines of the German Society of Hygiene and Microbiology, the serological diagnosis should follow the principle of a two-step procedure. A sensitive ELISA differentiating IgM and IgG is recommended as the first step. In case the ELISA is reactive, it is followed by immunoblots (IgM and IgG) as the second step. The reactive diagnostic bands should be clearly identified, which is easy if recombinant antigens are used. The sensitivity and standardization of immunoblots has been considerably enhanced by use of recombinant antigens instead of whole cell lysates. Improved sensitivity resulted from use of recombinant proteins that are expressed primarily *in vivo* (e.g., VlsE) and combination of homologous proteins from different strains of borrelia (e.g., DbpA). It also appears promising to use recombinant proteins (DbpA, VlsE, others) or synthetic peptides (the conserved C6 peptide derived from VlsE) as ELISA antigens. At present, detection rates for serum antibodies are 20–50% in stage I, 70–90% in stage II, and nearly 100% in stage III Lyme disease. The main goals for the future are to improve specificity in general and sensitivity for diagnosis of early manifestations (stage I and II). Detection of the etiological agent by culture or PCR should be confined to specific indications and specialised laboratories. Recommended specimens are skin biopsy specimens, CSF and synovial fluid. The best results are obtained from skin biopsies with culture or PCR (50–70%) and synovial tissue or fluid (50–70% with PCR). CSF yields positive results in only 10–30% of patients. Methods that are not recommended for diagnostic purposes are antigen tests in body fluids, PCR of urine, and lymphocyte transformation tests. Key Words: Lyme borreliosis—*Borrelia burgdorferi*—Diagnosis. Vector-Borne Zoonotic Dis. 3, 215–227.

INTRODUCTION

LYME BORRELIOSIS is a multisystem disease involving many organs such as the skin, the nervous system, the joints, and the heart (Steere et al. 1989, Pfister et al. 1994). This condition is the most frequent tick-borne disease in the northern hemisphere. Due to the diversity of clinical symptoms, Lyme disease is often considered in a differential diagnosis. Examinations for antibodies against *Borrelia burgdorferi* are thus in high demand, and are among the most frequently requested serological tests

in microbiological laboratories. Microbiological diagnosis in European patients must consider the heterogeneity of Lyme disease borreliae in Europe.

HETEROGENEITY OF LYME DISEASE BORRELIAE IN EUROPE AND ITS IMPACT FOR MICROBIOLOGICAL DIAGNOSIS

In Europe, Lyme borreliosis is caused by at least three species: *B. burgdorferi* sensu stricto,

B. afzelii, and *B. garinii*. In contrast, *B. burgdorferi* sensu stricto is the only human-pathogenic species in the United States (Wang et al. 1999b). The three human-pathogenic species comprise at least seven OspA-serotypes in Europe (Fig. 1) (Wilske et al. 1993c). Skin isolates primarily belong to *B. afzelii* (OspA-type 2), especially those from patients with acrodermatitis chronica atrophicans, a chronic skin disease not present in America (Canica et al. 1993, Ohlenbusch et al. 1996, Wilske et al. 1993c) (see also legend of Fig. 1). Isolates from CSF and ticks are heterogeneous with a predominance of *B. garinii* (Eiffert et al. 1995, van Dam et al. 1993, Wilske et al. 1996, Wilske et al. 1993c). Sequence analysis of polymerase chain reaction (PCR) *ospA* amplicons from synovial fluid of Lyme arthritis patients revealed heterogeneity (Eiffert et al. 1998, Vasiliu et al. 1998), whereas other studies found mainly *B. burgdorferi* s.s. using PCR based on the 5S/23S rRNA intergenic spacer region (Lünemann et al. 2001) or the flagellin gene (Jaulhac et al. 1996 and 2000). The most frequent genomic groups in Europe *B. afzelii* and *B. garinii* occur across the continent and the islands, whereas the third frequent group *B. burgdorferi* s.s. has only rarely been isolated in Eastern Europe (for a survey, see Hubalek et al. 1997). Strains may be very heterogeneous even within small areas (Eiffert et al. 1995, Gern et al. 1999, Michel et al. 2003, Rauter et al. 2002, Rijpkema et al. 1996). On the other side a focal prevalence of certain species or subtypes was also observed (Michel et al. 2003, Peter et al. 1995). Mixed infections have been repeatedly observed in ixodid ticks (for a survey, see Hubalek et al. 1997) and sometimes also in specimens from patients (Demaerschalck et al. 1995, Vasiliu et al. 1998, Wilske et al. 1996). The heterogeneity of the causative strains (Fig. 1) is a challenge for the microbiological diagnosis of Lyme borreliosis in Europe and must be kept in mind for development of diagnostic tools such as PCR primers and diagnostic antigens. For example, *ospA* PCR has been widely used. Here, it is important to be sure that not only representatives of the three species are detected, but also the different *ospA*-types of the heterogeneous *B. garinii* group (Eiffert et al. 1995). In addition, PCR should detect *B. valaisiana* and the re-

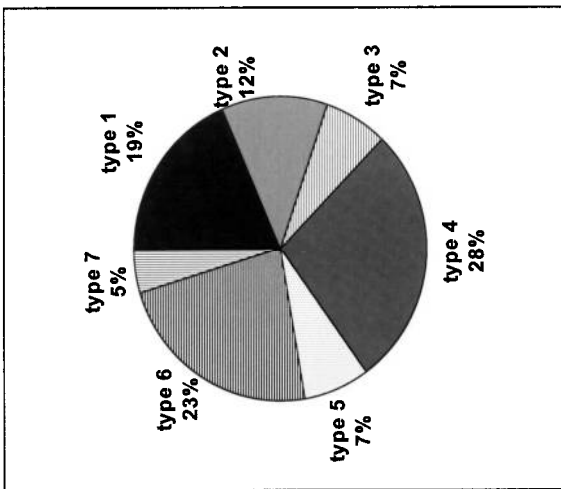
cently detected new genotype A14S since *B. valaisiana* and genotype A14S might also be pathogenic for humans, as suggested by positive PCR results or cultures obtained from skin biopsy specimens in a few studies (Rijpkema et al. 1997, Wang et al. 1999a, Wilske et al. 2002). An *ospA* PCR for detection and differentiation of the various European species and OspA-types has been described by Michel (2003).

Most of the proteins relevant for serodiagnosis are heterogeneous. Interspecies amino acid sequence identities are for example only 40–44% for DbpA (Osp17) and 54–68% for OspC for representative strains of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* (strains B31, PKo, and PBi, respectively) (Table 1). Especially DbpA has a much higher amino acid sequence heterogeneity compared to the DNA sequence heterogeneity indicating immune selection. However, highly heterogeneous proteins sometimes have conserved immunogenic epitopes (e.g., the C6 peptide of VlsE) (Liang et al. 1999, Liang et al. 2000).

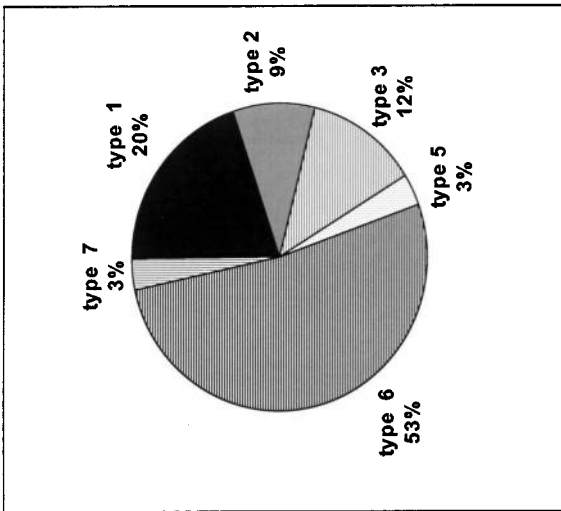
GUIDELINES FOR THE MICROBIOLOGICAL DIAGNOSIS OF LYME BORRELIOSIS

The German Society of Hygiene and Microbiology (DGHM) has recently published guidelines for the microbiological diagnosis of Lyme borreliosis written by an expert committee (MiQ 12 Lyme-Borreliose) (Wilske et al. 2000). The English version is accessible via internet (www.dghm.org/red/index.html?cname=MIQ). Except in cases with the pathognomic clinical manifestation erythema migrans, the diagnosis of Lyme borreliosis usually requires confirmation by means of a microbiological diagnostic assay. Antibody detection methods mainly are used for this purpose, whereas detection of the causative agent by culture isolation and nucleic acid techniques is confined to special situations, such as to clarify clinically and serologically ambiguous findings. Application of these methods should be reserved to laboratories specialized in this type of examination.

