(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2003 (05.06.2003)

PCT

(10) International Publication Number
WO 03/046561 A1

(51) International Patent Classification[7]: G01N 33/53
// 33/569

(21) International Application Number: PCT/FI02/00951

(22) International Filing Date:
26 November 2002 (26.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
20012310 26 November 2001 (26.11.2001) FI

(71) Applicant *(for all designated States except US)*: **BORTECH OY** [FI/FI]; Museokatu 15 A 7, FIN-00100 HELSINKI (FI).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LAHDENNE, Pekka** [FI/FI]; Museokatu 15 A 7, FIN-00100 HELSINKI (FI). **HEIKKILÄ, Tero** [FI/FI]; Kousatie 17 B 4, FIN-00430 HELSINKI (FI). **SEPPÄLÄ, Ilkka** [FI/FI]; Äestäjäntie 8 C 5, FIN-00390 HELSINKI (FI). **SAXEN, Harri** [FI/FI]; Tiilimäki 29, FIN-00330 HELSINKI (FI).

(74) Agent: **OY JALO ANT-WUORINEN AB**; Iso Roobertinkatu 4-6 A, FIN-00120 HELSINKI (FI).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/046561 A1

(54) Title: METHOD FOR DIAGNOSING EARLY AND LATE LYME BORRELIOSIS

(57) Abstract: The present invention relates to a method for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, to a diagnostic kit useful in said method and to an immunoassay method for diagnosing early and late Lyme borreliosis, especially for diagnosing erythema migrans. The methods according to the invention are characterized in that recombinant BBK32 proteins, optionally other immunogenic borrelial proteins, or their fragments are used as antigens.

Method for diagnosing early and late Lyme borreliosis

Field of the invention

The present invention relates to a method for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, to a diagnostic kit useful in said method and to an immunoassay method for diagnosing early and late Lyme borreliosis (LB), especially for diagnosing erythema migrans (EM). The methods according to the invention are characterized in that recombinant BBK32 proteins, other immunogenic borrelial proteins and/or their fragments are used as antigens.

Background of the invention

LB is a tick-transmitted spirochetal infectious disease, caused by *Borrelia burgdorferi*, which is characterized by multistage skin, joint, neurologic and cardiac manifestations [1]. The diagnosis of LB is based on clinical evaluation of the patients, but serologic assays, most frequently the enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB), are often used to provide supporting evidence of infection with *B. burgdorferi*. In the current routine LB serodiagnostic tests, the antigens predominantly used are borrelial flagellin protein or whole-cell lysate (WCL) of the *in vitro*-cultured microbes. A two-step approach with ELISA and a confirmatory WCL WB has been recommended by the Centers for Disease Control (USA) for positive or borderline results [2]. Especially in Europe the applicability of this procedure has remained questionable [3], mainly due to the existence of three species of *B. burgdorferi* sensu lato causing human LB, *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* [4]. One of the main reasons for these problems is the antigenic diversity due to variations in the sequences and expression of immunogenic proteins in these different borrelial species [3, 5].

Several other difficulties complicate current LB serology. In LB patients with early manifestations, *e.g.* erythema migrans (EM) and facial palsy, antibody responses to the current antigens may be weak or delayed [6]. EM, which appears at the site of the tick bite days to weeks after exposure, is the earliest and most common manifestation of LB. Tick bites may easily be unrecognised, and the clinician has to rely on the appearance of the skin lesion. In a routine clinical setting, EM is considered to be pathognomonic for early LB. The classical appearance of EM is an enlarging, ring-like erythema with a central clearing. However, early in the course of LB, atypical lesions may occur and cause diagnostic problems. Recent reports from the US and Europe have shown that during the first few days of infection the pathognomonic expanding peripheral erythema with a central clearing appears to be far less common than lesions with homogeneous redness [33, 34]. The gold standard for the diagnosis of early LB is microbiologic confirmation of LB by culture from biopsies taken from EM lesions and/or from blood. PCR-based methods can also be used to detect *B. burgdorferi* DNA in skin biopsies. However, in routine clinical practice, these methods are not feasible. Serologic confirmation of early LB is also problematic due to sensitivity problems of the current serodiagnostic assays. The sensitivity of the IgM or IgG enzyme-linked immunosorbent assay (ELISA) using borrelial flagella or whole-cell lysate antigens seldom exceeds 40-50% [6, 25]. Even at late stage LB, up to 5-10 % of patients may not have elevated antibody levels [7]. Further, viral infections cause false positivity in several LB tests for IgM antibodies [8]. Furthermore, in a subgroup of patients after a successful treatment of LB, antibody levels may stay high even for prolonged periods.

The expression of borrelial proteins also varies at different stages in the life cycle of *Borrelia* in ticks and in the mammalian hosts. Several genes, e.g. *bbk32*, *bbk50*, *vls* and *ospE/F* homologs [9-11], have been shown to be selectively expressed *in vivo*. Moreover, *bbk32* expression is detectable in spirochetes during tick feeding even before transmission to the host but not in unfed ticks [12]. In two previous studies, antibodies to BBK32 were observed in the sera of *B. burgdorferi* sensu stricto - infected mice and human patients with disseminated LB [9, 13]. So far, antigenic

properties of the BBK32 proteins in other species of *B. burgdorferi* sensu lato are not known.

The application of specific recombinant proteins of *B. burgdorferi*, such as outer surface protein A (OspA), OspB, OspC, OspE, OspF, P22, P39, P100, VlsE, and flagellin have improved the performance of ELISA assays [14-16]. However, the immune responses of patients infected with different *B. burgdorferi* subspecies vary greatly, and certain antigens may not always be expressed in hosts or be recognized immunologically. Thus, the sensitivity with single recombinant antigens has remained insufficient, so far. Another approach to improve the serodiagnosis of LB has been the use of peptides from certain borrelial proteins as antigens, e.g. a C-terminal decapeptide from OspC [17] and a peptide corresponding to a central invariable region in the VlsE protein, invariable region 6, IR6 [18]. Very recently, chimeric proteins comprising of epitopes of OspA, OspB, OspC, flagellin, and p93 have also been suggested for serodiagnostic antigens [19].

In the literature, the BBK32 protein has also been called P35 [9, 13, 20] and 47 kilodalton fibronectin binding (FBP) protein [21].

Detailed description of the invention

The purpose of the inventors was to test the antigenic potential of borrelial protein BBK32 and develop a reliable immunoassay method for the serodiagnosis of early and disseminated LB, especially for the serodiagnosis of erythema migrans. In order to cover all pathogenic borrelial species causing human LB, the inventors sequenced and cloned the *bbk32* from eight isolates of the three pathogenic borrelial species, *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*. The identity between the amino acid sequences of BBK32s in *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* isolates was 71-100%. The respective variant BBK32 recombinant proteins were tested in LB serology using serum samples from patients with early- and late-stage LB. In IgG Western blotting (WB) or enzyme-linked immunosorbent assay

(ELISA), up to 74% and 100% of acute and convalescent samples from 23 patients with erythema migrans (EM) were positive for recombinant BBK32 protein from *B. afzelii*. In the serology of disseminated LB, the three variant BBK32 antigens cross-reacted. In total, the sensitivity of IgG ELISA reached 100%. The results show that the BBK32 proteins are useful serodiagnostic antigens for early and disseminated LB, but in order to cover all the relevant borrelial species variant BBK32 proteins or fragments thereof should be used in parallel or combined in an immunoassay for LB.

It is therefore an object of the present invention to provide a novel method for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, in which method recombinant BBK32 proteins or their fragments containing antigenic epitopes from one or more *Borrelia* species are used as antigens in an immunoassay. The method according to the invention can be used for the serodiagnosis of early and late Lyme borreliosis, especially for the serodiagnosis of erythema migrans.

Within the scope of this invention and this specification, a 'fragment' is intended to mean a recombinantly produced fragment containing antigenic epitopes of the immunogenic protein(s) in question.

In a preferred embodiment according to the invention, recombinant BBK32 proteins or their fragments together with any other immunogenic proteins or their fragments derived from one or more *Borrelia* species are used as antigens in an immunoassay. Examples of the other immunogenic borrelial proteins which can be used in the method according to the invention include, but are not limited to, outer surface protein A (OspA), OspB, OspC, OspE, OspF, P22, P39, P100, VlsE, DbpA, and/or flagellin, preferably DbpA.

Preferably the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are derived from at least two *Borrelia* species selected from the

group consisting of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*, more preferably from all the three *Borrelia* species mentioned above.

It is apparent to those skilled in the art that instead of borrelial proteins or their fragments, or together with them, also peptides or polypeptides (referred here as peptides), *i.e.* shorter amino acid stretches comprising at least two, usually several amino acids, can be used as antigens in the method according to the invention. It is also possible to take a combination of several fragments or peptides from various immunogenic borrelial proteins and to use them as antigens.

The recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are used as antigens either in parallel or combined in an immunoassay. When recombinant BBK32 proteins are used as 'parallel' antigens, antibodies are measured separately against the recombinant BBK32 antigens from different *Borrelia* species in the same assay. It is also possible to combine the recombinant BBK32, other immunogenic borrelial antigens, or their fragments from different *Borrelia* species and measure antibodies against the 'combined' BBK32 antigen. For example in a parallel assay, three recombinant BBK32 protein antigens from three *Borrelia* species are preferably used in the same assay. In a combined assay, preferably three recombinant BBK32 proteins from three *Borrelia* species are combined to form the combined BBK32 protein antigen.

Within the scope of this invention, an 'immunoassay' is intended to cover all immunoassay methods known to persons skilled in the art.

The method according to the invention for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal comprises preferably the steps of

a) contacting the body fluid with recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments derived from one or more *Borrelia*

species under conditions effective to allow the formation of antigen-antibody complexes; and

b) detecting the complexes formed.

The body fluid is preferably a serum, plasma, whole blood, cerebrospinal fluid, or synovial fluid sample.

The conditions effective to allow the formation of antigen-antibody complexes as well as the means for detecting the complexes formed are chosen according to the antibodies and other reagents used in the assay and are known to a person skilled in the art.

It is a further object of the invention to provide a diagnostic kit useful for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, said kit comprising in a suitable container

a) recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments from one or more *Borrelia* species and a detectable label or marker linked to said proteins and/or their fragments, or

b) recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments from one or more *Borrelia* species and a second antibody linked to any detectable label or marker.

Detectable labels or markers and methods to link them to antigens or second antibodies are well disclosed in the literature and are also known to persons skilled in the art of immunoassays.

A further object of the invention is an immunoassay method for diagnosing early and late Lyme borreliosis, especially for the serodiagnosis of erythema migrans, comprising the steps of

a) contacting a body fluid from a human or other mammal with recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments derived from one or more *Borrelia* species under conditions effective to allow the formation of antigen-antibody complexes; and

b) detecting the complexes formed.

A still further object of the invention is to provide a novel method for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, in which method recombinant BBK32 proteins and recombinant decorin binding protein As (DbpAs) from at least two, preferably three, *Borrelia* species are used together as antigens in an immunoassay. Decorin binding protein A is a borrelial outer surface protein, which has been suggested to act as a species-specific serodiagnostic antigen for LB. In a preferred embodiment according to the invention, the recombinant BBK32 proteins and the recombinant DbpAs are derived from *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*.

Antibodies to the BBK32 protein seem to appear very early during human LB. In up to 74% of the patients at acute-phase EM, IgG antibodies to BBK32 were detectable by ELISA and/or WB. At follow-up, after successful antibiotic treatment, all the patients were anti-BBK32 antibody-positive. A recent study reported cloning of the *bbk32* from a *B. burgdorferi* sensu stricto isolate and showed early antibody responses to the recombinant BBK32 protein during experimental murine borreliosis [9]. Reverse transcriptase-PCR studies have also demonstrated *bbk32* expression in EM lesions of three patients, indicating that, during human LB, *bbk32* is expressed early [20]. However, antibody responses to BBK32 in patients with early local EM have not previously been studied.

With the current LB serology based mainly on flagellin as an antigen, IgM and IgG antibodies are not detectable by ELISA or immunoblot assays until 2-4 or 6-8 weeks after the onset of the disease [6]. During early local LB, the sensitivity of IgM

ELISA seldom exceeds 50% [6, 22-24]. A study on patients with culture-confirmed EM showed that positive serology at presentation and the rate of seroconversion correlated directly with disease duration [25]. If the EM lesion had emerged less than 7 days prior to sampling, only 10% of the patients showed antibodies in ELISA, whereas, of the patients whose EM had occurred 7 to 14 days earlier, 58% had detectable antibodies. A recent study reporting on anti-BBK32 antibodies in disseminated LB also showed IgG BBK32 antibodies in 84% of patients with EM [13]. However, in these patients, the EM lesion had been present for 2 weeks to 3 months after disease onset, suggesting dissemination of LB. In the present series, the time of occurrence of the EM lesion could not be accurately assessed. However, given the low proportion of seropositivity to flagellin at presentation of EM (17-26%) and the broad awareness of LB among the general population in regions where LB is endemic in Finland, it can be presumed that, in most cases, the EM lesions represented early disease. Hence, the present results imply that assessment of IgG, although not of IgM antibodies, to BBK32 proteins affords a major improvement in the serodiagnosis of early LB. The antibody response to the BBK32 protein also seems to precede the humoral response to *in vitro* -grown microbes (WCL), which do not necessarily synthesize this *in vivo* -expressed protein [9, 21].

