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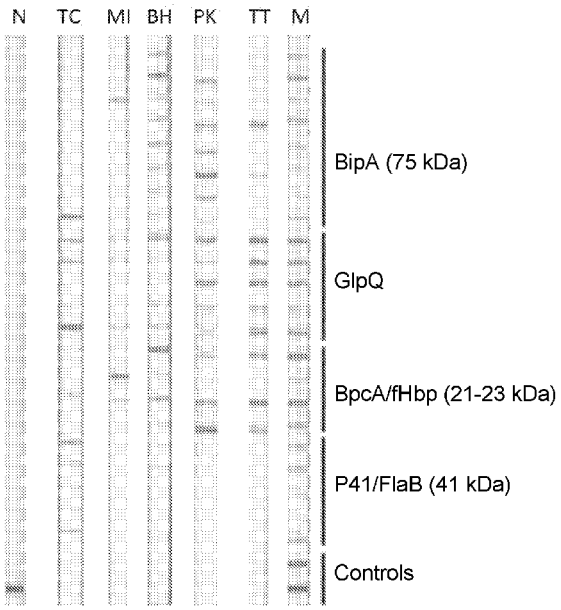
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(54) Title: SPECIES-SPECIFIC ANTIGEN SEQUENCES FOR TICK-BORNE RELAPSING FEVER (TBRF) AND METHODS OF USE

Fig. 1



(57) Abstract: The disclosure, in some aspects, provides antigen-specific amino acid sequences for tick-borne relapsing fever (TBRF) Borrelia species. Specifically, the disclosure provides compositions and methods that may be used to identify antibodies to infection by one or more TBRF Borrelia species in samples from subjects suspected of having TBRF. Further disclosed are compositions that include one or more labeled and/or tagged and/or bound amino acid sequences.

[Continued on next page]

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SPECIES-SPECIFIC ANTIGEN SEQUENCES FOR TICK-BORNE RELAPSING FEVER (TBRF) AND METHODS OF USE

FIELD OF THE INVENTION

Aspects of the present invention provide novel compositions and methods for diagnosing Tick-Borne Relapsing Fever (TBRF) resulting from infection by diverse TBRF *Borrelia* species.

SEQUENCE LISTING

The instant application incorporates by reference the Sequence Listing in the ASCII text file filed January 15, 2021, entitled "ID-FISH 0153-2015US01_ST25.txt", which file was created on January 7, 2021, the size of which file is 27,388 bytes.

BACKGROUND OF THE INVENTION

Borreliosis is caused by two groups of *Borrelia*, the *B. burgdorferi* group and the Tick-Borne Relapsing Fever (TBRF) *Borrelia* group (also referred to herein as RFB (relapsing fever *Borrelia*)). The *B. burgdorferi* group was once thought to be the only group that caused Lyme-like symptoms in infected subjects, but it is now known that TBRF *Borrelia* also causes Lyme-like symptoms. Several *Borrelia* species cause TBRF, and these are usually associated with specific species of ticks. For instance, *B. hermsii* is transmitted by *Ornithodorus hermsi* ticks, *B. parkerii* by *O. parkeri* ticks, and *B. turicatae* by *O. turicata* ticks. Each tick species has a preferred habitat and preferred set of hosts. Typically, hard (*Ixodes*) ticks transmit Lyme disease pathogens whereas soft (*Ornithodorus*) ticks transmit TBRF pathogens, but there are exceptions to this rule: several RFB agents are transmitted only by hard ticks (e.g., *B. miyamotoi*) and one species, *B. recurrentis*, is louse transmitted.

The main symptoms of TBRF are high fever (*e.g.*, 103°F), headache, and muscle and joint aches. Symptoms can reoccur, producing a telltale pattern of fever lasting roughly three days, followed by 7 days without fever, followed by another three days of fever. Without antibiotic treatment, this process can repeat several times.

Currently, the standard for identification is by identification of TBRF spirochetes in blood smears of a subject presenting symptoms consistent with TBRF. After obtaining a blood draw, a sample must be cultured for at least 24 hours to facilitate identification. However, even early in the disease when spirochetes are highest, positive identification is only made about 70% of the time. (*See*, www.cdc.gov/relapsing-fever/clinicians/index.html).

Thus, materials and methods of the current state of the art result in a delay in diagnosis and provide a relatively low level of sensitivity and specificity. New materials and methods are needed that are suitable for the identification of TBRF causative agents with decreased assay time and increased sensitivity and specificity.

SUMMARY

The invention, in part, relates to compositions and methods that may be used to identify antibodies to infection by one or more TBRF *Borrelia* species in samples from subjects suspected of having TBRF. With compositions and methods of the present invention, identification of TBRF *Borrelia* in subject samples may be performed with greater speed, sensitivity, and specificity than with existing compositions and methods. Antigen-specific amino acid sequences of the present invention may be used in diagnostic and scientific assays. Non-limiting examples of suitable assays include immunoblots, ELISA (enzyme-linked immunosorbent assay), etc. Amino acid sequences of the present invention may be used for detecting TBRF *Borrelia* specific T-cells (*e.g.*, the IgXSPOT test; IGeneX, Palo Alto, CA).