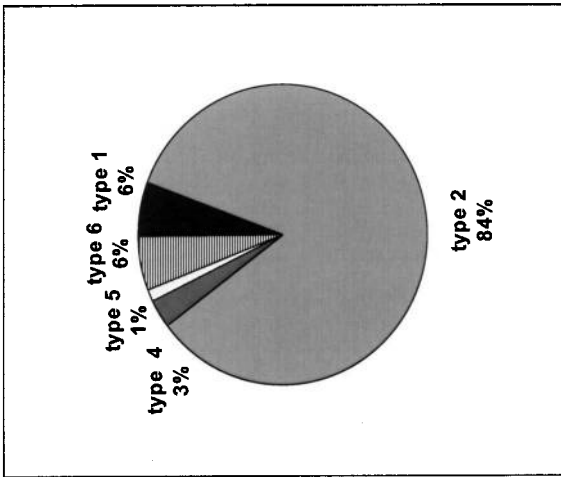
CSF (n=43)



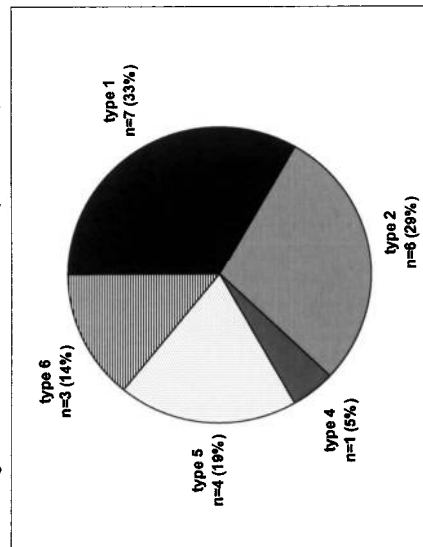
Ticks (n=90)



Skin (n=68)



Synovial fluid (n=32)



	CSF	Ticks	Skin	Syn.Fl.
<i>B. burgdorferi</i> s.s.	19%	20%	6%	33%
<i>B. afzelii</i>	12%	9%	84%	29%
<i>B. garinii</i>	69%	71%	10%	38%

FIG. 1. Heterogeneity of Lyme disease *Borrelia* species and OspA-serotypes in Europe. Data for skin, CSF, and ticks are based on analysis of culture isolates; data for synovial fluid are based on analysis of *ospA* PCR amplicons; source of skin specimens is known in 46 patients (30 cases with erythema migrans, thereof were 1, 2, 6, 1, and 2 cases infected with OspA-types 1, 2, 4, and 6, respectively; 16 cases with ACA, thereof were 1 and 15 cases infected with OspA-types 1 and 2, respectively). (Modified from Figures 5 and 6 in Wilske et al., 2002, with permission of the publisher.)

TABLE 1. SEQUENCE IDENTITIES AMONG MAJOR IMMUNODOMINANT PROTEINS OF THE THREE GENOSPECIES OF *B. BURGDORFERI* SENSU LATO (COMPARISON OF STRAINS B31, PKO AND PBI)

Protein	DNA sequences, range (in %)	Amino acid sequences, range (in %)
DbpA ^a	51–63	40–44
OspC ^a	61–77	54–68
OspA ^a	85–86	78–81
p35 ^a	74–85	65–80
BmpA (p39) ^a	91–93	89–90
p58 ^a	90–97	90–97
Flagellin	94–95	96–97
Flagellin fragment (aa 129–251)	nd	93–96
p83/100 ^a	87–89	81–87

^aSequence identities were calculated without the leader sequence of the lipoproteins.

SPECIMENS FOR THE MICROBIOLOGICAL DIAGNOSIS

For culture and PCR, skin biopsy samples are the most promising specimens. In general poor results are obtained from body fluids with the exception of PCR of synovial fluid. Examination of urine (PCR, antigen detection) is not recommended nor the examination (PCR or IFA) of ticks removed from patients in order to decide antibiotic prophylaxis (Brettschneider et al. 1998, Kaiser et al. 1998, Klempner et al. 2001, Wilske et al. 2000). Examination of ticks should be performed only for epidemiological or other scientific studies. For antibody determination, serum or CSF can be investigated. CSF examination should always be done together with serum antibody analysis (determination of the CSF/serum antibody index).

DIRECT DETECTION METHODS

Culture

B. burgdorferi can be cultivated in modified Kelly's medium (Preac-Mursic et al. 1991,

Wilske and Schriefer 2002). This, however, is a very time-consuming method (generation time of *B. burgdorferi* is about 7–20 h) characterised by low sensitivity, especially in body fluids (Arnez et al. 2001, Åsbrink et al. 1985, Karlsson et al. 1990, Strle et al. 1999, Zore et al. 2002) (Table 2). Culturing may be of help in individual cases if the clinical picture suggests Lyme borreliosis despite a negative antibody assay (seronegative Lyme borreliosis), for example, in atypical erythema migrans, suspected acute neuroborreliosis without detection of intrathecal antibodies or in the case of suspected Lyme borreliosis in patients with immune deficiencies.

PCR

There is no standardized method for the preparation of specimens nor for performing the PCR itself. For DNA amplification under experimental conditions various target sequences have been used by specialised laboratories, for example, from plasmid-borne genes such as *ospA* and *ospB*, or chromosomal genes such as the genes for the flagellar protein or p66 (clone 2H1), or from gene segments of the 16S rRNA or the 5S/23S rRNA intergenic

TABLE 2. SENSITIVITY OF DIRECT PATHOGEN DETECTION METHODS IN LYME BORRELIOSIS

Specimen	Sensitivity
Skin (erythema migrans, acrodermatitis)	50–70% when using culture or PCR
CSF (neuroborreliosis, stage II)	10–30% when using culture or PCR
Synovial fluid ^a (Lyme arthritis)	50–70% when using PCR (culture is only extremely seldom positive)

^aHigher sensitivity within synovial tissue compared to synovial fluid.

spacer region (for a survey, see Schmidt et al. 1997). *Borrelia* PCR should allow diagnosis of the *Borrelia* species, that is, the medical report should contain information as to which of the three species pathogenic for humans has been found. The diagnostic sensitivity of PCR is about the same as the sensitivity of culture. *Borreliae* are detected with much more difficulty from body fluids than from tissue specimens by either PCR or culture (Arnez et al. 2001, Jaulhac et al. 1996, Karlsson et al. 1990). Solely PCR of synovial fluid seems to surpass culture significantly in sensitivity (Nocton et al. 1994).

Sensitivity of culture and PCR

Table 2 gives a survey about sensitivity of direct detection methods in clinical specimens

from patients with Lyme borreliosis. Culture and PCR have the highest detection rates (50–70%) in skin biopsies from patients with erythema migrans or acrodermatitis chronica atrophicans (Åsbrink et al. 1985a, van Dam et al. 1993, von Stedingk et al. 1995, Weber et al. 1990, Zore et al. 2002). In contrast *borreliae* are detected by PCR or culture in the CSF of only 10–30% of patients with neuroborreliosis (Eiffert et al. 1995, Karlsson et al. 1990, Wilske and Preac-Mursic 1993b). CSF isolates are more frequently obtained from patients with short duration of disease than from patients with disease of long duration (Karlsson et al. 1990). It is surprising that *borreliae* are detected by PCR in 50–70% in the synovial fluids of Lyme arthritis patients, but culture is rarely successful (Eiffert et al. 1998, Vassiliu et al. 1998). The best PCR results are obtained from synovial tissue, not fluid (Jaulhac et al. 1996).