Only a few studies have evaluated the antigenic properties of the BBK32 protein. In these studies, the BBK32 proteins originated from American *B. burgdorferi* sensu stricto strains [13, 20]. In a preferred embodiment of the present invention variant recombinant proteins from the three pathogenic borrelial species, *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*, are used in the serodiagnosis of LB, especially in the serodiagnosis of erythema migrans. Recombinant BBK32 originating from a local *B. afzelii* isolate appeared to be superior to the other rBBK32 proteins for diagnosing EM. This finding agrees with the PCR results of the EM skin biopsies, where the majority of the infecting species proved to be *B. afzelii*. These observations are also in accord with two recent European studies, where over 90% of *Borrelia* isolates from EM lesions were *B. afzelii*, less than 10% *B. garinii*, and none were *B. burgdorferi* sensu stricto [26, 27]. The present results suggest that in

diverse epidemiological situations, for the serodiagnosis of early local EM, variant BBK32 proteins are preferred.

Although the immunoreactivity of EM patient sera to variant BBK32 proteins diverged, the three recombinant BBK32 antigens "cross-reacted" in the serology of disseminated LB. Furthermore, the intensities of the serologic responses against variant BBK32 proteins, as measured by OD values in ELISA, correlated well. Therefore, these results indicate that variant BBK32 proteins may have both specific and common antigenic epitopes. In European epidemiological studies, the most prevalent *Borrelia* species have been *B. afzelii* and *B. garinii* [28], *B. burgdorferi* sensu stricto occurring infrequently, especially in Scandinavia [27, 29]. In cerebrospinal fluid samples of European neuroborreliosis cases, the species predominantly isolated has been *B. garinii* [30]. The hypothesis that epitope specificity varies in early and late LB is in line with the analysis of BBK32 sequences of eight Finnish isolates of *B. burgdorferi* sensu lato, showing over 90% identity between the sequences of *B. burgdorferi* sensu stricto and *B. garinii*. In contrast, the identity between the BBK32 sequences of *B. afzelii* strains and of other species was approximately 70%.

In the serodiagnosis of disseminated LB, the BBK32 antigen has been evaluated in a limited number of patients only, so far [9, 13]. Fikrig *et al.* [9] reported high IgG antibodies to BBK32 in 3 of 3 patients with neuroborreliosis and in 3 of 7 patients with Lyme arthritis. Akin *et al.* [13] showed IgG responses to BBK32 in 83-92% of 25 Lyme arthritis patients. The preferred embodiment of the present invention with the three variant BBK32 proteins as antigens improved the sensitivity of ELISA up to 100%. In the serodiagnosis of neuroborreliosis, all but one case would have been detected irrespective of the origin of the BBK32 antigen. Instead, especially in ELISA for Lyme arthritis, use of a single BBK32 antigen would have left the sensitivity at 80-90%. The occasional discrepancies between the results of WB and ELISA may be due to differences in the orientation of the antigen and/or antigen-antibody complex formation.

In summary, the inventors have shown that the BBK32 proteins are useful antigens for both early and late LB serology. The BBK32 from *B. afzelii* proved to be a sensitive antigen of EM already at presentation. During the course of infection, the sensitivity increased being up to 100% in convalescence samples for EM patients. However, it is evident that variant BBK32 proteins should be used either in parallel or combined with an immunoassay for LB to cover all the relevant borrelial species, whose prevalence differs regionally in Europe.

The following examples further illustrate the invention without, however, limiting the same.

Examples

Bacterial strains. Finnish borrelial strains were obtained from the National Public Health Institute, Turku, Finland. *B. burgdorferi* sensu stricto strain ia (here referred to as Bbia) was isolated from the cerebrospinal fluid of a Finnish patient with neuroborreliosis. Of the *B. afzelii* strains, A91 and 1082 (referred to as BaA91 and Ba1082) were isolated from skin biopsy samples of Finnish patients with erythema migrans (EM), and 570 and 600 (referred to as Ba570 and Ba600) were isolated from ticks. *B. garinii* strains 40, 46, and 50 (referred to as Bg40, Bg46, and Bg50, respectively) were isolated from skin biopsy samples of Finnish patients with EM. The genotypes of culture-positive *Borreliae* were confirmed by sequencing a fragment of the flagellin gene [29]. *Borreliae* were cultivated in BSK-H (Barbour-Stoenner-Kelly) medium (Sigma, USA) at 33°C in 5% CO_2. The *B. afzelii* strain SK1 was used in an in-house ELISA for detecting antibodies against borrelial WCL. *Escherichia coli* host cells for cloning and for expression of recombinant proteins were INFαF (Invitrogen, Netherlands) and BL21 (Amersham Pharmacia Biotech, Sweden), respectively.

DNA purification. Borrelial genomic DNA was purified with a Dneasy Tissue Kit (Qiagen, Germany). Purified DNA was used in PCR and in cloning experiments. Plasmid DNA was purified with a QIAprep-spin plasmid kit (Qiagen, USA).

PCR and DNA sequencing. A PCR-based approach was employed to amplify and sequence the *bbk32* alleles from eight different isolates of *B. burgdorferi* sensu lato. Primers for *bbk32* sequencing were designed on the basis of published *bbk32* sequences (Table 1). Several primer pairs were designed and tested to ensure that the entire coding sequence of the *bbk32* was obtained. To eliminate possible errors caused by Taq-polymerase, the two strands for each *bbk32* were sequenced independently at least twice. Expression primers for each strain encoding the mature portion of the BBK32 protein after cysteine at the site of posttranslational acylation were chosen from the sequences analyzed. For each borrelial strain, the *bbk32* sequences were generated by PCR amplification of *B. burgdorferi* genomic DNA. Approximately 1 ng of template DNA was used in standard PCR conditions: 30 cycles of 94°C denaturing for 1 min, 50°C annealing for 1 min, and 72°C extension for 1 min 30 s with AmpliTaqGold DNA polymerase (Perkin Elmer, USA). The PCR-amplified full-length or partial *bbk32*s were cloned to the pCR 2.1-TOPO vector (Invitrogen, Netherlands) for sequencing. DNA sequencing was performed at the Core Facility of the Haartman Institute, University of Helsinki, with DyePrimer (T7, M13Rev) cycle sequencing kit (Applied Biosystems Inc., USA). Sequencing reactions were run and analyzed by the automated sequencing apparatus model 373A (Applied Biosystems Inc., USA). DNA and protein sequences were analyzed with Lasergene software (DNASTAR, USA).

TABLE 1. Primers used for PCR amplification of the *bbk32* genes

No.	Species	Primer 5′-3′	Location	Source
1	B.burgdorferi	CAC CCT CTT GAT AGC ACT TA	-203 - -184	B31(AF000788)
25	sensu stricto	CTT TAA AGG AGA GAA AGC ATG	-18-3	Bbia(AF472532)
3		CCG GAT CCG ATT TAT TCA TAA GAT ATG AAA T	60-82	Bbia
4		GCA ATC TGA GAC TAG AAA AG	329-348	Bbia
5		TGC AGT CTT TAC ACT TAC TT	879-860	Bbia
6		CCC TCG AGA TTA GTA CCA AAC GCC ATT	1084-1065	Bbia
10		ACA TAT TAT GTA GCC TGT TTT A	1122-1101	B31
2	B. garinii	CTT TAA AGG AGA GAA AGC ATG	-18-3	Bbia
3		CCG GAT CCG ATT TAT TCA TAA GAT ATG AAA T	60-82	Bg40(AF472529)
4		GCA ATC TGA GAC TAG AAA AG	329-348	Bg40
5		TGC AGT CTT TAC ACT TAC TT	879-860	Bg40
15		CCC TCG AGA GTA CCA AAT GCC ATT CT	1084-1064	Bg40
7		ACA TAT TAT GTA GCC TGT TTT A	1122-1101	B31
2	B. afzelii	CTT TAA AGG AGA GAA AGC ATG	-18-3	Bbia
9		CCG GAT CCG ATT TAT TCA TAA GAG ATG AAA T	57-79	BaA91(AF472525)
10		TGA GCA TAA AAG GAT GCT TC	369-387	BaA91
20		GCA GTC CTT GCA CTC ACT	855-838	BaA91
12		CCC TCG AGC AAA GAT TAG TAC CAA ACA C	1065-1046	BaA91
7		ACA TAT TAT GTA GCC TGT TTT A	1122-1101	B31

Restriction enzyme sites for BamHI and XhoI in expression primers are underlined

Primers 2 and 7 were used in all strains

Primers 3, 4, and 5 were used both in *B. burgdorferi* sensu stricto and *B. garinii* PCR amplifications

Cloning and expression of recombinant BBK32. For expression of the recombinant BBK32 (rBBK32), glutathione S-transferase (GST) fusion protein constructs were generated. The PCR-amplified DNA encoding the mature portion of BBK32 was cloned into the pCR 2.1-TOPO plasmid (Invitrogen, Netherlands). The recombinant plasmid was purified and digested with BamHI and XhoI restriction enzymes. The cleaved *bbk32* was then ligated to a similarly digested pGEX-4T-1 expression plasmid (Amersham Pharmacia Biotech, Sweden) and transformed into *E. coli* BL21

host cells. The expression of recombinant GST-BBK32 protein was generated according to the manufacturer's instructions (Amersham Pharmacia Biotech, Sweden). The expression and purity of the GST-rBBK32 fusion protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Western blotting. GST-rBBK32s originating from Bbia, BaA91, and Bg40 (referred to here as $rBBK32_{Bbia}$, $rBBK32_{BaA91}$, or $rBBK32_{Bg40}$, respectively) were fractionated in 10% SDS-PAGE and transferred to a nitrocellulose membrane (BioRad, 0.2 μm pore size, USA) by semi-dry transfer with 40 mM glycine-50 mM Tris (pH 9.0)- 0.375% (w/v) SDS- 20% (v/v) methanol buffer. Equal amounts of each GST-rBBK32 were used for one 7-cm-wide nitrocellulose membrane. Two-mm strips of the nitrocellulose membranes were soaked in 0.1% Tween 20, 0.9% NaCl. Serum samples were diluted in 0.1% Tween 20, 0.9% NaCl, 0.1 g/l fat-free bovine milk powder (Valio, Finland). Samples were incubated at 1:100 dilution for 2 h. After four buffer rinses, the Western blots were incubated with alkaline phosphatase-conjugated rabbit anti-human IgG (Jackson Immuno Research Laboratories Inc., USA) at 1:5000 for 2 h. After washing, the bands were visualized with 5-bromo-4-chloro-3-indolylphosphate nitro blue tetrazolium (Sigma Chemical Co., USA). The reaction was terminated 10-15 min later by washing with distilled water. The WB results were analyzed with MacBAS 2.5 (Fuji, Japan) software, and the cut-off for a positive IgG WB result was defined as the mean + 3 standard deviations (SD) of the values of healthy blood donors. For detection of GST, monoclonal anti-GST antibodies (Sigma, USA) were used.

ELISA. ELISA analyses for anti-flagellin antibodies were done as described earlier [31]. Briefly, IgG antibodies against *B. burgdorferi* were measured with a commercial flagellin-based ELISA kit (Dako, Denmark) modified by titrating the antibodies. Sera were diluted serially in three-fold steps for the test and applied to the plates for overnight incubation. The bound antibodies were detected with biotin-labeled goat anti-human IgG (Zymed, USA). An end-point titer was obtained at an optical density level determined by a cut-off control provided by the kit. The titer limit for a positive IgG antibody level was 500. The cut-off control material con-

formed with the level of the mean + 3 SD of the reference population living in central Finland, an area with low prevalence of LB [31].

For ELISA assays measuring anti-BBK32 antibodies, the wells in a microtiter plate were coated with 100 µl (2 µg/ml) of variant BBK32 recombinant proteins overnight. After washing, 100 µl of diluted serum samples were added to the wells and incubated overnight. Serum samples were diluted 1:10 (EM) or 1:100 (neuroborreliosis and Lyme arthritis) in 5 mg/ml bovine serum albumin (BSA) in 0.155 M NaCl-0.04% Tween 20 buffer (BSA-NaCl-Tween). After washing, the wells were incubated with alkaline phosphatase-conjugated rabbit anti-human IgG or IgM (Jackson Immuno Research Laboratories Inc., USA) 1:5000 in BSA-NaCl-Tween for 2 h. The reactions were visualized with 4-nitrophenylphosphate (Boehringen Mannheim GmbH, Germany) 1 mg/ml in diethanolamine buffer pH 10.0. The optical density (OD) measurements were made after 10 to 20 minutes at wavelength 405 nm using a Multiscan photometer (Thermo Labsystems, Finland).

Samples. 1. For serological analyses, human serum samples were collected from Finnish patients with culture- or PCR-positive EM, with neuroborreliosis (NB), and with Lyme arthritis (LA). Samples were collected from EM patients at diagnosis (acute) and 1 to 3 months after treatment (convalescent). Of the 23 patients with EM, genotyping by PCR analysis [29] showed *B. afzelii* in 17, and *B. garinii* in 4 of the skin biopsies. In two biopsies, genotyping was not feasible. In the patients with disseminated LB, the clinical manifestations agreed with the CDC criteria for LB [32]. The clinical diagnosis was confirmed in ELISA by demonstrating serum antibodies (and CSF anti-flagellin antibodies in NB patients) against flagellin and *B. burgdorferi* WCL. Serum samples from patients with syphilis, Epstein-Barr virus (EBV) infection, systemic lupus erythematosus (SLE), rheumatoid factor (RF) positivity, anti-streptolysin (ASO) positivity, and sera from healthy blood donors were used as controls.

2. Serum samples were also collected from patients with clinically documented or culture- or PCR-confirmed EM from Germany, Slovenia, and USA. German samples were collected in Northern Bavaria as part of a regional study on LB

[35]. Sera were collected from 22 patients with physician-diagnosed EM at the time of diagnosis and during the convalescence phase. The clinical diagnosis of EM was confirmed by especially trained physicians. In addition, from 10 US patients with culture-positive EM sera were collected at the time of diagnosis and during convalescence 3-4 weeks after treatment [36]. Serum samples were available from 20 Slovenian patients in the acute stage of EM. Two out of 9 Slovenian patients from whom skin biopsies were taken were culture-positive (*B. burgdorferi* sensu lato) [37]. All patients with EM were treated with oral antimicrobials.

Serum samples from 40 Finnish healthy blood donors were used as negative controls and to define the cut-off value for all ELISAs (cut-off = mean plus 3 SD).

Nucleotide sequence accession numbers. The nucleotide sequences of the *bbk32* were submitted to GenBank under accession numbers AF472525 for *B. afzelii* A91 (SEQ ID NO. 1), AF472527 for *B. afzelii* 1082 (SEQ ID NO. 5), AF472526 for *B. afzelii* 570 (SEQ ID NO. 3), AF472528 for *B. afzelii* 600 (SEQ ID NO. 7), AF472529 for *B. garinii* 40 (SEQ ID NO. 9), AF472530 for *B. garinii* 46 (SEQ ID NO. 11), AF472531 for *B. garinii* 50 (SEQ ID NO. 13), and AF472532 for *B. burgdorferi* sensu stricto ia (SEQ ID NO. 15).

Statistical analyses. The Microsoft Excel 2000 program (Microsoft, USA) was used for calculations of standard statistics.

Results

Sequence analysis of BBK32 in the Finnish borrelial isolates. The deduced amino acid sequences of $BBK32_{BaA91}$, $BBK32_{Ba1082}$, $BBK32_{Ba570}$, $BBK32_{Ba600}$, $BBK32_{Bg40}$, $BBK32_{Bg46}$, $BBK32_{Bg50}$, and $BBK32_{Bbia}$ contained 352 to 360 residues. The sequences of all the BBK32 proteins revealed a hydrophobic leader sequence of 18 to 19 residues and a phenylalanine-X-Y-cysteine motif, consistent with a lipoprotein. Characteristically for borrelial lipoproteins, the greater part of the mature por-

tion of the BBK32 protein was hydrophilic (data not shown). The BBK32 leader sequences in the *B. garinii* strains and the *B. burgdorferi* sensu stricto strain were identical, but differed by three amino acids from the leader sequences in the identical *B. afzelii* strains. The inter-species identity of the deduced amino acid sequences of the BBK32 proteins ranged from 71 to 95% (Figure 1). The differences in the amino acid sequences were distributed evenly along the sequence. The identity of the BBK32 amino acid sequences within the borrelial subspecies ranged from 94 to 100%. The calculated molecular mass of the mature BBK32 proteins (without putative lipid acylation) ranged from 38.7 to 39.5 kDa.

Sequence analysis of BBK32 in B. afzelii strains. The inventors sequenced the *bbk32* from two human (BaA91 and Ba1082) and two tick isolates (Ba570 and Ba600). The identity of the deduced amino acid sequences was from 99 to 100%. The BBK32 sequences of the two human *B. afzelii* isolates were identical. In the GenBank search, one *bbk32* sequence from *B. afzelii* was found. The BBK32 sequence of the ACA1 strain (AF213179) is only partial, corresponding to the sequence between amino acid positions 34 and 336 of the deduced sequence of $BBK32_{BaA91}$. In the matching regions of the four studied BBK32 sequences from *B. afzelii* and the partial $BBK32_{ACA1}$ sequence, identity was from 99 to 100%.

Sequence analysis of BBK32 in B. garinii strains. The deduced amino acid sequences of $BBK32_{Bg40}$ and $BBK32_{Bg50}$ were identical, and $BBK32_{Bg46}$ was 94% identical with them. In the GenBank search, one partial *bbk32* sequence from *B. garinii* strain Ip90 (AF213178) was found. This sequence matched the $BBK32_{Bg40}$ sequence between amino acid residues 35 and 343, but in the $BBK32_{Ip90}$ sequence a six amino acid deletion was observed from positions 201 to 206. Sequence identity in the corresponding regions of the BBK32 sequences from *B. garinii* strains in this study and the partial $BBK32_{Ip90}$ sequence was from 92 to 93%.

Sequence analysis of BBK32 in B. burgdorferi sensu stricto strains. The *bbk32* sequence from the local strain Bbia was compared with two sequences published in

the GenBank. The *bbk32* sequence of the B31 strain (AE000788) was complete and that of strain N40 (U82107) was a partial sequence, lacking the first 86 amino acids. The BBK32 amino acid sequences from Bbia and B31 were 96% identical. Identity in the corresponding regions of BBK32 sequences of Bbia, B31, and N40 was from 91 to 94%. In the $BBK32_{B31}$ sequence there was a six amino acid deletion at positions 201 to 206 of $BBK32_{Bbia}$. In the same region, three tyrosine residues were deleted from the $BBK32_{N40}$ sequence.

Western blotting. In IgG Western blots using rBBK32 proteins from *B. afzelii* (BaA91) as antigen with serum samples from Finnish patients with culture- or PCR-positive EM, 10/15 (67%) at the acute and 15/15 (100%) at the convalescent phase were positive (Figure 2). With $rBBK32_{Bg40}$ as an antigen, 4/15 and 8/15, and with $rBBK32_{Bbia}$, 7/15 and 3/15 were positive at the acute and convalescent phases, respectively. Of the 10 control patients (five with syphilis and five healthy blood donors), one with syphilis had a weak antibody response to $rBBK32_{BaA91}$ (data not shown). In ELISA for anti-flagellin antibodies (Dako, Denmark), 6/15 patients had IgG or IgM antibodies at the acute or convalescent phase (Figure 2). One patient had IgM antibodies in the in-house ELISA (WCL as antigen).

In another WB experiment, serum samples from 10 patients with NB and 10 with LA were analyzed for the presence of anti-BBK32 IgG antibodies. All 20 patients with disseminated LB reacted positively with the three rBBK32 proteins as determined with the MacBAS program, using the blood donors' mean + 3 SD as a cut-off value (Table 2). Minor differences were observed in the immunoreactivity of serum samples against different rBBK32 proteins. Specifically, in four serum samples, weaker reactivities were observed against $rBBK32_{BaA91}$ than against the other rBBK32s (data not shown). A few of the control samples were low positives (Table 2). None of the patient or control sera recognized pure GST (data not shown).

TABLE 2. IgG Western blotting reactivity against recombinant BBK32 proteins from *B. afzelii* (BaA91), *B. garinii* (Bg40), and *B. burgdorferi* sensu stricto (Bbia) of LB patients and controls. The intensities of WB bands were analyzed with MacBas 2.5 software.

Patient/control group	No. of positive samples/ no. of samples recombinant BBK32		
	BaA91	Bg40	Bbia
Neuroborreliosis	10/10	10/10	10/10
Lyme arthritis	10/10	10/10	10/10
Syphilis	1/5	0/5	2/5
Positive for RF*	0/5	0/5	1/5
Blood donors	0/5	0/5	0/5

* RF, rheumatoid factor

ELISA. 1. Serum samples from Finnish LB patients and controls were tested in IgG ELISA, using all three rBBK32 proteins individually as antigens. In patients with NB, 14/14, 13/14, and 14/14 samples were positive when $rBBK32_{BaA91}$, $rBBK32_{Bg40}$, and $rBBK32_{Bbia}$, respectively, were used as antigens. In serum samples from patients with LA, these figures were 12/15, 11/15, and 14/15, respectively. In total, 14 of 14 (100%) samples from patients with NB, and 15 of 15 (100%) samples from patients with LA were positive for one or more rBBK32 proteins (Figure 3). The ELISA OD values of NB patients correlated well between variant BBK32 proteins as antigens, the correlation coefficients being 0.91, 0.78, and 0.83, between BBK32 from BaA91 and Bg40, BaA91 and Bbia, and Bg40 and Bbia, respectively. In LA patients, the respective correlation coefficients were 0.89, 0.90, and 0.93.

Serum samples from patients with EM were analyzed in both IgG and IgM ELISA. In IgG ELISA with $rBBK32_{BaA91}$ as an antigen, 17/23 (74%) samples taken at the acute and 15/23 (65%) at the convalescent phase were positive (Figure 4). On the other hand, when $rBBK32_{Bg40}$ and $rBBK32_{Bbia}$ were used as antigens, 6/23 (26%) and 5/23 (22%) of acute samples, and 7/23 (30%), and 4/23 (17%) of convalescent samples, respectively, were positive. Of the control samples, 2/10 with syphilis, 2/8

RF-positive, 4/10 EBV-positive, and 2/20 healthy blood donor samples showed low positive OD values. In IgM ELISA with samples from EM patients, 4 to 13% of the acute or convalescent samples were positive, depending on the rBBK32 antigen used.

In the ELISA tests for anti-flagellin antibodies, 6/23 (26%) patients had IgG antibodies in either acute or convalescent samples. In IgM ELISA assays for anti-flagellin antibodies, 4/23 (17%) were positive.

2. *Antibodies in EM patients from different countries.* At presentation of EM, 65 of the 75 (87%) patients (Finnish, German, Slovenian, and American patients included) had IgG antibodies to one or more variants of rBBK32, 29/75 (39%) to flagella, and 29/75 (39%) to the IR_6 peptide antigen (Table 3, Figure 5). In samples from different regions, reactivity to the rBBK32 variants diverged. The majority of patients from Finland had antibodies to rBBK32 from *B. afzelii*, whereas, in the German and Slovenian sera, the most sensitive antigen was rBBK32 from *B. garinii*. All 10 samples from the USA reacted positively with BBK32 from *B. burgdorferi* sensu stricto and from *B. garinii*.

In the different regions, the proportion of patients with IgM anti-flagella antibodies at the time of diagnosis varied from 13% to 45%. Of the total of 75 patients, 29% had IgM anti-flagella antibodies. IgM and/or IgG anti-flagella antibodies were detected in 46% of the patients.

Antibodies in the convalescent phase of EM patients. Forty of the 55 patients (73%) in the convalescent phase had IgG antibodies to one or more rBBK32s, 25/55 (45%) to flagella and 19/55 (35%) to the IR_6 peptide. The pattern of seropositivity to variant rBBK32s in the convalescent and the acute sera was similar. In the convalescence samples from Finland and Germany, the overall rate of IgG antibody positivity to rBBK32s had slightly decreased from that at diagnosis (Table 3, Figure 5).

The study with sera from epidemiologically diverse regions provides supporting evidence that the BBK32 proteins may be useful antigens in EM serology. At presentation of EM, the sensitivity of the BBK32 ELISAs appeared better than the anti-flagella or the new anti-IR_6 tests.

5

TABLE 3. Number of positive sera (%) for any of the three BBK32 variant antigens (BBK32 - all), IR_6 peptide antigen, and for flagella antigen (Dako) in patients with EM from Finland (FIN), Germany (GER), USA, and Slovenia (SLO).

	AT DIAGNOSIS					AT CONVALESCENCE			
Antigen	FIN n=23	GER n=22	USA n=10	SLO n=20	TOTAL n=75	FIN n=23	GER n=22	USA n=10	TOTAL n=55
BBK32 - all*	17 (74%)	21 (95%)	10 (100%)	17 (82%)	65 (87%)	15 (65%)	15 (68%)	10 (100%)	40 (73%)
IR6*	7 (30%)	7 (32%)	4 (40%)	11 (55%)	29 (39%)	6 (26%)	6 (27%)	7 (70%)	19 (35%)
Flagella*	6 (26%)	9 (41%)	4 (40%)	10 (50%)	29 (39%)	6 (26%)	11 (50%)	8 (80%)	25 (45%)
Flagella**	3 (13%)	8 (36%)	2 (20%)	9 (45%)	22 (29%)	4 (17%)	7 (32%)	8 (80%)	19 (34%)
Flagella***	6 (26%)	13 (59%)	4 (40%)	12 (60%)	35 (46%)	8 (35%)	15 (68%)	10 (80%)	33 (60%)

*IgG class antibodies; **IgM class antibodies; *** IgG and/or IgM antibodies

Antibodies to BBK32 in cerebrospinal fluid (CSF) samples. Antibodies to BBK32 have also been measured in the cerebrospinal fluid (CSF). CSF samples were obtained from 85 patients who had been treated for neuroborreliosis. The clinical diagnosis of neuroborreliosis was based on the clinical guidelines for diagnosis presented by the Centers for Disease Control and Prevention, USA. As a control assay, CSF antibodies to purified intact flagella of *B. afzelii* (Dako, Denmark) were determined. The CSF samples had also been studied for pleocytosis. Based on the anti-flagella antibodies and CSF findings, the patients were divided into three groups: confirmed (elevated anti-flagella IgG antibodies and pleocytosis in CSF), probable (elevated anti-flagella IgG antibodies but no pleocytosis in CSF), and possible (no anti-flagella IgG antibodies in CSF but either serum anti-flagella antibodies or pleocytosis in CSF) neuroborreliosis. In the classification, CSF anti-flagella antibodies were defined as significant if the IgG anti-flagella antibody titer in the CSF was higher than that of the serum divided by 400 (the approximate serum IgG/CSF IgG ratio in healthy persons). According to this definition, CSF anti-flagella antibody titers > 20 were regarded as significant. Furthermore, patients were defined to have neuroborreliosis of short duration if the symptoms had lasted less than 3 months and of long duration if the symptoms had lasted longer than 3 months. As controls, we used CSF samples from 14 patients with syphilis, from 32 patients with other neurological diseases such as confirmed viral meningitis or convulsions/epilepsy, and from 20 patients without any proven infection. All the CSF samples from the controls were negative for anti-flagella antibodies.

IgG ELISAs were performed with rBBK32 antigens, including three variants originating from *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*, as described above. For rBBK32 ELISA, each well was coated overnight at +4°C with 200 ng of protein, rBBK32 diluted in 3 M urea. The CSF samples were used at 1:10 dilution in NaCl-0.04% Tween 20 buffer (BNT). Samples of 20 CSF controls without proven infection were used to define the cutoff value (mean plus 3 SD).