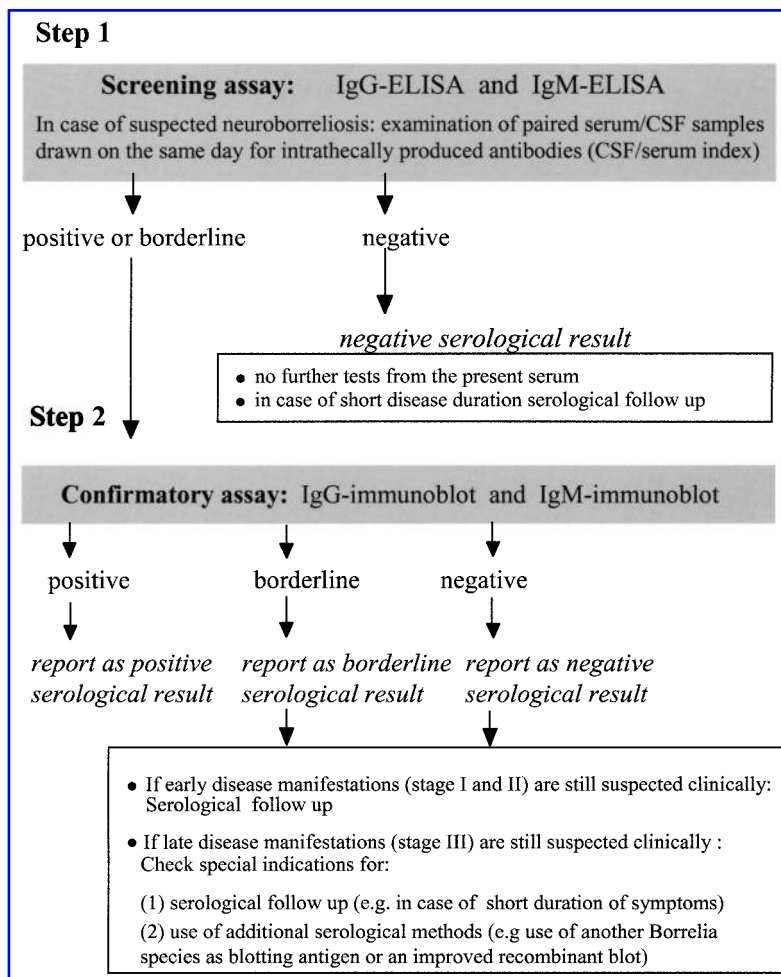


FIG. 2. Two-step approach in serodiagnosis. For criteria for positive, borderline, and negative blot, see text. (Modified from Figure 6 in Wilske et al., 2000.)

ANTIBODY DETECTION

It is generally accepted that serological examination should follow the principles of a two step approach (Centers for Disease Control and Prevention 1995, Johnson et al. 1996, Wilske et al. 2000, Wilske and Schriefer 2002): (1) A serological screening assay and (2) in the event of a positive or equivocal result a confirmatory assay. A sensitive ELISA is recommended, which—in case it is reactive—should be confirmed by the immunoblot (Fig. 2).

ELISA

The ELISA tests used for screening should be at least second generation tests (Wilske et al. 2000), which have been improved with respect to cross reactivity with other bacteria (e.g., extract antigen with previous Reiter treponeme adsorption) (Wilske et al. 1993a) or purified intact flagella as antigen (Hansen et al. 1988). Strains used as antigen source should express OspC the immunodominant antigen of the IgM response and DbpA an immunodominant antigen of the IgG response (Wilske et al. 2000). Recently specific recombinant antigens (i.e., VlsE) or synthetic peptides (i.e., the C6 peptide derived from VlsE) have been successfully used in the United States (Bacon et al. 2003, Lawrenz et al. 1999, Liang et al. 1999) and in a study with European sera from patients with erythema migrans, acrodermatitis, and arthritis (C6 peptide) (Liang et al. 2000). Very recently also patients with neuroborreliosis stage II have been investigated with the C6 ELISA (IgG test) and compared to the recombinant immunoblot (Fingerle et al. 2002). Of 36 sera 31 were positive by immunoblot and 34 by the C6-ELISA. Two of the 31 immunoblot positive sera were only borderline in the C6-ELISA, these sera had antibodies against recombinant DbpA and p58 and DbpA and VlsE respectively. The C6-ELISA appears to be sufficiently sensitive as a screening test for IgG antibodies in patients with neuroborreliosis if also borderline results are included. However, VlsE has other immunodominant epitopes besides the C6 region that could improve diagnostic sensitivity; heterogeneity of those immunodominant epitopes especially must be considered in Europe (Göt-

ner et al. 2002). The IgM and IgG immune responses of Lyme borreliosis patients in recombinant immunoblots should suggest the best combination of antigens for the development of recombinant ELISAs.

Immunoblot

As a confirmatory assay the immunoblot should have high specificity (at least 95%). If a whole cell lysate is used as antigen, diagnostic bands must be defined by monoclonal antibodies (Fig. 3). In case of recombinant antigens, identification of diagnostic bands is much easier. For the whole cell lysate blot, strains expressing immunodominant variable antigens (OspC, DbpA=Osp17) in culture should be used (i.e., strain PKo) (Wilske et al. 2000).

The immunoblot criteria recommended by the Centers of Disease Control (CDC) for use in the United States can not be used for Europe (Hauser et al. 1997, Hauser et al. 1998, Robertson et al. 2000). Dressler et al. (1994) have shown in an immunoblot study that the immune response of European patients is re-

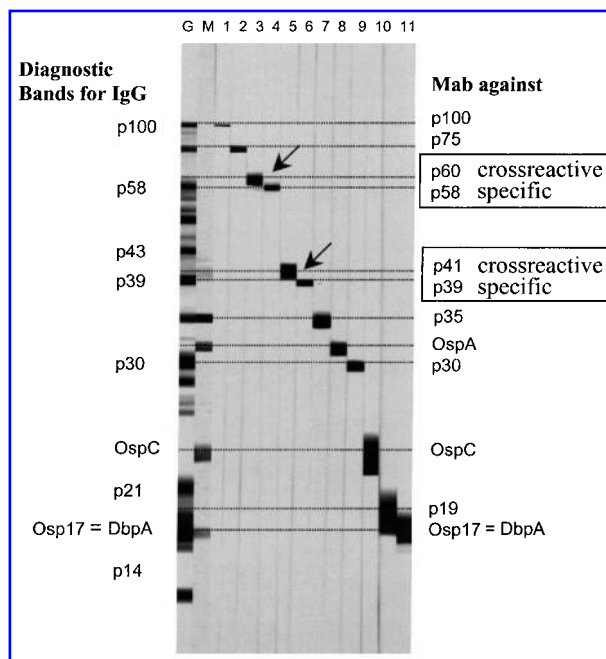


FIG. 3. Standardization of the whole cell lysate immunoblot with monoclonal antibodies (antigen, *B. afzelii* strain PKo; control sera, G=IgG, M=IgM; monoclonal antibodies (1–11). Arrows indicate closely neighbored proteins difficult to distinguish. (Modified from Figure 3 in Wilske et al., 2000, with permission of the publisher.)

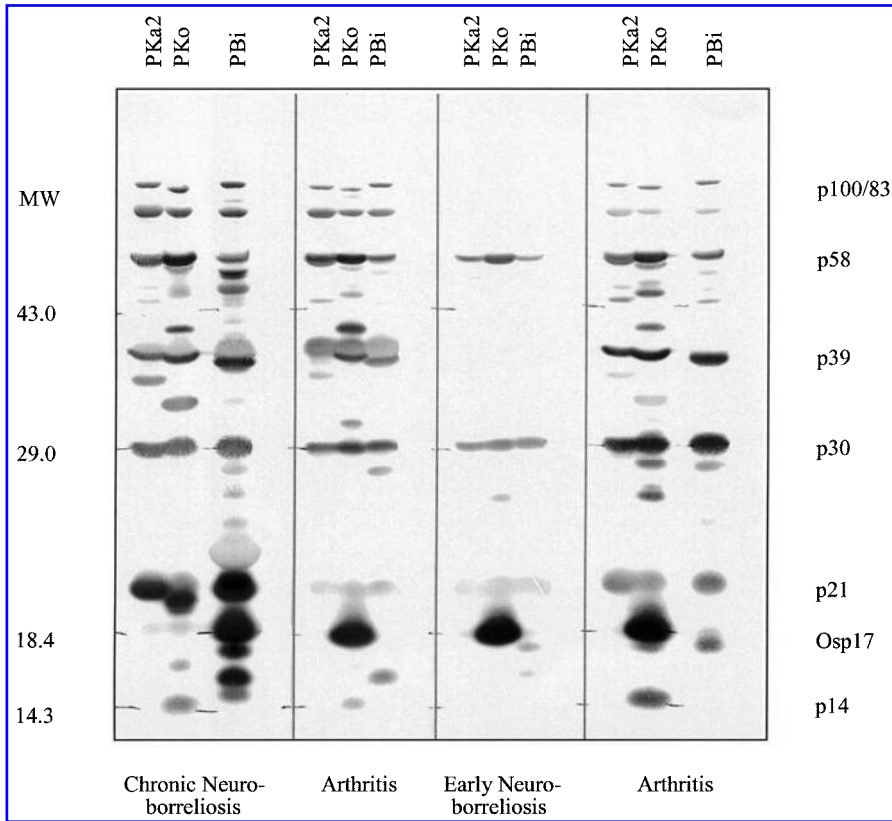


FIG. 4. Heterogeneous IgG reactivity of sera from Lyme borreliosis patients in the immunoblot with different strains of *B. burgdorferi* s.l. as antigen. Strain PKa2 is *B. burgdorferi* s.s., strain PKo is *B. afzelii*, and strain PBi is *B. garinii*. (Modified from Figure 2 in Hauser et al., 1997, with permission of the publisher.)

stricted to a narrower spectrum of *Borrelia* proteins, compared with that shown by American patients. Using different serum panels (first serum panel from Germany, second serum panel from various European countries), Hauser et al. demonstrated in two studies that strain-specific interpretation rules must be defined (Hauser et al. 1997, Hauser et al. 1998). Figure 4 shows that immunoblot antibody binding patterns vary considerably by strain used as antigen. Thus different interpretation rules are required in order to achieve equal sensitivity and specificity when different genospecies of *Borrelia* are used in preparing the blot antigen.