Of all the 85 patients, 70% had CSF antibodies to rBBK32, whereas anti-flagella antibodies were observed in 53% of the patients. Of the 40 patients with negative or borderline anti-flagella antibodies (possible neuroborreliosis), 43% had anti-BBK32 antibodies (Figure 6).

Of the 60 patients with duration of the disease < 3 months, 73% had CSF antibodies to rBBK32. Of the 25 patients with disease of longer duration (> 3 months), 58% had antibodies to rBBK32, mostly at low level (Figure 7). In the patients with a longer duration of the disease, the antibody levels to rBBK32 in the CSF were lower than in the patients with a short duration ($p<0.012$) (Figure 7).

Recombinant BBK32 fragment as an antigen. In another experiment, a 91-amino acid hydrophilic fragment of the BBK32 protein (from *B. afzelii*) (fragment) was initially cloned and expressed as a recombinant fusion protein (GST-fusion protein), purified and subsequently tested as an antigen in ELISA. As a comparison, antibodies to the BBK32 whole protein (GST-fusion protein) and to a commercial whole cell lysate antigen (Institut Virion/Serion GmbH, Germany) were assessed. The serum samples were from culture- or PCR-positive EM patients (n=23) or from patients with Lyme arthritis (n=7), neuroborreliosis (n=7) or acrodermatitis chronica atrophicans (n=3) and from control patients (syphilis, rheumatoid factor positive-patients, salmonella-, yersinia-infection, anti-streptolysin positive patients). In the serology of disseminated borreliosis, the performance of the BBK32 fragment in ELISA was as good as the BBK32 whole protein and whole cell lysate antigen but in the serology of erythema migrans, ELISA with the BBK32 fragment performed better than the control assays (Table 4).

TABLE 4.

		IgG BBK32	IgG fragment	Virion, IgG	Virion, IgM
EM I (n=23)	Positive	4	10	5	7
	Borderline			3	5
	Negative	19	13	15	11
EM II (n=23)	Positive	6	12	5	6
	Borderline			3	6
	Negative	17	11	15	11
Syfilis (n=5)	Positive	1	1	3	1
	Borderline		1		2
	Negative	4	3	2	2
RF+ (n=5)	Positive	1	2	2	1
	Borderline				1
	Negative	4	3	3	3
Salmonella (n=5)	Positive				
	Borderline				1
	Negative	5	5	5	4
Yersinia (n=5)	Positive		1		1
	Borderline			1	
	Negative	5	4	4	4
AST+ (n=5)	Positive	1			
	Borderline			1	1
	Negative	4	5	4	4
ACA (n=3)	Positive	3	3	3	2
	Borderline				1
	Negative				
NB (n=7)	Positive	6	5	6	1
	Borderline				4
	Negative	1	2	1	2
LA (n=7)	Positive	7	6	7	4
	Borderline				1
	Negative		1		2

Brief description of the figures

Figure 1. Identities of deduced amino acid sequences of BBK32 among the isolates of Finnish *B. burgdorferi* sensu stricto (Bbia), *B. garinii* (Bg40, Bg46, and Bg50), and *B. afzelii* (BaA91, Ba1082, Ba570, and Ba600). The identities (%) were calculated from the sequences of the entire proteins including the leader peptides with Multiple sequence alignment, Jotun Hein method, Lasergene software.

Figure 2. Evaluation of sensitivity of BBK32 in IgG Western blotting (WB) for serodiagnosis of early Lyme borreliosis. Serum samples were collected from culture or PCR-positive patients with erythema migrans at diagnosis (acute) and 1-3 months after antibiotic treatment (convalescent). Immunoreactivity was assessed by densitometry with MacBas 2.5 software. The cut-off for positive WB was defined as the mean value plus 3 SD of 5 healthy blood donors. In IgM and IgG anti-flagellin ELISA (Dako, Denmark), the cut-off value was based on the mean OD plus 3 SD of healthy controls. Ba = *B. afzelii*; Bg = *B. garinii*; ND = not performed. +,- = positive or negative WB or ELISA, respectively, by the method indicated.

Figure 3. IgG ELISA OD values with recombinant BBK32 as an antigen from *B. afzelii* (panel A), *B. garinii* (panel B), and *B. burgdorferi* sensu stricto (Bbia, panel C) with serum samples from patients with neuroborreliosis (NB) or Lyme arthritis (LA). Control samples were from patients with syphilis (SY), systemic lupus erythematosus (SLE), Epstein-Barr virus (EBV) infection, positive rheumatoid factor (RF+), positive for anti-streptolysin (ASO), and samples from healthy blood donors (BD). The cut-off level (mean + 3 SD of BD samples) is indicated with a line.

Figure 4. IgG ELISA OD values with recombinant BBK32 as an antigen from *B. afzelii* (panel A), *B. garinii* (panel B), and *B. burgdorferi* sensu stricto (Bbia, panel C) with serum samples from erythema migrans patients at the acute (EM I) and convalescent (EM II) phases. Control samples were from patients with syphilis (SY), Epstein Barr virus (EBV) infection, positive rheumatoid factor (RF+), and samples from healthy blood donors (BD). The cut-off level (mean + 3 SD of BD samples) is indicated with a line.

Figure 5. ELISA OD/cut-off values of patients with erythema migrans from Finland (FIN), Germany (GER), USA, and Slovenia (SLO). Serum samples were drawn at diagnosis (a) and after antibiotic treatment in convalescence (c). IgG antibodies to rBBK32 from *B. afzelii* (BBK32-afz), *B. garinii* (BBK32-gar), *B. burgdorferi* sensu

stricto (BBK32-sensu stricto), and to IR_6 peptide were assessed. Control samples (CO) were from 40 healthy blood donors. The level of positivity for OD/cut-off values (>1) is indicated with a horizontal line.

Figure 6. IgG antibodies (ELISA) to the recombinant BBK32 in the CSF of patients with confirmed, probable or possible neuroborreliosis, and of controls, including patients with syphilis, other neurological diseases, and with no proven infection. The level of positivity for OD/cutoff values (>1) is indicated by horizontal lines. The highest OD value of individual CSF sample with the BBK32 variants in ELISAs was used in the analyses.

Figure 7. IgG antibodies to the recombinant BBK32 in the CSF of patients with duration of neurologic symptoms < 3 months (acute) and > 3 months (chronic). The level of positivity for the OD/cutoff values (>1) is indicated by a horizontal line. The highest OD value of individual CSF sample with the BBK32 variants in ELISAs was used in the analyses.

References

1. Steere AC. Lyme disease. N Engl J Med **1989;** 321:586-596.

2. Johnsson BJB, Robbins, Bailey 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.

3. Robertson J, Guy E, Andrews N, et al. A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. J Clin Microbiol **2000;** 38:2097-2102.

4. Baranton G, Postic D, Saint Girons I, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. Nov., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol **1992;** 42:378-383.

5. Roessler D, Hauser U, Wilske B. Heterogeneity of BmpA (P39) among European isolates of *Borrelia burgdorferi* sensu lato and influence of interspecies variability on serodiagnosis. J. Clin. Microbiol. **1997;** 35:2752-2758.

6. Mitchell PD, Reed KD, Aspeslet TL, Vandermause MF, Melski JW. Comparison of four immunoserologic assays for detection of antibodies to *Borrelia burgdorferi* in patients with culture-positive erythema migrans. J Clin Microbiol **1994;** 32:1958-1962.

7. Oksi J, Uksila J, Marjamäki M, et al. Antibodies against whole sonicated *Borrelia burgdorferi* spirochetes, 41-kilodalton flagellin, and P39 protein in patients with PCR- or culture-proven late Lyme borreliosis. J Clin Microbiol **1995;** 33:2260-2264.

8. Brown SL, Hansen SL, Langone JJ. Role of serology in the diagnosis of Lyme disease. JAMA **1999;** 282:62-66.

9. Fikrig E, Barthold SW, Sun W, et al. *Borrelia burgdorferi* P35 and P37 proteins, expressed in vivo, elicit protective immunity. Immunity **1997;** 6:531-539.

10. Zhang JR, Hardman JM, Barbour AG, Norris SJ. Antigenic variation in Lyme disease borreliae by promiscuous recombinant VMP-like sequence cassettes. Cell **1997;** 89:275-285.

11. Hefty PS, Jolliff SE, Caimano M, et al. Regulation of OspE-related, OspF-related, and Elp lipoproteins of *Borrelia burgdorferi* strain 297 by mammalian host-specific signals. Infect Immun **2001;** 69:3618-3627.

12. Fikrig E, Feng W, Barthold SW, Telford III SR, Flavell RA. Arthropod- and host-specific *Borrelia burgdorferi bbk32* expression and the inhibition of spirochete transmission. J Immunol **2000;** 164:5344-5351.

13. Akin E, McHugh GL, Flavell RA, Fikrig E, Steere AC. The immunoglobulin (IgG) antibody response to OspA and OspB correlates with severe and prolonged Lyme arthritis and the IgG response to P35 correlates with mild and brief arthritis. Infect Immun **1999;** 67:173-181.

14. Magnarelli LA, Ijdo JW, Padula SJ, et al. Serologic diagnosis of Lyme borreliosis by using enzyme-linked immunosorbent assays with recombinant antigens. J Clin Microbiol **2000;** 38:1735-1739.

15. Rauer S, Spohn N, Rasiah C, et al. Enzyme-linked immunosorbent assay using recombinant OspC and the internal 14-kDa flagellin fragment for serodiagnosis of early Lyme disease. J Clin Microbiol **1998;** 36:857-861.

16. 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.

17. Mathiesen MJ, Christiansen M, Hansen K, et al. Peptide-based OspC enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. J Clin Microbiol **1998;** 36:3474-3479.

18. Liang FT, Steere AC, Marques AR, et al. Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* VlsE. J Clin Microbiol **1999;** 37:3990-3996.

19. Gomes-Solecki MJ, Wormser GP, Persing DH, et al. A first-tier rapid assay for the serodiagnosis of *Borrelia burgdorferi* infection. Arch Intern Med **2001;** 161:2015-2020.

20. Fikrig E, Feng W, Aversa J, Schoen RT, Flavell RA. Differential expression of *Borrelia burgdorferi* genes during erythema migrans and Lyme arthritis. J Inf Dis **1998;** 178:1198-1201.

21. Probert WS, Johnson BJ. Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* isolate B31. Mol Microbiol **1998;** 30:1003-1015.

22. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol **1995;** 33:419-427.

23. Aguero-Rosenfeld ME, Nowakowski J, McKenna DF, Carbonaro CA, Wormser GP. Serodiagnosis in early Lyme disease. J Clin Microbiol **1993;** 31:3090-3095.

24. Grodzicki RL, Steere AC. Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. J Inf Dis **1988;** 157:790-797.

25. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. J Clin Microbiol **1996;** 34:1-9.

26. Arnez M, Ruzic-Sabljic E, Ahcan J, Radsel-Medvescek A, Pleterski-Rigler D, Strle F. Isolation of *Borrelia burgdorferi* sensu lato from blood of children with solitary erythema migrans. Pediatr Infect Dis J **2001;** 20:251-255.

27. Ornstein K, Berglund J, Nilsson I, Norrby R, Bergström S. Characterization of Lyme borreliosis isolates from patients with erythema migrans and neuroborreliosis in southern Sweden. J Clin Microbiol **2001;** 39:1294-1298.

28. Wang G, van Dam AP, Schwartz I, Dankert J. Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev **1999;** 12:633-653.

29. Junttila J, Peltomaa M, Soini H, Marjamäki M, Viljanen MK. Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks in urban recreational areas of Helsinki. J Clin Microbiol **1999;** 37:1361-1365.

30. Hubalek Z, Halouzka J. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. Eur J Epidemiol **1997;** 13:951-957.

31. Seppälä IJT, Kroneld R, Schauman K, Forsen KO, Lassenius R. Diagnosis of Lyme borreliosis: non-specific serological reactions with *Borrelia burgdorferi* sonicate antigen caused by IgG2 antibodies. J Med Microbiol **1994;** 40:293-302.

32. Wharton M, Chorba TL, Vogt RL, Morse DL, Buehler JW. Case definitions for public health surveillance. Morbid Mortal Weekly Report **1990;** 39:19-21.

33. Oksi J, Marttila H, Soini H, Aho H, Uksila J, Viljanen MK. Early dissemination of *Borrelia burgdorferi* without generalized symptoms in patients with erythema migrans. APMIS 2001; **109**: 581-588.

34. Smith RP, Schoen RT, Rahn DW, et al. Clinical characteristics and treatment outcome of early Lyme disease in patients with microbiologically confirmed erythema migrans. Ann Intern Med 2002; **136**: 421-428.

35. Huppertz HI, Böhme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme borreliosis in the Wurzburg region of Germany. Eur J Clin Microbiol Infect Dis 1999; **18**: 697-703.

36. Vaz A, Glickstein L, Field JA, et al. Cellular and humoral immune responses to *Borrelia burgdorferi* antigens in patients with culture-positive early Lyme disease. Infect Immun 2001; **69**: 7437-7444.

37. Strle F, Nelson JA, Ruzic-Sabljic E, et al. European Lyme borreliosis; 231 culture-confirmed cases involving patients with erythema migrans. Clin Inf Dis 1996; **23**: 61-65.

Claims

1. A method for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, characterized in that recombinant BBK32 proteins or fragments thereof from one or more *Borrelia* species are used as antigens in an immunoassay.

2. The method according to claim 1 for the serodiagnosis of early and late Lyme borreliosis.

3. The method according to claim 2 for the serodiagnosis of erythema migrans.

4. The method according to any one of claims 1 to 3, characterized in that recombinant BBK32 proteins or their fragments and any other immunogenic protein(s) or their fragments from one or more *Borrelia* species are used as antigens in an immunoassay.

5. The method according to claim 4, characterized in that recombinant BBK32 proteins or their fragments and recombinant decorin binding protein As (DbpAs) or their fragments from one or more *Borrelia* species are used as antigens in an immunoassay.

6. The method according to any one of claims 1 to 5, characterized in that the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are derived from at least two *Borrelia* species selected from the group consisting of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*.

7. The method according to claim 6, wherein the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are derived from *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*.

8. The method according to claim 7 wherein the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are used as antigens either in parallel or combined in an immunoassay.

9. The method according to claim 8 wherein borrelial peptides are used instead of or together with recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments.

10. The method according to any one of the preceding claims, comprising the steps of

a) contacting the body fluid with recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments derived from one or more *Borrelia* species under conditions effective to allow the formation of antigen-antibody complexes; and

b) detecting the complexes formed.

11. The method according to claim 10, characterized in that the body fluid is a serum, plasma, whole blood, cerebrospinal fluid, or synovial fluid sample.

12. A diagnostic kit useful for detecting *Borrelia burgdorferi* sensu lato infection or the presence of antibodies against *Borrelia* species in a body fluid from a suspected infected or vaccinated human or other mammal, said kit comprising in a suitable container

a) recombinant BBK32 proteins or their fragments and optionally other immunogenic proteins or their fragments from one or more *Borrelia* species and a detectable label or marker linked to said proteins and/or fragments, or

b) recombinant BBK32 proteins or their fragments and optionally other immunogenic proteins or their fragments from one or more *Borrelia* species and a second antibody linked to any detectable label or marker.

13. The diagnostic kit according to claim 12 for the serodiagnosis of early and late Lyme borreliosis, especially for the serodiagnosis of erythema migrans.

14. The diagnostic kit according to claim 12 or 13, wherein the kit comprises recombinant decorin binding protein As (DbpAs).

15. The diagnostic kit according to any one of claims 12 to 14 wherein the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are derived from at least two *Borrelia* species selected from the group consisting of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*.

16. An immunoassay method for diagnosing early and late Lyme borreliosis comprising the steps of

a) contacting a body fluid from a human or other mammal with recombinant BBK32 proteins or their fragments and optionally with other immunogenic proteins or their fragments derived from one or more *Borrelia* species under conditions effective to allow the formation of antigen-antibody complexes; and

b) detecting the complexes formed.

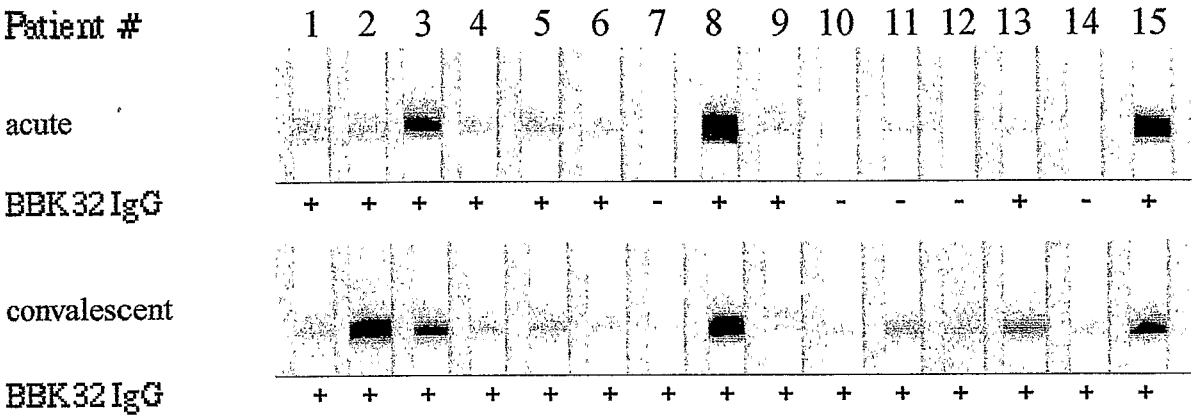
17. The immunoassay method according to claim 16 for the serodiagnosis of erythema migrans.

18. The immunoassay method according to claim 16 or 17, wherein recombinant BBK32 proteins and recombinant decorin binding protein As (DbpAs) are used as antigens.

19. The immunoassay method according to any one of claims 16 to 18, wherein the recombinant BBK32 proteins, optional other immunogenic proteins, or their fragments are derived from at least two Borrelia species selected from the group consisting of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*.

Figure 1.

	Bbia	Bg40	Bg46	Bg50	BaA91	Ba1082	Ba570	Ba600
Bbia	-	95.3	92.5	95.3	73.1	73.1	72.8	73.1
Bg40		-	93.9	100	71.7	71.7	71.4	71.7
Bg46			-	93.9	72.2	72.2	72.5	72.8
Bg50				-	71.7	71.7	71.4	71.7
BaA91					-	100	99.2	99.4
Ba1082						-	99.2	99.4
Ba570							-	99.7
Ba600								-



Patient #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
acute															
BBK32 IgG	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+
convalescent															
BBK32 IgG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PCR	Ba	Ba	Bg	Ba	Ba	Ba	Bg	Bg	ND	Bg	Ba	Ba	Ba	Ba	Ba
Fla IgG acute	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+
Fla IgG convalescent	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+
Fla IgM acute	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+
Fla IgM convalescent	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+

Fig. 2

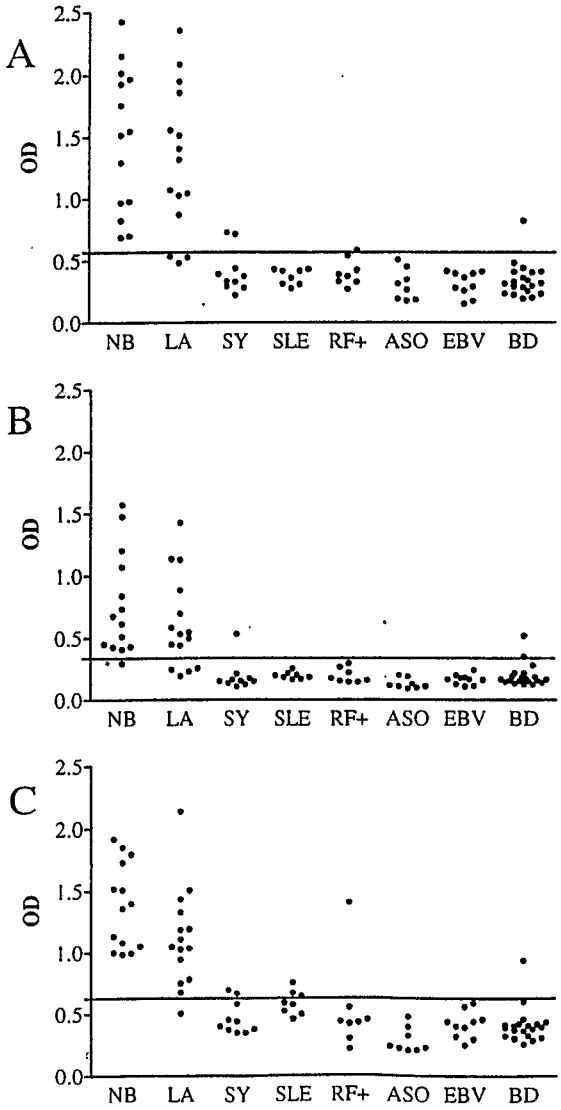


Fig. 3

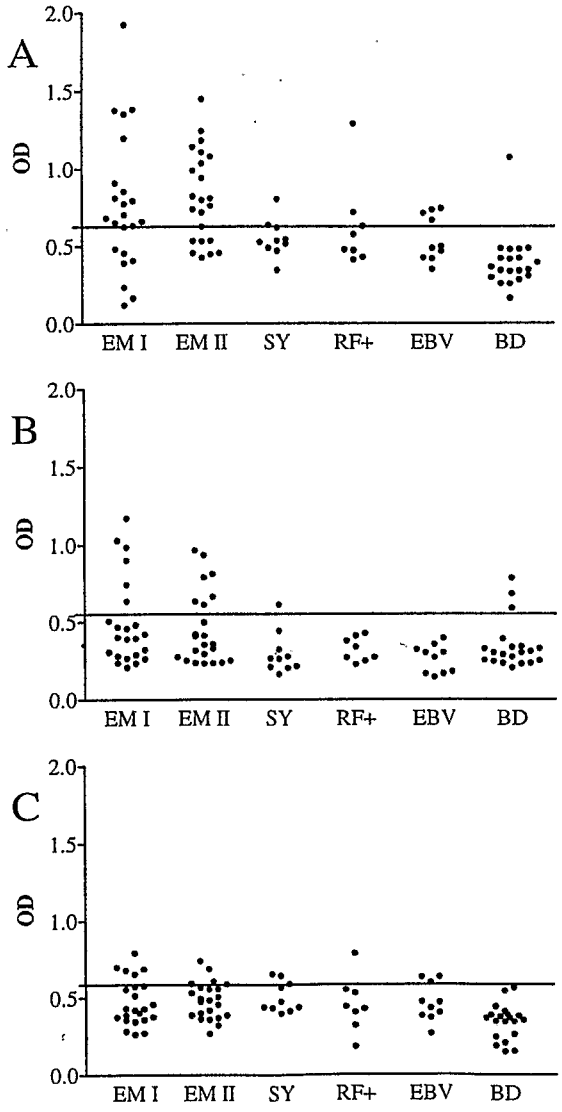


Fig. 4

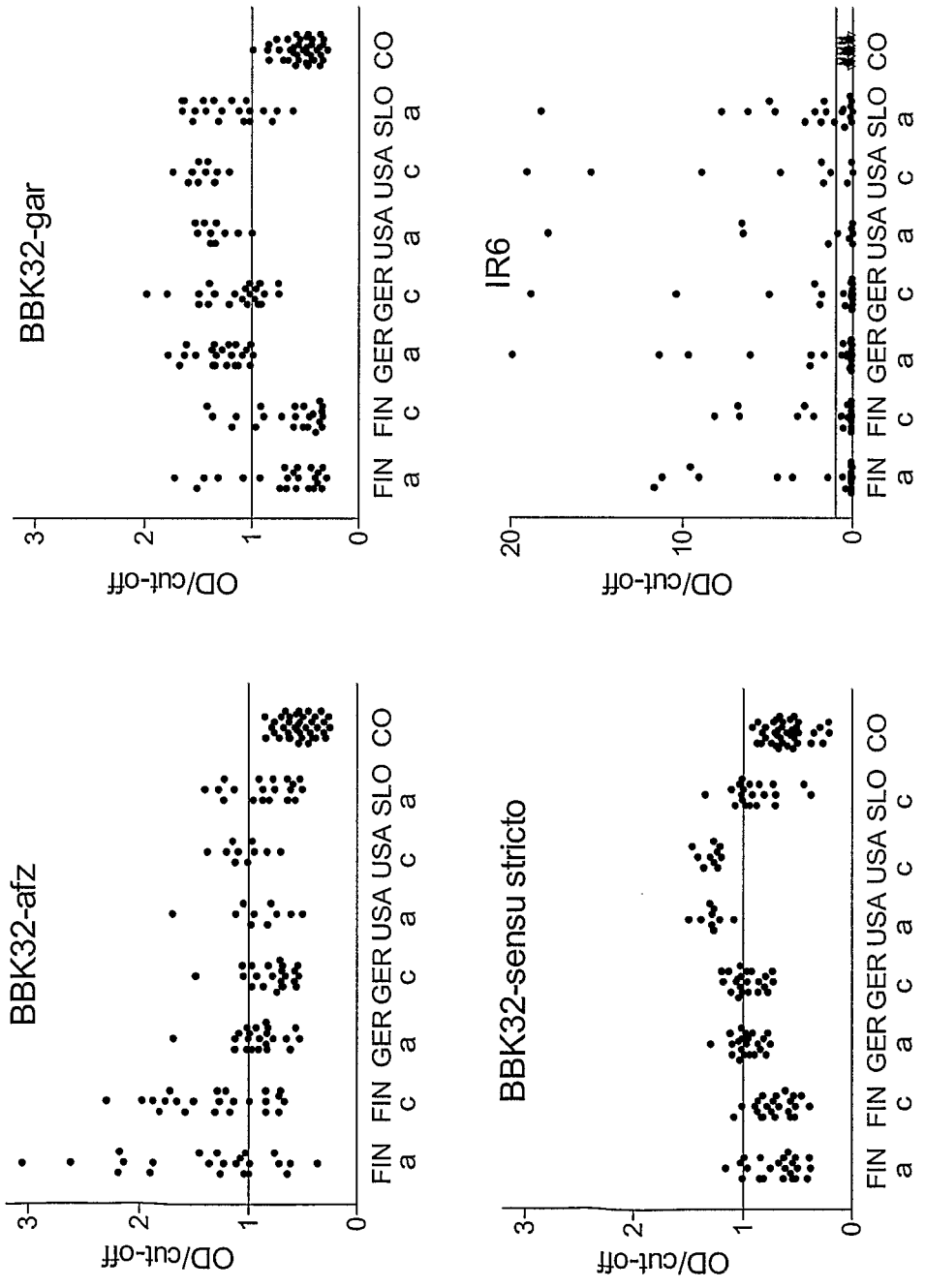

BBK32-gar
BBK32-afz
IR6
BBK32-sensu stricto
OD/cut-off
0
1
2
3
10
20
FIN FIN GER GER USA USA SLO CO
a c a c a c a c

Fig. 5

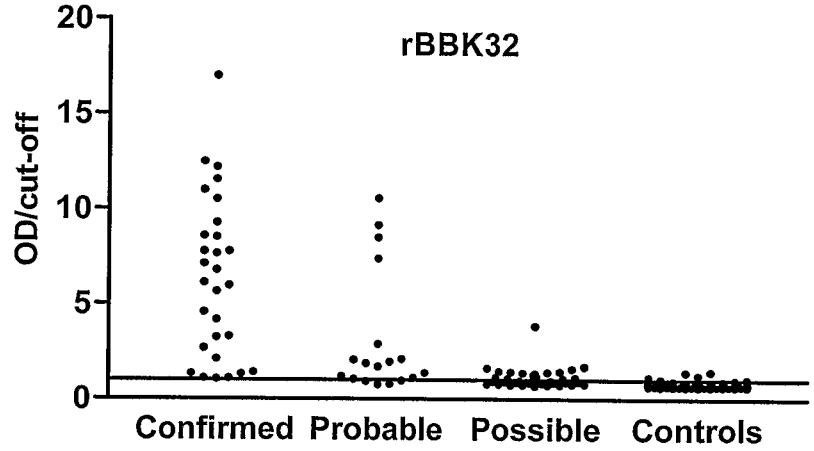


FIGURE 6.