Interpretation criteria for the immunoblot recommended by the DGHM are published in the MiQ 12 Lyme-Borreliose (Wilske et al. 2000). These are if *B. afzelii* strain PKo is used as antigen source the following: The IgG blot is positive if ≥ 2 bands of the following are present: p83/100, p58, p43, p39, p30, OspC, p21,

Osp17, p14; the IgM blot is positive if ≥ 1 band of the following is present: p41 (strong), p39, OspC, DbpA (Osp17). Further interpretation criteria (other strains, recombinant blot) are available via internet (www.dghm.org/red/index.html?cname=MIQ). Borderline results are reported if diagnostic bands are visible but the criteria for a positive blot are not fulfilled. A blot is negative if no diagnostic bands are visible.

Examples for IgM and IgG immunoblots are shown in Figure 5. Patients with early manifestations of acute neuroborreliosis have an immune response restricted to only a few proteins. Patients with late disease such as acrodermatitis or arthritis have IgG antibodies to a broad spectrum of antigens. Using recombinant antigens for the immunoblot has several advantages compared to the immunoblot using whole cell lysate antigen: (a) specific antigens can be selected (i.e., p83/100, BmpA), (b) homologous antigens derived from different

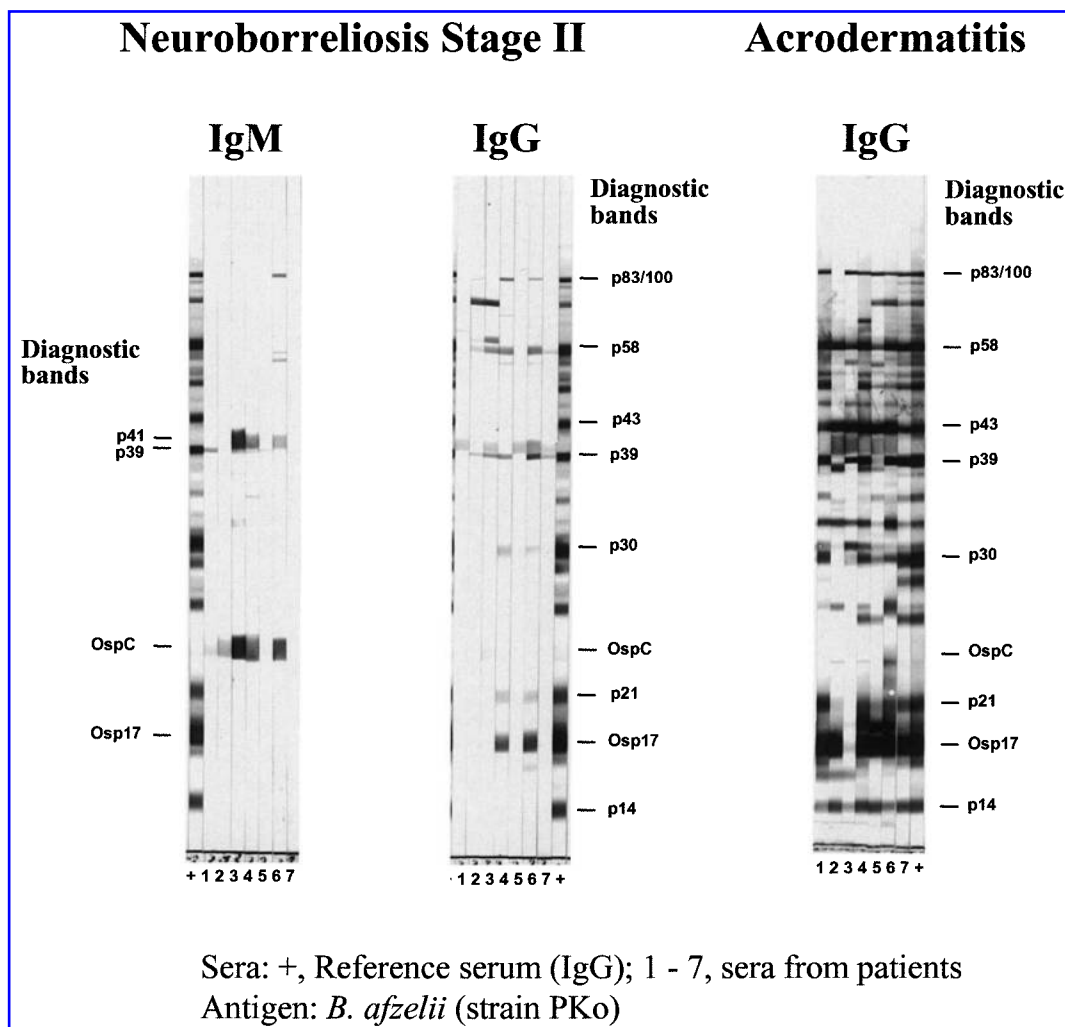


FIG. 5. Whole cell lysate immunoblot: IgM- and IgG immune response in patients with neuroborreliosis (lanes 1–7, respectively), IgG immune response in patients with acrodermatitis (lanes 1–7). Lanes designated with + are IgG blots to demonstrate a broad panel of diagnostic bands. (Modified from Figure 4 in Wilske et al., 2000, with permission of the publisher.)

strains can be combined (i.e., DbpA (Osp17), OspC, BmpA), (c) truncated antigens with higher specificity can be designed (internal flagellin fragments), and (d) antigens primarily expressed *in vivo* can be used (i.e., DbpA, VlsE) (Heikkilä et al. 2002, Schulte-Spechtel et al. 2002, Wilske et al. 1999). Commercial recombinant antigen immunoblots are better standardised than the conventional ones. If a broad panel of recombinant antigens (including the recently described VlsE) is used the recombinant blot is at least as sensitive as the conventional one. An in house recombinant IgG immunoblot (Wilske et al. 1999) shown in Figure 6 could be significantly improved by addition

of recombinant VlsE and an additional DbpA homologue (Schulte-Spechtel et al. 2003). Purified proteins and immunoblot reactivity with sera from patients with acute neuroborreliosis are shown in Figure 7. By addition of VlsE and the DbpA homologue, sensitivity increased from 52.7% to 86.1% in 36 cases of neuroborreliosis stage II, while specificity remained unchanged. Sensitivity was also increased compared to the whole cell lysate immunoblot (86.1% versus 63.8%). Thus the new recombinant immunoblot is a considerable step towards better standardisation and in addition is more sensitive than the whole cell lysate blot since homologous proteins from different