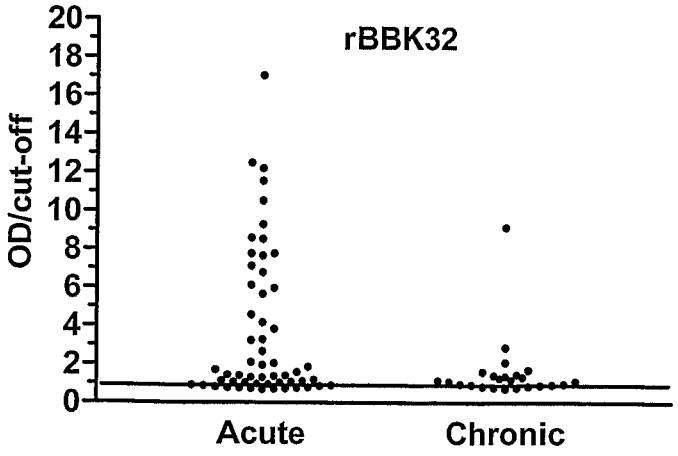


FIGURE 7.

SEQUENCE LISTING

<110> Bortech Oy

<120> Method for diagnosing early and late Lyme borreliosis

<130> 38916

<140>
<141>

<160> 16

<170> PatentIn Ver. 2.1

<210> 1
<211> 1059
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1059)

<400> 1

```
atg aaa att aaa agt aaa tgc tta gct ttg gga ttg ctt ttt ggt ttt   48
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
  1               5                  10                  15

atc agt tgt gat tta ttc ata aga gat gaa ata aaa gag aaa tct ctt   96
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
             20                  25                  30

ggc ttg tgt gat gag gaa agt tct att tta gag act ggt gac aaa tct   144
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
         35                  40                  45

gtt aaa aag tct ctt aat aag aaa ggc aaa gat aag gtt gct aga aag   192
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
     50                  55                  60

aaa gtt gaa ggt aat gct gtt aaa aaa gac ccg ttt aat cat cat gta   240
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
 65                  70                  75                  80

aag agg gag tct gtt aat aat agt aat cta tca caa aaa aat gtg ata   288
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                 85                  90                  95

tcg gaa gaa gaa att ttg aaa act aaa tta tta aga gaa cga cct gag   336
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110

act aga aaa gaa gaa ata caa aaa cag caa gat gag cat aaa agg atg   384
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
        115                 120                 125

ctt caa gga agt tta agt ttt ctt agt ggt gaa agt ggt gaa ttg aag   432
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140
```

```
gat act att gaa agc aat gaa att gat ttt act ata gat tct gat tta   480
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160

aga ccg aag agt gat tta caa gct att tca ggc tca aat tct att tca   528
Arg Pro Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
                165                 170                 175

cat act gat gaa ata gaa gaa gaa gat tat gat cag tat tct tta gaa   576
His Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190

gaa gat tat tat tat gat gag gaa aca aga tta agt aat aga tat gaa   624
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205

tct tat cta gag ggt gtt aaa tat aat gta agt tca gca att aaa aca   672
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220

att gtt aag ata tat gat aat tat acc tta ctt tca aca aag caa acc   720
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225                 230                 235                 240

caa atg tat tct aca cgt ctt gac aac ctt gct aaa gcc aaa gct aga   768
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255

gaa gaa gct aaa aag ttt aca aaa gaa gaa ctt gaa aaa gat ctt aag   816
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270

acc tta ttg aac tat att caa gtg agt gca agg act gcg aca aat ttt   864
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285

gta tat gca aga gaa ata tat tca aaa aga aaa tta gat gcc att gaa   912
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300

aca gaa ata aaa aat tta att tta aag atc aaa gga caa tct gat tta   960
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320

tac gag gca tat aaa gca ata gta agg tca atc tta tta atg aaa gat  1008
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335

tct ctt aaa ata atc gaa ata gtc att gat aag aat ggt gtt tgg tac  1056
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350

taa                                                             1059
```

<210> 2
<211> 352
<212> PRT
<213> Borrelia burgdorferi

<400> 2
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
1 5 10 15
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
20 25 30
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
35 40 45
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
50 55 60
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
65 70 75 80
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
85 90 95
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
100 105 110
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
115 120 125
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
130 135 140
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145 150 155 160
Arg Pro Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
165 170 175
His Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
180 185 190
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
195 200 205
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
210 215 220

Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225 230 235 240
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
245 250 255
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
260 265 270
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
275 280 285
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
290 295 300
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305 310 315 320
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
325 330 335
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
340 345 350

<210> 3
<211> 1059
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1059)

<400> 3
atg aaa att aaa agt aaa tgc tta gct ttg gga ttg ctt ttt ggt ttt 48
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe

```
  1               5                  10                  15

atc agt tgt gat tta ttc ata aga gat gaa ata aaa gag aaa tct ctt   96
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
            20                  25                  30

ggc ttg tgt gat gag gaa agt tct att tta gag act ggt gac aaa tct   144
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
        35                  40                  45

gtt aaa aag tct ctt aat aag aaa ggc aaa gat aag gtt gct aga aag   192
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
    50                  55                  60

aaa gtt gaa ggt aat gct gtt aaa aaa gac ccg ttt aat cat cat gta   240
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
65                  70                  75                  80

aag agg gag tct gtt aat aat agt aat cta tca caa aaa aat gtg ata   288
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                85                  90                  95

tcg gaa gaa gaa att ttg aaa act aaa tta tta aga gaa cga cct gag   336
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110

act aga aaa gaa gaa ata caa aaa cag caa gat gag cat aaa agg atg   384
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
        115                 120                 125

ctt caa gga agt tta agt ttt ctt agt ggt gaa agt ggt gaa ttg aag   432
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140

gat act att gaa agc aat gaa att gat ttt act ata gat tct gat tta   480
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160

aga ctg aag agt gat tta caa gct att tca ggc tca aat tct att tca   528
Arg Leu Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
                165                 170                 175

tat act gat gaa ata gaa gaa gaa gat tat gat cag tat tct tta gaa   576
Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190

gaa gat tat tat tat gat ggg gaa aca aga tta agt aat aga tat gag   624
Glu Asp Tyr Tyr Tyr Asp Gly Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205

tct tat cta gag ggt gtt aaa tat aat gta agt tca gca att aaa aca   672
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220

att gtt aag ata tat gat aat tat acc tta ctt tca aca aag caa acc   720
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225                 230                 235                 240

caa atg tat tct aca cgt ctt gac aac ctt gct aaa gcc aaa gct aga   768
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255
```

```
gaa gaa gct aaa aag ttt aca aaa gaa gaa ctt gaa aaa gat ctt aag   816
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270

acc tta ttg aac tat att caa gtg agt gca agg act gcg aca aat ttt   864
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285

gta tat gca aga gaa ata tat tca aaa aga aaa tta gat gcc att gaa   912
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300

aca gaa ata aaa aat tta att tta aag atc aaa gga caa tct gat tta   960
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320

tac gag gca tat aaa gca ata gta agg tca atc tta tta atg aaa gat   1008
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335

tct ctt aaa ata atc gaa ata gtc att gat aag aat ggt gtt tgg tac   1056
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350

taa                                                              1059
```

<210> 4
<211> 352
<212> PRT
<213> Borrelia burgdorferi

<400> 4

```
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
  1               5                  10                  15
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
             20                  25                  30
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
         35                  40                  45
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
     50                  55                  60
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
 65                  70                  75                  80
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                 85                  90                  95
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
        115                 120                 125
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160
Arg Leu Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
                165                 170                 175
Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190
Glu Asp Tyr Tyr Tyr Asp Gly Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205
```

```
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225                 230                 235                 240
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350
```

<210> 5
<211> 1059
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1059)

<400> 5

```
atg aaa att aaa agt aaa tgc tta gct ttg gga ttg ctt ttt ggt ttt   48
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
  1               5                  10                  15

atc agt tgt gat tta ttc ata aga gat gaa ata aaa gag aaa tct ctt   96
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
             20                  25                  30

ggc ttg tgt gat gag gaa agt tct att tta gag act ggt gac aaa tct   144
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
         35                  40                  45

gtt aaa aag tct ctt aat aag aaa ggc aaa gat aag gtt gct aga aag   192
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
     50                  55                  60

aaa gtt gaa ggt aat gct gtt aaa aaa gac ccg ttt aat cat cat gta   240
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
 65                  70                  75                  80

aag agg gag tct gtt aat aat agt aat cta tca caa aaa aat gtg ata   288
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                 85                  90                  95

tcg gaa gaa gaa att ttg aaa act aaa tta tta aga gaa cga cct gag   336
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110

act aga aaa gaa gaa ata caa aaa cag caa gat gag cat aaa agg atg   384
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
```

```
        115                 120                 125

ctt caa gga agt tta agt ttt ctt agt ggt gaa agt ggt gaa ttg aag   432
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140

gat act att gaa agc aat gaa att gat ttt act ata gat tct gat tta   480
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160

aga ccg aag agt gat tta caa gct att tca ggc tca aat tct att tca   528
Arg Pro Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
                165                 170                 175

cat act gat gaa ata gaa gaa gaa gat tat gat cag tat tct tta gaa   576
His Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190

gaa gat tat tat tat gat gag gaa aca aga tta agt aat aga tat gaa   624
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205

tct tat cta gag ggt gtt aaa tat aat gta agt tca gca att aaa aca   672
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220

att gtt aag ata tat gat aat tat acc tta ctt tca aca aag caa acc   720
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225                 230                 235                 240

caa atg tat tct aca cgt ctt gac aac ctt gct aaa gcc aaa gct aga   768
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255

gaa gaa gct aaa aag ttt aca aaa gaa gaa ctt gaa aaa gat ctt aag   816
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270

acc tta ttg aac tat att caa gtg agt gca agg act gcg aca aat ttt   864
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285

gta tat gca aga gaa ata tat tca aaa aga aaa tta gat gcc att gaa   912
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300

aca gaa ata aaa aat tta att tta aag atc aaa gga caa tct gat tta   960
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320

tac gag gca tat aaa gca ata gta agg tca atc tta tta atg aaa gat  1008
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335

tct ctt aaa ata atc gaa ata gtc att gat aag aat ggt gtt tgg tac  1056
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350

taa                                                             1059
```

<210> 6
<211> 352
<212> PRT
<213> Borrelia burgdorferi

<400> 6
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
1 5 10 15
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
20 25 30
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
35 40 45
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
50 55 60
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
65 70 75 80
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
85 90 95
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
100 105 110
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
115 120 125
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
130 135 140
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145 150 155 160
Arg Pro Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
165 170 175
His Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
180 185 190
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
195 200 205
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
210 215 220
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225 230 235 240
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
245 250 255
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
260 265 270
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
275 280 285
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
290 295 300
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305 310 315 320
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
325 330 335
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
340 345 350

<210> 7
<211> 1059
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS

<222> (1)..(1059)

<400> 7

```
atg aaa att aaa agt aaa tgc tta gct ttg gga ttg ctt ttt ggt ttt   48
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
  1               5                  10                  15

atc agt tgt gat tta ttc ata aga gat gaa ata aaa gag aaa tct ctt   96
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
             20                  25                  30

ggc ttg tgt gat gag gaa agt tct att tta gag act ggt gac aaa tct  144
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
         35                  40                  45

gtt aaa aag tct ctt aat aag aaa ggc aaa gat aag gtt gct aga aag  192
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
     50                  55                  60

aaa gtt gaa ggt aat gct gtt aaa aaa gac ccg ttt aat cat cat gta  240
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
 65                  70                  75                  80

aag agg gag tct gtt aat aat agt aat cta tca caa aaa aat gtg ata  288
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                 85                  90                  95

tcg gaa gaa gaa att ttg aaa act aaa tta tta aga gaa cga cct gag  336
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110

act aga aaa gaa gaa ata caa aaa cag caa gat gag cat aaa agg atg  384
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
        115                 120                 125

ctt caa gga agt tta agt ttt ctt agt ggt gaa agt ggt gaa ttg aag  432
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140

gat act ata gaa agc aat gaa att gat ttt act ata gat tct gat tta  480
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160

aga ctg aag agt gat tta caa gct att tca ggc tca aat tct att tca  528
Arg Leu Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
                165                 170                 175

tat act gat gaa ata gaa gaa gaa gat tat gat cag tat tct tta gaa  576
Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190

gaa gat tat tat tat gat gag gaa aca aga tta agt aat aga tat gaa  624
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205

tct tat cta gag ggt gtt aaa tat aat gta agt tca gca att aaa aca  672
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220

att gtt aag ata tat gat aat tat acc tta ctt tca aca aag caa acc  720
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
```

```
225                 230                 235                 240

caa atg tat tct aca cgt ctt gac aac ctt gct aaa gcc aaa gct aga   768
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255

gaa gaa gct aaa aag ttt aca aaa gaa gaa ctt gaa aaa gat ctt aag   816
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270

acc tta ttg aac tat att caa gtg agt gca agg act gcg aca aat ttt   864
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285

gta tat gca aga gaa ata tat tca aaa aga aaa tta gat gcc att gaa   912
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300

aca gaa ata aaa aat tta att tta aag atc aaa gga caa tct gat tta   960
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320

tac gag gca tat aaa gca ata gta agg tca atc tta tta atg aaa gat   1008
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335

tct ctt aaa ata atc gaa ata gtc att gat aag aat ggt gtt tgg tac   1056
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350

taa                                                              1059
```