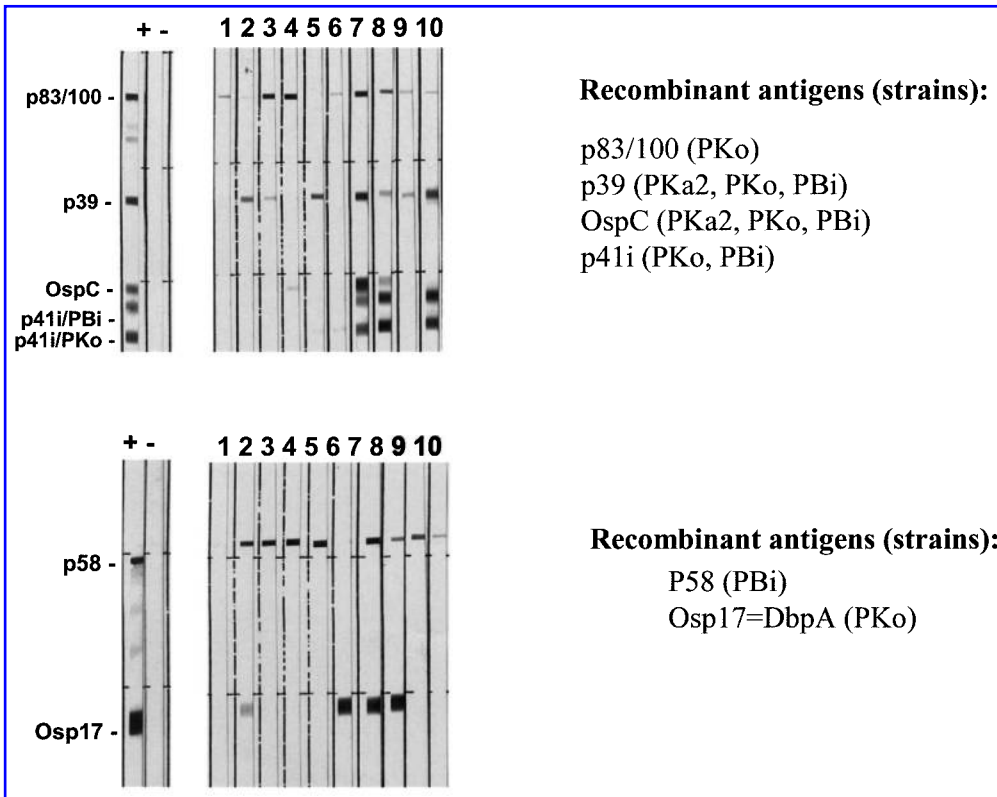


FIG. 6. Recombinant IgG immunoblot with sera from patients with neuroborreliosis stage II (old immunoblot); top and bottoms panels are the same. (Modified from Figure 2 in Wilske et al., 1999, with permission of the publisher.)

strains (especially those with low sequence identities as DbpA, see Table 1) and *in vivo* expressed proteins (as VlsE) are used as antigens.

Determination of the CSF/serum index

Methods taking into account potential dysfunction of the blood-CSF barrier are suitable for the detection of intrathecal antibody production (Wilske et al. 1986, Hansen et al. 1990, Hansen et al. 1991). Determination of the CSF/serum index should be performed if neuroborreliosis is considered, since a positive CSF/serum index confirms involvement of the CNS. It may be positive in some cases when serum antibody tests are negative or equivocal, especially if the patient's illness has been of short duration (Wilske et al. 2000). Depending on the time elapsed since the first manifestation of neurological symptoms, the IgG CSF/serum index is positive for 80–90% of patients (8–41 days after onset of the disease) up to 100% of patients (>41 days after onset) (Hansen et al. 1991). Detection of intrathecally

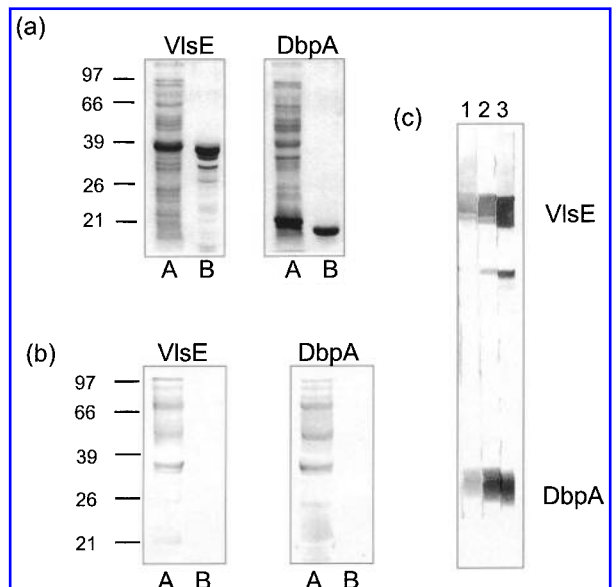


FIG. 7. New recombinant antigens for an improved immunoblot: VlsE from *B. burgdorferi* s.s. strain PKa2 and DbpA from *B. garinii* strain PBr. (a) SDS-PAGE: A—recombinant *E. coli* whole cell lysate; B—purified protein. (b) Immunoblot with immune serum against *E. coli*, antigens as in a. (c) Immunoblot with sera from three patients with acute neuroborreliosis. (Modified from Figures 1 and 3 in Schulte-Spechtel et al., 2003, with permission of the publisher.)

produced IgM antibodies shows a high degree of sensitivity in neuroborreliosis with short duration of symptoms, especially in children (Christen et al. 1993, Hansen et al. 1991).

CSF/serum index determination is especially important for diagnosis of chronic neuroborreliosis. A positive IgG CSF/serum index is essential for the diagnosis of chronic borreliosis of the central nervous system (see EUCALB case definitions, Stanek et al. 1996), whereas chronic peripheral polyneuropathy is usually negative for intrathecal antibody production (Kristoferitsch et al. 1993).

Serological findings in various stages of the disease

Interpretation of serological test results must always be done in context with clinical data (Table 3). Here case definitions are helpful (Stanek et al. 1996, Wilske et al. 2000). In stage I (erythema migrans), only 20–50% of the patients are seropositive for IgM and/or IgG antibodies (Åsbrink et al. 1985b, Hansen and Åsbrink 1989, Weber et al. 1990). IgM antibodies usually prevail. An exception might be the immune response against the recently detected VlsE. In American patients with erythema migrans IgG responses against VlsE are observed earlier than IgM responses (in acute erythema migrans, in 44% versus 19%, in convalescent erythema migrans in 59% versus 43%) (Bacon et al. 2003). In European patients with erythema migrans, an early IgG response to VlsE was observed in 20 of 23 (87%) culture-confirmed EM cases, the IgM response has not been investigated (Liang et al. 2000). In stage II (acute neuroborreliosis) seropositivity (IgM and/or IgG antibodies) increases to 70–90% (Hansen et al. 1988, Wilske et al. 1993a). In principle, patients with early man-

ifestations may be seronegative especially in case of short duration of symptoms. Then serological follow-up is recommended. Six weeks or more after onset of symptoms, 100% of the patients with stage II neuroborreliosis were seropositive (Hansen et al. 1988). In cases with late disease (stage III, acrodermatitis and arthritis), IgG antibodies were detectable in all patients tested (Hansen and Åsbrink 1989, Johnson et al. 1996, Wilske et al. 1993a). A negative IgG test argues against late Lyme borreliosis. Thus, a positive IgM test without a positive IgG test is not diagnostic for late disease manifestations (Wilske et al. 2000). An exception could be the situation of a patient who received inadequate antibiotic therapy for early disease, but sufficient drug to abrogate IgM to IgG class switch or very short duration of clinical symptoms. Since serological findings vary considerably and antibodies may persist for long time in successfully treated individuals, serological follow up is not suitable for determining whether further antibiotic therapy is warranted. The presence of specific antibodies does not prove the presence of disease; a positive antibody test may also be due to clinical or subclinical infections in the past. The more non-specific the symptoms, the lower is the predictive value of a positive serological test. Seropositivity in the normal healthy population varies with age and increased outdoor activities (e.g., in one study in Bavaria <5% up to 20%) (Reimer et al. 1999).

METHODS WHICH ARE NOT RECOMMENDED FOR MICROBIOLOGICAL DIAGNOSIS

Recently, various methods have been used in commercially oriented laboratories that are not

TABLE 3. SENSITIVITY OF ANTIBODY DETECTION METHODS IN THE DIAGNOSIS OF LYME DISEASE

<i>Stage</i>	<i>Sensitivity</i>	<i>Remarks</i>
I	20–50%	Predominance of IgM
II	70–90%	In cases of short disease duration predominance of IgM, in cases of long disease duration predominance of IgG
III	Nearly 100%	Usually solely IgG ^a

^aThe presence of IgM antibodies without IgG antibodies is not diagnostic for late disease; for possible exceptions, see text.

sufficiently evaluated for diagnostic purposes. Among them are the antigen tests in body fluids, PCR of urine, and lymphocyte transformation tests. These tests are not recommended for microbiological diagnosis. They are unreliable and some of them are in addition very expensive, especially if used for therapy control (Brettschneider et al. 1998, Kalish et al. 2003, Klempner et al. 2001).

REFERENCES

- Arnez, M, Ruzic-Sabljic, E, Ahcan, J, et al. Isolation of *Borrelia burgdorferi* sensu lato from blood of children with solitary erythema migrans. *Pediatr Infect Dis J* 2001; 3:251-255.
- Åsbrink, E, Hovmark, A. Successful cultivation of spirochetes from skin lesions of patients with erythema chronicum migrans Afzelius and acrodermatitis chronica atrophicans. *Acta Pathol Microbiol Immunol Scand B* 1985; 93:161-163.
- Åsbrink, E, Hovmark, A, Hederstedt, B. Serologic studies of erythema chronicum migrans Afzelius and acrodermatitis chronica atrophicans with indirect immunofluorescence and enzyme-linked immunosorbent assays. *Acta Derm Venereol* 1985; 65:509-514.
- Bacon, RM, Biggerstaff, BJ, Schriefer, M, et al. Improved serodiagnosis of Lyme disease by kinetic ELISAs using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with two-tiered testing. *J Infect Dis* 2003; 187:1187-1199.
- Brettschneider, S, Bruckbauer, H, Klugbauer, N, et al. Diagnostic value of PCR for detection of *Borrelia burgdorferi* in skin biopsy and urine samples from patients with skin borreliosis. *J Clin Microbiol* 1998; 36:2658-2665.
- Canica, MM, Nato, F, Du Merle, L, et al. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. *Scand J Infect Dis* 1993; 25:441-448.
- Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the second National Conference on Serologic Diagnosis of Lyme Disease. *Morbidity Mortality Weekly Report* 1995; 44:590.
- Christen, H-J, Hanefeld, F, Eiffert, H, et al. Epidemiology and clinical manifestations of Lyme borreliosis in childhood. A prospective multicentre study with special regard to neuroborreliosis. *Acta Paediatr* 1993; 386:1-76.
- Demaerschalck, I, Messaoud, AB, De Kesel, M, et al. Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. *J Clin Microbiol* 1995; 33:602-608.
- Dressler, F, Ackermann, R, Steere, AC. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. *J Infect Dis* 1994; 169:313-318.
- Eiffert, H, Karsten, A, Thomssen, R, et al. Characterization of *Borrelia burgdorferi* strains in Lyme arthritis. *Scand J Infect Dis* 1998; 30:265-268.
- Eiffert, H, Ohlenbusch, A, Christen, H-J, et al. Nondifferentiation between Lyme disease spirochetes from vector ticks and human cerebrospinal fluids. *J Infect Dis* 1995; 171:476-479.
- Gern, L, Hu, CM, Kocianova, E, et al. Genetic diversity of *Borrelia burgdorferi* sensu lato isolates obtained from *Ixodes ricinus* ticks collected in Slovakia. *Eur J Epidemiol* 1999; 15:665-669.
- Göttner, G, Schulte-Spechtel, U, Wilske, B. Antigenic and genetic heterogeneity of the immunodominant surface protein VlsE among European *B. burgdorferi* s.l. strains. *Int J Med Microbiol* 2002; 292:105.
- Goossens, HAT, van den Bogaard, AE, Nohlmans, MKE. Evaluation of fifteen commercially available serological tests for diagnosis of Lyme borreliosis. *Eur J Clin Microbiol Infect Dis* 1999; 18:551-560.
- Fingerle, V, Schulte-Spechtel, U, Wilske, B. Evaluation of an ELISA based on the C6-peptide of VlsE for diagnosis of early neuroborreliosis. *Int J Med Microbiol* 2002; 292 (Suppl. 34): 217.
- Hansen, K, Åsbrink, E. Serodiagnosis of erythema migrans and acrodermatitis chronica atrophicans by the *Borrelia burgdorferi* flagellum enzyme-linked immunosorbent assay. *J Clin Microbiol* 1989; 27:545-551.
- Hansen, K, Cruz, M, Link, H. Oligoclonal *Borrelia burgdorferi*-specific IgG antibodies in cerebrospinal fluid in Lyme neuroborreliosis. *J Infect Dis* 1990; 161:1194-1202.
- Hansen, K, Hinderesson, P, Pedersen, NS. Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis in Lyme disease. *J Clin Microbiol* 1988; 26:338-346.
- Hansen, K, Lebech, A-M. Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal synthesis of *Borrelia burgdorferi*-specific immunoglobulin G, A, and M. *Ann Neurol* 1991; 30:197-205.
- Hauser, U, Lehnert, G, Lobentanzer, R, et al. Interpretation criteria for standardized western blots for three European species of *Borrelia burgdorferi* sensu lato. *J Clin Microbiol* 1997; 35:1433-1444.
- Hauser, U, Lehnert, G, Wilske, B. Diagnostic value of proteins of three *Borrelia* species (*Borrelia burgdorferi* sensu lato) and implications for development and use of recombinant antigens for serodiagnosis of Lyme borreliosis in Europe. *Clin Diagn Lab Immunol* 1998; 5:456-462.
- Heikkilä, T, Seppälä, I, Saxen, H, et al. Species-specific serodiagnosis of Lyme arthritis and neuroborreliosis due to *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* by using decorin binding protein A. *J Clin Microbiol* 2002; 40:453-460.
- Hubalek, Z, Halouzka, J. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. *Eur J Epidemiol* 1997; 13: 951-957.
- Johnson, BJB, Robbins, KE, Balley, RE, et al. Serodiagnosis of Lyme disease: accuracy of a two-step approach using a flagella-based ELISA and immunoblotting. *J Infect Dis* 1996; 174:346-353.
- Jaulhac, B, Chary-Valckenaere, I, Sibilia, J, et al. Detection of *Borrelia burgdorferi* by DNA amplification in synovial tissue samples from patients with Lyme arthritis. *Arthritis Rheum* 1996; 39:736-745.
- Jaulhac, B, Heller, R, Limbach, FX, et al. Direct molecular

- typing of *Borrelia burgdorferi* sensu lato species in synovial samples from patients with Lyme arthritis. *J Clin Microbiol* 2000; 38:1895–1900.
- Kaiser, R. Teilnehmer der Expertenkonferenz: Frühsommermeningoenzephalitis und Lyme-Borreliose-Prävention vor und nach Zeckenstich. *Dtsch med Wochenschr* 1998; 123:847–853.
- Karlsson, M, Hovind-Hougen, K, Svenungsson, B, et al. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. *J Clin Microbiol* 1990; 28:473–479.
- Kalish, RS, Wood, J A, Golde, W, et al. Human T lymphocyte response to *Borrelia burgdorferi* infection: no correlation between human leukocyte function antigen type 1 peptide response and clinical status. *J Infect Dis* 2003; 187:102–108.
- Klempner, MS, Schmid, CH, Hu, L, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. *Am J Med* 2001; 110:217–219.
- Kristoferitsch, W. Chronical peripheral neuropathy. In: Weber, K, Burgdorfer, W, eds. *Aspects of Lyme Borreliosis*. Berlin: Springer, 1993:219–227.
- Lawrenz, MB, Hardham, JM, Owens, RT, et al. Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi*. *J Clin Microbiol* 1999; 37:3997–4004.
- Liang, FT, Alvarez, AL, Gu, Y, et al. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of *Borrelia burgdorferi*. *J Immunol* 1999; 163:5566–5573.
- Liang, FT, Aberer, E, Cinco, M, et al. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi* sl. *J Infect Dis* 2000; 182:1455–1462.
- Lünemann, JD, Zarmas, S, Priem, S, et al. Rapid typing of *Borrelia burgdorferi* sensu lato species in specimens from patients with different manifestations of Lyme borreliosis. *J Clin Microbiol* 2001; 39:1130–1133.
- Michel, H, Wilske, B, Hettche, G, et al. An ospA-polymerase chain reaction/restriction fragment length polymorphism-based method for sensitive detection and reliable differentiation of all European *Borrelia burgdorferi* sensu lato species and OspA types. *Med Microbiol Immunol* 2003 (in press).
- Nocton, JJ, Dressler, F, Rutledge, BJ, et al. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. *N Engl J Med* 1994; 330:229–234.
- Ohlenbusch, A, Matuschka, F-R, Richter, D, et al. Etiology of the acrodermatitis chronica atrophicans lesion in Lyme disease. *J Infect Dis* 1996; 174:421–423.
- Péter, O, Bretz, AG, Bee, D. Occurrence of different genospecies of *Borrelia burgdorferi* sensu lato in ixodid ticks of Valais, Switzerland. *Eur J Epidemiol* 1995; 11:463–467.
- Pfister, H-W, Wilske, B, Weber, K. Lyme borreliosis: basic science and clinical aspects. *Lancet* 1994; 343:1013–1016.
- Preac-Mursic, V, Wilske, B, Reinhardt, S. Culture of *Borrelia burgdorferi* on six solid media. *Eur J Microbiol Infect Dis* 1991; 10:1076–1079.
- Rauter, C, Oehme, R, Diterich, I, et al. Distribution of clinically relevant *Borrelia* genospecies in ticks assessed by a novel, single-run, real-time PCR. *J Clin Microbiol* 2002; 40:36–43.
- Reimer, B, Marschang, A, Fingerle, V, et al. Epidemiology of Lyme borreliosis in South-Eastern Bavaria (Germany). *Zent Bl Bakteriol* 1999; 289:653–654.
- Rijpkema SGT, Tazelaar, DJ, Molkenboer, MJCH, et al. Detection of *Borrelia afzelii*, *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. *Clin Microbiol Infect* 1997; 3:109–116.
- Rijpkema, S, Golubic, D, Molkenboer, M, et al. Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. *Exp Appl Acarol* 1996; 20:23–30.
- Robertson, J, Guy, E, Andrews, N, et al. European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. *J Clin Microbiol* 2000; 38:2097–2102.
- Schmidt, BL. PCR in laboratory diagnosis of human *Borrelia burgdorferi* infections. *Clin Microbiol Rev* 1997; 10:185–201.
- Schulte-Spechtel, U, Lehnert, G, Liegl, G, et al. Significant improvement of the recombinant *Borrelia* IgG immunoblot by addition of VlsE and a DbpA homologue derived from *B. garinii* for the diagnosis of early neuroborreliosis. *J Clin Microbiol* 2003; 41:1299–1303.
- Stanek, G, O'Connell, S, Cimmino, M, et al. European Union concerted action on risk assessment in Lyme borreliosis: clinical case definitions for Lyme borreliosis. *Wien Klin Wochenschr* 1996; 108/23:741–747.
- Steere, AC. Medical progress—Lyme disease. *N Engl J Med* 1989; 321:586–596.
- Strle, F, Nadelman, RB, Cimperman, J, et al. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. *Ann Intern Med* 1999; 130:32–36.
- Van Dam, AP, Kuiper, H, Vos, K, et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. *Clin Infect Dis* 1993; 17:708–717.
- Vasiliu, V, Herzer, P, Rössler, D, et al. Heterogeneity of *Borrelia burgdorferi* sensu lato demonstrated by an ospA-type-specific PCR in synovial fluid from patients with Lyme arthritis. *Med Microbiol Immunol* 1998; 187:97–102.
- Von Stedingk, LV, Olsson, I, Hanson, HS, et al. Polymerase chain reaction for detection of *Borrelia burgdorferi* DNA in skin lesions of early and late Lyme borreliosis. *Eur J Clin Microbiol Infect Dis* 1995; 14:1–5.
- Wang, G, van Dam, AP, Dankert, J. Phenotypic and genetic characterization of a novel *Borrelia burgdorferi* sensu lato isolate from a patient with Lyme borreliosis. *J Clin Microbiol* 1999; 37:3025–3028.
- Wang, G, van Dam, AP, Schwartz, I, et al. Molecular typing of *Borrelia burgdorferi* sensu lato taxonomic, epi-