<210> 8
<211> 352
<212> PRT
<213> Borrelia burgdorferi

<400> 8

```
Met Lys Ile Lys Ser Lys Cys Leu Ala Leu Gly Leu Leu Phe Gly Phe
  1               5                  10                  15
Ile Ser Cys Asp Leu Phe Ile Arg Asp Glu Ile Lys Glu Lys Ser Leu
             20                  25                  30
Gly Leu Cys Asp Glu Glu Ser Ser Ile Leu Glu Thr Gly Asp Lys Ser
         35                  40                  45
Val Lys Lys Ser Leu Asn Lys Lys Gly Lys Asp Lys Val Ala Arg Lys
     50                  55                  60
Lys Val Glu Gly Asn Ala Val Lys Lys Asp Pro Phe Asn His His Val
 65                  70                  75                  80
Lys Arg Glu Ser Val Asn Asn Ser Asn Leu Ser Gln Lys Asn Val Ile
                 85                  90                  95
Ser Glu Glu Glu Ile Leu Lys Thr Lys Leu Leu Arg Glu Arg Pro Glu
            100                 105                 110
Thr Arg Lys Glu Glu Ile Gln Lys Gln Gln Asp Glu His Lys Arg Met
        115                 120                 125
Leu Gln Gly Ser Leu Ser Phe Leu Ser Gly Glu Ser Gly Glu Leu Lys
    130                 135                 140
Asp Thr Ile Glu Ser Asn Glu Ile Asp Phe Thr Ile Asp Ser Asp Leu
145                 150                 155                 160
Arg Leu Lys Ser Asp Leu Gln Ala Ile Ser Gly Ser Asn Ser Ile Ser
```

```
                165                 170                 175
Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Gln Tyr Ser Leu Glu
            180                 185                 190
Glu Asp Tyr Tyr Tyr Asp Glu Glu Thr Arg Leu Ser Asn Arg Tyr Glu
        195                 200                 205
Ser Tyr Leu Glu Gly Val Lys Tyr Asn Val Ser Ser Ala Ile Lys Thr
    210                 215                 220
Ile Val Lys Ile Tyr Asp Asn Tyr Thr Leu Leu Ser Thr Lys Gln Thr
225                 230                 235                 240
Gln Met Tyr Ser Thr Arg Leu Asp Asn Leu Ala Lys Ala Lys Ala Arg
                245                 250                 255
Glu Glu Ala Lys Lys Phe Thr Lys Glu Glu Leu Glu Lys Asp Leu Lys
            260                 265                 270
Thr Leu Leu Asn Tyr Ile Gln Val Ser Ala Arg Thr Ala Thr Asn Phe
        275                 280                 285
Val Tyr Ala Arg Glu Ile Tyr Ser Lys Arg Lys Leu Asp Ala Ile Glu
    290                 295                 300
Thr Glu Ile Lys Asn Leu Ile Leu Lys Ile Lys Gly Gln Ser Asp Leu
305                 310                 315                 320
Tyr Glu Ala Tyr Lys Ala Ile Val Arg Ser Ile Leu Leu Met Lys Asp
                325                 330                 335
Ser Leu Lys Ile Ile Glu Ile Val Ile Asp Lys Asn Gly Val Trp Tyr
            340                 345                 350
```

<210> 9
<211> 1083
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1083)

<400> 9

```
atg aag aaa gtt aaa agt aaa tat tta gct ttg gga tta ctt ttt ggt   48
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15

ttt ata agt tgt gat tta ttc ata aga tat gaa atg aaa gag gaa tcc   96
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30

cct ggc tta ttt gat aag gga aac tct att tta gag act agc gag gaa   144
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45

tct att aaa aag cct atg aat aag aaa ggc aaa ggt aag att gcc aga   192
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg
     50                  55                  60

aag aac gga aaa agc aag gtt tct gga aaa gaa ccg ttt att cat agt   240
Lys Asn Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Phe Ile His Ser
 65                  70                  75                  80

ttt aaa aga gac gct gct aat aaa agc aat ttt tta caa aaa aat gta   288
Phe Lys Arg Asp Ala Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95

atg tta gag gaa gaa agt tta aaa act gaa tta tta aaa gag caa tct   336
```

```
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110

gag act aga aaa gaa aaa ata caa aaa caa caa gat gaa tat aaa ggg   384
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125

atg act aaa gga agt tta aat tcc ctt agc ggt gaa agt ggt gaa ttg   432
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140

aag gag act att gaa agc aat gaa att gat att act ata gat tct gat   480
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160

tta agg cca aag agt tcc tta caa gac att gca gga tca aac tct att   528
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175

tca tac act gat gaa ata gag gaa gag gat tat gct cgg tat tat tta   576
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190

gat gaa gat gat gaa gat gat gaa tat tat gaa gat gat tat gag gaa   624
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205

ata aga tta agc aat cga tat caa tct tat cta gaa ggt gtt aaa tat   672
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220

aat gta gat tca gca att aac aca att aat aag ata tat gat act tat   720
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240

aca tta ttc tca aca aag cta acc caa atg tat tct aca cgc ctt gac   768
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255

aac ctt gct aaa gcc aaa gct aaa gaa gaa gct gca aag ttt aca aaa   816
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270

gaa gac ctt gaa aaa aat ttc aag acc tta ttg aat tac att caa gta   864
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285

agt gta aag act gca aca aat ttt gta tac ata aat gaa atg cat gca   912
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Glu Met His Ala
    290                 295                 300

aaa agg aaa tta gag aac att gaa gca aaa ata aaa act tta att gca   960
Lys Arg Lys Leu Glu Asn Ile Glu Ala Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320

aag atc aaa gaa aaa tct aat tta tac tca gca tat aaa gca ata gta  1008
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335

agt tca atc tta tta atg agg gat tct ctt aaa gaa gtg caa tat gcc  1056
```

```
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Tyr Ala

            340                 345                 350

att gac aag aat ggc att tgg tac taa                                1083
Ile Asp Lys Asn Gly Ile Trp Tyr
        355                 360
```

<210> 10
<211> 360
<212> PRT
<213> Borrelia burgdorferi

<400> 10

```
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg

     50                  55                  60
Lys Asn Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Phe Ile His Ser
 65                  70                  75                  80
Phe Lys Arg Asp Ala Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Glu Met His Ala
    290                 295                 300
Lys Arg Lys Leu Glu Asn Ile Glu Ala Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Tyr Ala
            340                 345                 350
Ile Asp Lys Asn Gly Ile Trp Tyr
```

355 360

<210> 11
<211> 1083
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1083)

<400> 11

```
atg aag aaa gtt aaa agt aaa tat tta gct ttg gga tta ctt ttt ggt   48
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15

ttt ata agt tgt gat tta ttc ata aga tat gaa atg aaa gag gaa tcc   96
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30

cct ggc tta ttt gat aag gaa aac tct att tta gag act ggc gag gaa   144
Pro Gly Leu Phe Asp Lys Glu Asn Ser Ile Leu Glu Thr Gly Glu Glu
         35                  40                  45

tct att aaa aag cct atg aat aag aaa gat aaa ggt aag att gcc aga   192
Ser Ile Lys Lys Pro Met Asn Lys Lys Asp Lys Gly Lys Ile Ala Arg
     50                  55                  60

aag aaa ggc aaa agc aag gtt tct gga aaa gaa cca tct att cat agt   240
Lys Lys Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Ser Ile His Ser
 65                  70                  75                  80

tta aaa agg gac tct gct aat aaa agc aat ttt tta caa aaa aat gta   288
Leu Lys Arg Asp Ser Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95

atg tta gag gaa gaa agt tta aaa act gaa tta tta aaa gag caa tct   336
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110

gag act aga aaa gaa aaa ata caa aaa caa caa gat gaa tat aaa ggg   384
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125

atg act aaa gga agt tta aat tcc ctt agc ggt gaa agt ggt gaa ttg   432
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140

aag gag act att gaa agt aat gaa att gat att act ata gat tct gat   480
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160

tta agg cta aaa agt gcc tta caa ggc att gca gga tca aac tct att   528
Leu Arg Leu Lys Ser Ala Leu Gln Gly Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175

tca tac act gat gaa ata gag gaa gag gat tat gat cgg tat tat tta   576
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Arg Tyr Tyr Leu
            180                 185                 190
```

```
gaa gaa tat gat gaa gat gtt gaa tat tat gaa gat gat tat gag gaa   624
Glu Glu Tyr Asp Glu Asp Val Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205

ata aga tta agc aat cga tat caa tct tat cta gaa ggt gtt aaa tat   672
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220

aat gta gat tca gca att aaa aca att aat aag ata tat gat act tat   720
Asn Val Asp Ser Ala Ile Lys Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240

aca tta ttt tca aca aag cta acc caa atg tat tct aca cgc ctt gac   768
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255

aac ctt gct aaa gcc aaa gct aaa gaa gaa tct gca aag ttt aca aaa   816
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ser Ala Lys Phe Thr Lys
            260                 265                 270

gaa gac ctt gaa aaa aat ttc aag acc tta ttg aat tac att caa gta   864
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285

agt gta aag act gca aca aat ttt gta tac ata aat gac acg acc gca   912
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Asp Thr Thr Ala
    290                 295                 300

aaa agg aaa tta gag aac att gaa aca aaa ata aaa act tta att gca   960
Lys Arg Lys Leu Glu Asn Ile Glu Thr Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320

aag atc aaa gaa aaa tct aat tta tac tca gca tat aaa gca ata gta  1008
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335

agt tca atc tta tta atg agg gat tct ctt aaa gaa gtg caa agt gcc  1056
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Ser Ala
            340                 345                 350

att gac gag aat ggc att tgg tac taa                             1083
Ile Asp Glu Asn Gly Ile Trp Tyr
        355                 360
```

<210> 12
<211> 360
<212> PRT
<213> Borrelia burgdorferi

```
<400> 12
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30
Pro Gly Leu Phe Asp Lys Glu Asn Ser Ile Leu Glu Thr Gly Glu Glu
         35                  40                  45
Ser Ile Lys Lys Pro Met Asn Lys Lys Asp Lys Gly Lys Ile Ala Arg
     50                  55                  60
Lys Lys Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Ser Ile His Ser
```

```
 65                  70                  75                  80
Leu Lys Arg Asp Ser Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160
Leu Arg Leu Lys Ser Ala Leu Gln Gly Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Asp Arg Tyr Tyr Leu
            180                 185                 190
Glu Glu Tyr Asp Glu Asp Val Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220
Asn Val Asp Ser Ala Ile Lys Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ser Ala Lys Phe Thr Lys
            260                 265                 270
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Asp Thr Thr Ala
    290                 295                 300
Lys Arg Lys Leu Glu Asn Ile Glu Thr Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Ser Ala
            340                 345                 350
Ile Asp Glu Asn Gly Ile Trp Tyr
        355                 360
```

<210> 13
<211> 1083
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1083)

<400> 13

```
atg aag aaa gtt aaa agt aaa tat tta gct ttg gga tta ctt ttt ggt   48
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15

ttt ata agt tgt gat tta ttc ata aga tat gaa atg aaa gag gaa tcc   96
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30

cct ggc tta ttt gat aag gga aac tct att tta gag act agc gag gaa   144
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45
```

```
tct att aaa aag cct atg aat aag aaa ggc aaa ggt aag att gcc aga   192
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg
     50                  55                  60

aag aac gga aaa agc aag gtt tct gga aaa gaa ccg ttt att cat agt   240
Lys Asn Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Phe Ile His Ser
 65                  70                  75                  80

ttt aaa aga gac gct gct aat aaa agc aat ttt tta caa aaa aat gta   288
Phe Lys Arg Asp Ala Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                85                  90                  95

atg tta gag gaa gaa agt tta aaa act gaa tta tta aaa gag caa tct   336
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110

gag act aga aaa gaa aaa ata caa aaa caa caa gat gaa tat aaa ggg   384
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125

atg act aaa gga agt tta aat tcc ctt agc ggt gaa agt ggt gaa ttg   432
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140

aag gag act att gaa agc aat gaa att gat att act ata gat tct gat   480
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160

tta agg cca aag agt tcc tta caa gac att gca gga tca aac tct att   528
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175

tca tac act gat gaa ata gag gaa gag gat tat gct cgg tat tat tta   576
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190

gat gaa gat gat gaa gat gat gaa tat tat gaa gat gat tat gag gaa   624
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205

ata aga tta agc aat cga tat caa tct tat cta gaa ggt gtt aaa tat   672
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220

aat gta gat tca gca att aac aca att aat aag ata tat gat act tat   720
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240

aca tta ttc tca aca aag cta acc caa atg tat tct aca cgc ctt gac   768
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255

aac ctt gct aaa gcc aaa gct aaa gaa gaa gct gca aag ttt aca aaa   816
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270

gaa gac ctt gaa aaa aat ttc aag acc tta ttg aat tac att caa gta   864
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285
```

```
agt gta aag act gca aca aat ttt gta tac ata aat gaa atg cat gca   912
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Glu Met His Ala
    290                 295                 300

aaa agg aaa tta gag aac att gaa gca aaa ata aaa act tta att gca   960
Lys Arg Lys Leu Glu Asn Ile Glu Ala Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320

aag atc aaa gaa aaa tct aat tta tac tca gca tat aaa gca ata gta   1008
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335

agt tca atc tta tta atg agg gat tct ctt aaa gaa gtg caa tat gcc   1056
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Tyr Ala
            340                 345                 350

att gac aag aat ggc att tgg tac taa                              1083
Ile Asp Lys Asn Gly Ile Trp Tyr
        355                 360
```