- demiological, and clinical implications. *Clin Microbiol Rev* 1999; 12:633–653.
- Weber, K, Preac-Mursic, V, Wilske, B, et al. A randomized trial of ceftriaxone versus oral penicillin for treatment of early Lyme borreliosis. *Infection* 1990; 18:91–96
- Wilske, B. Microbiological diagnosis in Lyme borreliosis. *Int J Med Microbiol* 2002; 291:33:114–119.
- Wilske, B, Busch, U, Eiffert, H, et al. Diversity of OspA and OspC among cerebrospinal fluid isolates of *Borrelia burgdorferi* sensu lato from patients with neuroborreliosis in Germany. *Med Microbiol Immunol* 1996; 184:195–201.
- Wilske, B, Fingerle, V, Herzer, P, et al. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis, comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. *Med Microbiol Immunol* 1993; 182:255–270.
- Wilske, B, Habermann, C, Fingerle, V, et al. An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. *Med Microbiol Immunol* 1999; 188:139–144.
- Wilske, B, Preac-Mursic, V. Microbiological diagnosis of Lyme borreliosis. In: Weber, K, Burgdorfer, W, eds. *Aspects of Lyme Borreliosis*. Berlin: Springer; 1993:267–300.
- Wilske, B, Preac-Mursic, V, Göbel, UB, et al. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. *J Clin Microbiol* 1993; 31:340–350.
- Wilske, B, Schierz, G, Preac-Mursic, V, et al. Intrathecal production of specific antibodies against *Borrelia burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). *J Infect Dis* 1986; 153:304–314.
- Wilske, B, Schriefer, M. *Borrelia*. Murray, PR, Baron, EJ, Jorgensen, JH, eds. In: *Manual of Clinical Microbiology*, 8th ed. Washington, DC: ASM Press; 2003:937–954.
- Wilske, B, Zöller, L, Brade, V, et al. MIQ 12 Lyme-borreliose. In: *Qualitätsstandards in der mikrobiologisch infektologischen Diagnostik*. Mauch, H, Lütticken, R, eds. Munich: Urban & Fischer Verlag, 2000.
- Zore, A, Ruzic-Sabljić, E, Maraspin, V, et al. Sensitivity of culture and polymerase chain reaction for the etiologic diagnosis of erythema migrans. *Wien Klin Wochenschr* 2002; 114:606–609.

Address reprint requests to:

Bettina Wilske, M.D., Ph.D.

Max von Pettenkofer Institute

University of Munich

National Reference Center for *Borrelia*

Pettenkofer-Strasse 9a

D 80336 Munich, Germany

E-mail: Bettina.Wilske@mvp-bak.med.uni-

muenchen.de

This article has been cited by:

1. Albina Poljak, Pär Comstedt, Markus Hanner, Wolfgang Schüler, Andreas Meinke, Benjamin Wizel, Urban Lundberg. 2012. Identification and characterization of *Borrelia* antigens as potential vaccine candidates against Lyme borreliosis. *Vaccine* **30**:29, 4398-4406. [[CrossRef](#)]
2. Pär Comstedt, Tobias Jakobsson, Sven Bergström. 2011. Global ecology and epidemiology of *Borrelia garinii* spirochetes. *Infection Ecology & Epidemiology* **1**:0. . [[CrossRef](#)]
3. Magdalena Ligor, Paweł Olszowy, Bogusław Buszewski. 2011. Application of medical and analytical methods in Lyme borreliosis monitoring. *Analytical and Bioanalytical Chemistry* . [[CrossRef](#)]
4. Gary P. Wormser, Guiqing Wang The Role of Culture and Nucleic Acid Amplification in Diagnosis of Lyme Borreliosis 159-183. [[CrossRef](#)]
5. Viera Svihrova, Henrieta Hudeckova, Milos Jesenak, Katarina Schwarzova, Zina Kostanova, Ivan Ciznar. 2011. Lyme borreliosis—analysis of the trends in Slovakia, 1999–2008. *Folia Microbiologica* **56**:3, 270-275. [[CrossRef](#)]
6. Iwona Wojciechowska-Koszko, Iwona Mączyńska, Zbigniew Szych, Stefania Giedrys-Kalemba. 2011. Serodiagnosis of Borreliosis: Indirect Immunofluorescence Assay, Enzyme-Linked Immunosorbent Assay and Immunoblotting. *Archivum Immunologiae et Therapiae Experimentalis* **59**:1, 69-77. [[CrossRef](#)]
7. José J. Pereyra-Rodríguez, José Bernabeu-Wittel, Elías Cañas, Julián Conejo-Mir. 2011. Mácula eritematosa lentamente progresiva. *Enfermedades Infecciosas y Microbiología Clínica* **29**:1, 68-69. [[CrossRef](#)]
8. G. Stanek, V. Fingerle, K.-P. Hunfeld, B. Jaulhac, R. Kaiser, A. Krause, W. Kristoferitsch, S. O'Connell, K. Ornstein, F. Strle, J. Gray. 2011. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clinical Microbiology and Infection* **17**:1, 69-79. [[CrossRef](#)]
9. Iván Bárcena-Uribarri, Marcus Thein, Anna Sacher, Ignas Bunikis, Mari Bonde, Sven Bergström, Roland Benz. 2010. P66 porins are present in both Lyme disease and relapsing fever spirochetes: A comparison of the biophysical properties of P66 porins from six *Borrelia* species. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1798**:6, 1197-1203. [[CrossRef](#)]
10. Monica L. Vieira, Zenaide M. de Moraes, Amance P. Gonçalves, Eliete C. Romero, Silvio A. Vasconcellos, Ana L.T.O. Nascimento. 2010. Lsa63, a newly identified surface protein of *Leptospira interrogans* binds laminin and collagen IV. *Journal of Infection* **60**:1, 52-64. [[CrossRef](#)]
11. D. Hulínská, J. Votýpka, D. Vaňousová, J. Hercogová, V. Hulínský, H. Dřevová, Z. Kurzová, L. Uherková. 2009. Identification of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in patients with erythema migrans. *Folia Microbiologica* **54**:3, 246-256. [[CrossRef](#)]
12. A. Krause, V. Fingerle. 2009. Lyme-Borreliose. *Zeitschrift für Rheumatologie* **68**:3, 239-254. [[CrossRef](#)]
13. Barbro Hedin Skogman, Stefan Croner, Maria Nordwall, Mattias Eknefelt, Jan Ernerudh, Pia Forsberg. 2008. Lyme Neuroborreliosis in Children. *The Pediatric Infectious Disease Journal* **27**:12, 1089-1094. [[CrossRef](#)]
14. Gaia Codolo, Amedeo Amedei, Allen C. Steere, Elena Papinutto, Andrea Cappon, Alessandra Polenghi, Marisa Benagiano, Silvia Rossi Paccani, Vittorio Sambri, Gianfranco Del Prete, Cosima Tatiana Baldari, Giuseppe Zanotti, Cesare Montecucco, Mario Milco D'Elia, Marina de Bernard. 2008. *Borrelia burgdorferi* NapA-driven Th17 cell inflammation in Lyme arthritis. *Arthritis & Rheumatism* **58**:11, 3609-3617. [[CrossRef](#)]
15. N FOMENKO, N LIVANOVA, N CHERNOUSOVA. 2008. Diversity of *Borrelia burgdorferi* sensu lato in natural foci of Novosibirsk region. *International Journal of Medical Microbiology* **298**, 139-148. [[CrossRef](#)]
16. J ZAJKOWSKA, M KONDRUSIK, S GRYGORCZUK, S PANCEWICZ, A IZYCKA. 2008. The usefulness of 'in vivo' antigens in the diagnosis of human Lyme borreliosis. *International Journal of Medical Microbiology* **298**, 361-364. [[CrossRef](#)]
17. Barbro H. Skogman, Stefan Croner, Pia Forsberg, Jan Ernerudh, Pekka Lahdenne, Heidi Sillanpää, Ilkka Seppälä. 2008. Improved Laboratory Diagnostics of Lyme Neuroborreliosis in Children by Detection of