<210> 14
<211> 360
<212> PRT
<213> Borrelia burgdorferi

<400> 14

```
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg
     50                  55                  60
Lys Asn Gly Lys Ser Lys Val Ser Gly Lys Glu Pro Phe Ile His Ser
 65                  70                  75                  80
Phe Lys Arg Asp Ala Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95
Met Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125
Met Thr Lys Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270
```

```
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285
Ser Val Lys Thr Ala Thr Asn Phe Val Tyr Ile Asn Glu Met His Ala
    290                 295                 300
Lys Arg Lys Leu Glu Asn Ile Glu Ala Lys Ile Lys Thr Leu Ile Ala
305                 310                 315                 320
Lys Ile Lys Glu Lys Ser Asn Leu Tyr Ser Ala Tyr Lys Ala Ile Val
                325                 330                 335
Ser Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Tyr Ala
            340                 345                 350
Ile Asp Lys Asn Gly Ile Trp Tyr
        355                 360
```

<210> 15
<211> 1083
<212> DNA
<213> Borrelia burgdorferi

<220>
<221> CDS
<222> (1)..(1083)

<400> 15

```
atg aaa aaa gtt aaa agt aaa tat ttg gct ttg gga tta ctt ttt ggt   48
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15

ttt ata agt tgt gat tta ttc ata aga tat gaa atg aaa gag gaa tcc   96
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30

cct ggc tta ttt gat aag gga aac tct att tta gag act agc gag gaa   144
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45

tct att aaa aag cct atg aat aag aaa ggt aaa ggt aag att gct aga   192
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg
     50                  55                  60

aag aaa ggc aaa agc aag gtt tct aga aaa gaa ccg tat att cat agt   240
Lys Lys Gly Lys Ser Lys Val Ser Arg Lys Glu Pro Tyr Ile His Ser
 65                  70                  75                  80

tta aaa agg gac tct gct aat aaa agc aat ttt tta caa aaa aat gta   288
Leu Lys Arg Asp Ser Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95

att tta gag gaa gaa agt tta aaa act gaa tta tta aaa gag caa tct   336
Ile Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110

gag act aga aaa gaa aaa ata caa aaa caa caa gat gaa tat aaa ggg   384
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125

atg act caa gga agt tta aat tcc ctt agc ggt gaa agt ggt gaa ttg   432
Met Thr Gln Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140
```

```
aag gag act att gaa agc aat gaa att gat att act ata gat tct gat   480
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160

tta agg cca aag agt tcc tta caa gac att gca gga tca aac tct att   528
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175

tca tac act gat gaa ata gag gaa gag gat tat gct cgg tat tat tta   576
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190

gat gaa gat gat gaa gat gat gaa tat tat gaa gat gat tat gag gaa   624
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205

ata aga tta agc aat cga tat caa tct tat cta gaa ggt gtt aaa tat   672
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220

aat gta gat tca gca att aac aca att aat aag ata tat gat act tat   720
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240

aca tta ttc tca aca aag cta acc caa atg tat tct aca cgc ctt gac   768
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255

aac ctt gct aaa gcc aaa gct aaa gaa gaa gct gca aag ttt aca aaa   816
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270

gaa gac ctt gaa aaa aat ttc aag acc tta tta aat tat att caa gta   864
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285

agt gta aag act gca gca aat ttt gta tac ata aat gac acg cat gca   912
Ser Val Lys Thr Ala Ala Asn Phe Val Tyr Ile Asn Asp Thr His Ala
    290                 295                 300

aaa agg aaa tta gag aac att gaa gca gaa ata aaa act tta att gca   960
Lys Arg Lys Leu Glu Asn Ile Glu Ala Glu Ile Lys Thr Leu Ile Ala
305                 310                 315                 320

aag atc aaa gaa caa tct aat tta tac gaa gca tat aaa gca ata gta  1008
Lys Ile Lys Glu Gln Ser Asn Leu Tyr Glu Ala Tyr Lys Ala Ile Val
                325                 330                 335

acg tca atc tta tta atg agg gat tct ctt aaa gaa gtg caa ggt atc  1056
Thr Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Gly Ile
            340                 345                 350

att gac aag aat ggc gtt tgg tac taa                             1083
Ile Asp Lys Asn Gly Val Trp Tyr
        355                 360
```

<210> 16
<211> 360
<212> PRT
<213> Borrelia burgdorferi

<400> 16

```
Met Lys Lys Val Lys Ser Lys Tyr Leu Ala Leu Gly Leu Leu Phe Gly
  1               5                  10                  15
Phe Ile Ser Cys Asp Leu Phe Ile Arg Tyr Glu Met Lys Glu Glu Ser
             20                  25                  30
Pro Gly Leu Phe Asp Lys Gly Asn Ser Ile Leu Glu Thr Ser Glu Glu
         35                  40                  45
Ser Ile Lys Lys Pro Met Asn Lys Lys Gly Lys Gly Lys Ile Ala Arg
     50                  55                  60
Lys Lys Gly Lys Ser Lys Val Ser Arg Lys Glu Pro Tyr Ile His Ser
 65                  70                  75                  80
Leu Lys Arg Asp Ser Ala Asn Lys Ser Asn Phe Leu Gln Lys Asn Val
                 85                  90                  95
Ile Leu Glu Glu Glu Ser Leu Lys Thr Glu Leu Leu Lys Glu Gln Ser
            100                 105                 110
Glu Thr Arg Lys Glu Lys Ile Gln Lys Gln Gln Asp Glu Tyr Lys Gly
        115                 120                 125
Met Thr Gln Gly Ser Leu Asn Ser Leu Ser Gly Glu Ser Gly Glu Leu
    130                 135                 140
Lys Glu Thr Ile Glu Ser Asn Glu Ile Asp Ile Thr Ile Asp Ser Asp
145                 150                 155                 160
Leu Arg Pro Lys Ser Ser Leu Gln Asp Ile Ala Gly Ser Asn Ser Ile
                165                 170                 175
Ser Tyr Thr Asp Glu Ile Glu Glu Glu Asp Tyr Ala Arg Tyr Tyr Leu
            180                 185                 190
Asp Glu Asp Asp Glu Asp Asp Glu Tyr Tyr Glu Asp Asp Tyr Glu Glu
        195                 200                 205
Ile Arg Leu Ser Asn Arg Tyr Gln Ser Tyr Leu Glu Gly Val Lys Tyr
    210                 215                 220
Asn Val Asp Ser Ala Ile Asn Thr Ile Asn Lys Ile Tyr Asp Thr Tyr
225                 230                 235                 240
Thr Leu Phe Ser Thr Lys Leu Thr Gln Met Tyr Ser Thr Arg Leu Asp
                245                 250                 255
Asn Leu Ala Lys Ala Lys Ala Lys Glu Glu Ala Ala Lys Phe Thr Lys
            260                 265                 270
Glu Asp Leu Glu Lys Asn Phe Lys Thr Leu Leu Asn Tyr Ile Gln Val
        275                 280                 285
Ser Val Lys Thr Ala Ala Asn Phe Val Tyr Ile Asn Asp Thr His Ala
    290                 295                 300
Lys Arg Lys Leu Glu Asn Ile Glu Ala Glu Ile Lys Thr Leu Ile Ala
305                 310                 315                 320
Lys Ile Lys Glu Gln Ser Asn Leu Tyr Glu Ala Tyr Lys Ala Ile Val
                325                 330                 335
Thr Ser Ile Leu Leu Met Arg Asp Ser Leu Lys Glu Val Gln Gly Ile
            340                 345                 350
Ile Asp Lys Asn Gly Val Trp Tyr
        355                 360
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 02/00951

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/53 // G01N 33/569
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Abstracts of the General meeting of the American Society of Microbiology, Volume 100, 2000, A.P. Van Dam et al, "V-12. BBK32 is an Important Diagnostic Antigen in Lyme Borreliosis", page 661, abstract	1-4,6-13, 15-17,19
Y	--	5,14,18
X	Immunity, Volume 6, 1997, Erol Fikrig et al, "Borrelia burgdorferi P35 and P37 Proteins, Expressed In Vivo, Elicit Protective Immunity", pages 531-539, abstract	1-4,6-13, 15-17,19
Y	--	5,14,18

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 2003	11 -03- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer TERESE PERSSON/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 02/00951

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9742325 A1 (YALE UNIVERSITY), 13 November 1997 (13.11.97)	1-4,6-13, 15-17,19
Y		5,14,18
	--	
X	WO 0120325 A1 (IMMUNETICS, INC.), 22 March 2001 (22.03.01), page 15, line 7 - line 15	1-4,6-13, 15-17,19
Y		5,14,18
A		1-19
	--	
X	J. Med. Microbiol, Volume 50, 2001, Louis A. Magnarelli et al, "Reactivity of dog sera to whole-cell or recombinant antigens of Borrelia burgdorferi by ELISA and immunoblot analysis", pages 889-895, abstract; page 893, column 1, last paragraph	1-4,6-13, 15-17,19
Y		5,14,18
	--	
X	Journal of Clinical Microbiology, Volume 35, No. 1, 1997, Robert D. Gilmore et al, "Molecular Characterization of a 35-Kilodalton Protein of Borrelia burgdorferi, an Antigen of Diagnostic Importance in Early Lyme Disease", pages 86-91, abstract, discussion	1-4,6-13, 15-17,19
Y		5,14,18
	--	
X	Journal of Clinical Microbiology, Volume 38, No. 5, 2000, Louis A. Magnarelli et al, "Serologic Diagnosis of Lyme Borreliosis by Using Enzyme-Linked Immunosorbent Assays with Recombinant Antigen", pages 1735-1739, abstract, page 175, column 2, paragraph 3	1-4,6-13, 15-17,19
Y		5,14,18
	--	

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Infection and Immunity, Volume 69, No. 6, 2001, William S. Probert et al, "Mapping the Ligand-Binding Region of Borrelia burgdorferi Fibronectin-Binding Protein BBK32", pages 4129-4133, page 1738, column 1, lines 21-24	1-4,6-13, 15-17,19
Y		5,14,18
	--	
Y	WO 0077041 A2 (THE TEXAS A & M UNIVERSITY SYSTEMS), 21 December 2000 (21.12.00), page 26, line 14 - line 23	5,14,18
	--	
A	Infection and Immunity, Volume 67, No. 1, 1999, Evren Akin et al, "The Immunoglobulin (IgG) Antibody Response to OspA and OspB Correlates with Severe and Prolonged Lyme Arthritis and the IgG Response to P35 Correlates with Mild and Brief Arthritis", pages 173-181	1-19
	--	
A	Journal of Clinical Microbiology, Volume 37, No. 12, 1999, M.B. Lawrenz et al, "Human Antibody Responses to VlsE Antigenic Variation Protein of Borrelia burgdorferi", pages 3997-4004	1-19
	--	
P,X	Journal of Clinical Microbiology, Volume 40, No. 4, 2002, Tero Heikkilä et al, "Recombinant BBK32 Protein in Serodiagnosis of Early and Late Lyme Borreliosis", pages 11741180	1-4,6-13, 15-17,19
P,Y		5,14,18
	--	
P,X	WO 0220046 A1 (THE TEXAS A & M UNIVERSITY SYSTEM), 14 March 2002 (14.03.02)	1-4,6-13, 15-17,19
P,Y		5,14,18
	--	

INTERNATIONAL SEARCH REPORT

Inter ›nal application No.
PCT/FI02/00951

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 4,6-13,15-17 and 19 (all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Present claims 4, 6-13, 15-17 and 19 relate to a large number of possible proteins due to the expression "immunogenic proteins". Support within the meaning of Article 6PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the immunogenic proteins. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts related to the immunogenic proteins disclosed in the present application at page 4, line 25-27.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established will not be the subject of an international preliminary examination (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

Additional remark;

Having consulted the applicant's agent by telephone, 6 March 2003, the present claim 9 has been interpreted to relate to "borrelia peptides of BBK32" instead of "borrelia peptides".

INTERNATIONAL SEARCH REPORT

Information on patent family members

30/12/02

International application No.
PCT/FI 02/00951

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9742325 A1	13/11/97	EP 0915977 A JP 2000510339 T	19/05/99 15/08/00
WO 0120325 A1	22/03/01	AU 6054099 A	17/04/01
WO 0077041 A2	21/12/00	AU 5495500 A	02/01/01
WO 0220046 A1	14/03/02	AU 9262601 A	22/03/02

专利名称(译)	诊断早期和晚期莱姆病的方法		
公开(公告)号	EP1448989A1	公开(公告)日	2004-08-25
申请号	EP2002781347	申请日	2002-11-26
[标]申请(专利权)人(译)	BORTECH		
申请(专利权)人(译)	BORTECH OY		
当前申请(专利权)人(译)	BORTECH OY		
[标]发明人	LAHDENNE PEKKA HEIKKILA TERO SEPPALA ILKKA SAXEN HARRI		
发明人	LAHDENNE, PEKKA HEIKKILÄ, TERO SEPPÄLÄ, ILKKA SAXEN, HARRI		
IPC分类号	G01N33/569 G01N33/68 G01N33/53		
CPC分类号	G01N33/6854 G01N33/56911 G01N2333/20 Y02A50/57		
优先权	2001002310 2001-11-26 FI		
外部链接	Espacenet		

摘要(译)

本发明涉及一种检测伯氏疏螺旋体感染的方法或检测来自疑似感染或接种的人或其他哺乳动物的体液中的疏螺旋体属的抗体的方法，以及用于所述方法和免疫测定方法的诊断试剂盒用于诊断早期和晚期莱姆疏螺旋体病，特别是用于诊断迁移性红斑。根据本发明的方法的特征在于重组BBK32蛋白，任选其他免疫原性的borrelial蛋白或其片段用作抗原。