Antibodies to New Antigens in Cerebrospinal Fluid. *The Pediatric Infectious Disease Journal* 27:7, 605-612. [[CrossRef](#)]

18. T. Cerar, E. Ružič-Sabljić, U. Glinšek, A. Zore, F. Strle. 2008. Comparison of PCR methods and culture for the detection of *Borrelia* spp. in patients with erythema migrans. *Clinical Microbiology and Infection* 14:7, 653-658. [[CrossRef](#)]
19. Elizabeth Valentine-Thon, Karsten Ilseemann, Martin Sandkamp. 2008. Neuartiger Lymphozyten-Transformations-Test (LTT-MELISA®) zum Nachweis einer Lyme-Borreliose / A novel lymphocyte transformation test (LTT-MELISA®) for Lyme borreliosis. *LaboratoriumsMedizin* 32:1, 26-34. [[CrossRef](#)]
20. Vincent Staszewski, Karen D. McCoy, Torkild Tveraa, Thierry Boulinier. 2007. INTERANNUAL DYNAMICS OF ANTIBODY LEVELS IN NATURALLY INFECTED LONG-LIVED COLONIAL BIRDS. *Ecology* 88:12, 3183-3191. [[CrossRef](#)]
21. T. Skarpaas, U. Ljøstad, M. Søybye, Å. Mygland. 2007. Sensitivity and specificity of a commercial C6 peptide enzyme immuno assay in diagnosis of acute Lyme neuroborreliosis. *European Journal of Clinical Microbiology & Infectious Diseases* 26:9, 675-677. [[CrossRef](#)]
22. U. Ljøstad, T. Skarpaas, Å. Mygland. 2007. Clinical usefulness of intrathecal antibody testing in acute Lyme neuroborreliosis. *European Journal of Neurology* 14:8, 873-876. [[CrossRef](#)]
23. Gary P Wormser, Raymond J Dattwyler, Eugene D Shapiro, J Stephen Dumler, Susan O'Connell, Justin D Radolf, Robert B Nadelman. 2007. Single-dose prophylaxis against Lyme disease. *The Lancet Infectious Diseases* 7:6, 371-373. [[CrossRef](#)]
24. Andrew R Pachner, Israel Steiner. 2007. Lyme neuroborreliosis: infection, immunity, and inflammation. *The Lancet Neurology* 6:6, 544-552. [[CrossRef](#)]
25. Elisabeth Aberer. 2007. Lyme borreliosis ? an update. *JDDG* 5:5, 406-414. [[CrossRef](#)]
26. KAI E. KISAND, TIINA PRÜKK, KALLE V. KISAND, SIIRI-MERIKE LÜÜS, IRJA KALBE, RAIVO UIBO. 2007. Propensity to excessive proinflammatory response in chronic Lyme borreliosis. *APMIS* 115:2, 134-141. [[CrossRef](#)]
27. Elizabeth Valentine-Thon, Karsten Ilseemann, Martin Sandkamp. 2007. A novel lymphocyte transformation test (LTT-MELISA®) for Lyme borreliosis. *Diagnostic Microbiology and Infectious Disease* 57:1, 27-34. [[CrossRef](#)]
28. Stephen J. Traub, Gregory A. Cummins Tick-Borne Diseases 982-1008. [[CrossRef](#)]
29. Tjaša Cerar, Eva Ružič-Sabljić, Jože Cimperman, Franc Strle. 2006. Comparison of immunofluorescence assay (IFA) and LIAISON® in patients with different clinical manifestations of Lyme borreliosis. *Wiener klinische Wochenschrift* 118:21-22, 686-690. [[CrossRef](#)]
30. A. Smismans, V. J. Goossens, E. Nulens, C. A. Bruggeman. 2006. Comparison of five different immunoassays for the detection of *Borrelia burgdorferi* IgM and IgG antibodies. *Clinical Microbiology and Infection* 12:7, 648-655. [[CrossRef](#)]
31. H PFISTER, T RUPPRECHT. 2006. Clinical aspects of neuroborreliosis and post-Lyme disease syndrome in adult patients. *International Journal of Medical Microbiology* 296, 11-16. [[CrossRef](#)]
32. D LENCAKOVA, C HIZOTEUFEL, B PETKO, U SCHULTESPECHTEL, M STANKO, B WILSKE, V FINGERLE. 2006. Prevalence of *Borrelia burgdorferi* s.l. OspA types in *Ixodes ricinus* ticks from selected localities in Slovakia and Poland. *International Journal of Medical Microbiology* 296, 108-118. [[CrossRef](#)]
33. S Miertusova Tothova, S Bonin, G Trevisan, G Stanta. 2006. Mycosis fungoides: is it a *Borrelia burgdorferi*-associated disease?. *British Journal of Cancer* 94:6, 879-883. [[CrossRef](#)]
34. G. Piccolin, G. Benedetti, C. Doglioni, C. Lorenzato, S. Mancuso, N. Papa, L. Pitton, M.C. Ramon, C. Zasio, Dr. , G. Bertiato. 2006. A Study of the Presence of *B. burgdorferi*, *Anaplasma* (Previously *Ehrlichia*) *phagocytophilum*, *Rickettsia*, and *Babesia* in *Ixodes ricinus* Collected within the Territory of Belluno, Italy. *Vector-Borne and Zoonotic Diseases* 6:1, 24-31. [[Abstract](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]

35. Beatriz Fernández-Jorge, Manuel Almagro-Sánchez, Raquel Escudero-Nieto, Eduardo Fonseca-Capdevila. 2006. Eritema migratorio por *Borrelia afzelii*. *Medicina Clínica* **126**:6, 237-238. [[CrossRef](#)]
36. A. Krause, P. Herzer. 2005. Frühdiagnostik der Lyme-Arthritis. *Zeitschrift für Rheumatologie* **64**:8, 531-537. [[CrossRef](#)]
37. R ESCUDERONIETO. 2005. Enfermedades producidas por *Borrelia*. *Enfermedades Infecciosas y Microbiología Clínica* **23**:4, 232-240. [[CrossRef](#)]
38. Bettina Wilske. 2005. Epidemiology and diagnosis of Lyme borreliosis. *Annals of Medicine* **37**:8, 568. [[CrossRef](#)]
39. References 251-324. [[CrossRef](#)]