According to an aspect of the invention, compositions that include one or more labeled and/or tagged and/or bound amino acid sequences, wherein the one or more labeled and/or tagged and/or bound amino acid sequences comprise amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3 are provided. In some embodiments, the composition also includes amino acid sequences that are bound to a substance selected from the group consisting of nitrocellulose, nylon, polyvinylidene difluoride (PVDF), magnetic beads, and agarose. In some embodiments, each of said one or more amino acid sequences are tagged with an antibody with specificity for said amino acid sequence.

According to an aspect of the invention, methods are provided for detecting antibodies resulting from infection by one or more members of Relapsing Fever (TBRF) Borrelia genus, wherein the genus comprises *B. hermsii*, *B. turicatae*, *B. miyamotoi*, *B. duttonii*, and *B. parkeri*, if present in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), the methods including: providing a biological sample obtained from a subject suspected of having TBRF; mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group consisting

of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3; and detecting a positive immunobinding reaction which indicates the presence of TBRF specific antibodies in the sample. In some embodiments, two or more of the labeled and/or tagged and/or bound amino acid sequences are mixed with the biological sample and a sample is considered positive for TBRF if at least two amino acid sequences are detected. In some embodiments, the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG or anti-human IgM antibody linked to a detectable moiety. In some embodiments, the detectable moiety is selected from the group consisting of chromophores, radioactive moieties and enzymes. In some embodiments, the detectable moiety includes alkaline phosphatase. In some embodiments, the detectable moiety includes biotin.

According to an aspect of the invention, a vector including a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3, and a heterologous nucleic acid sequence is provided. In some embodiments, the nucleic acid sequence is operatively linked to a promoter sequence. In some embodiments, the vector is a prokaryotic vector. In some embodiments, a cell includes the vector. In some embodiments, the cell is a bacterial cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a photomicrographic image of a line immunoblot. TBRF ImmunoBlot strips were tested with rabbit anti-TBRF *Borrelia* serum samples. Antibodies to TBRF *Borrelia* species *B. turcica*, *B. miyamotoi*, *B. hermsii*, *B. parkeri,* and *B. turicatae* were detected by TBRF ImmunoBlot strips prepared from recombinant antigens from all five species, demonstrating that the TBRF ImmunoBlot detected TBRF *Borrelia* group antibodies. N, negative control; TC, *B. turcica*; MI, *B. miyamotoi*; BH, *B. hermsii*; PK, *B. parkeri*, TT, *B. turicatae*; and M, positive control.

Fig. 2 shows a photomicrographic image of TBRF ImmunoBlots of representative patient serum samples. Patient serum samples were tested on Tick-Borne Relapsing Fever (TBRF) IgM and IgG ImmunoBlots. These results illustrate how detection of antibodies to TBRF Borrelia species identified patients with Chronic Lyme Disease symptoms that would

otherwise have been missed by only testing for *Borrelia burgdorferi* species antigens. P, positive control; N, negative control; G, TBRF ImmunoBlot IgG; M, TBRF ImmunoBlot IgM; 1, *B. hermsii*-positive IgM; 2, *B. turicatae*-positive IgG; 3, *B. miyamotoi*-positive IgM; 4, *B. turcica*-positive IgG; 5, *B. parkeri*-positive IgM and IgG.

Fig. 3 presents a table showing representative results of TBRF ImmunoBlot IgM and IgG tests with SEQ ID NOs: 1–11 on patient serum samples. Pos, positive; I, indeterminate; Tt, *B. turicatae*; Pk, *B. parkeri*; Mia, *B. miyamotoi*; Du, *B. duttonii*; Bh, *B. hermsii*;

DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 *B. hermsii* Vlp7

MQQPEAGKTGVSGGVNGNLGNSLMELGRSAENAFYAFIELVSDVLGFTAKSDTTKQ
EVGGYFNSLGAKLGEASNDLEQVAVKAETGVDKSDSSKNPIREAVNEAKEVLGTLK
GYVESLGTIGDSNPVGYANNAAGSGTTAADDELRKAFKALQEIVKAATDAGVKALK
IGATTLQANGGADNKEGAKILATSGGNPAAADVAKAAAILSSVSGEEMLSSIVKSGE
NDAQLAAAADGNTSAISFAKGGSDAHLAGANTPKAAAVAGGIALRSLVKTGKLAA
GAADNATGGGKEVQGVGVAAANKLLRAVEDVIKKTVKNVLEKAKEKIDKARGSQE
PVSESSK

SEQ ID NO: 2 *B. turicatae* BipA-2H

MDNVMSGIDNVIQGAGTFATAAMQGVGTVIDVLQDVGTFVISDIQNMGARMLFGT
GENSSVASEGSESVMSLSSNDSSEAKDVTVVLSSDVTSKGNDVAVGLSSDETQVIGR
LEKYLQSAIKINGRSDSDQSNLESGRKKFFNWLKTSDTNASKRKELVQDLQKVFDLI
KEKSSDSTELKHWVQSIVDRIEDKSTIVDIDSDDELNNDKEVDFLIENTLASRDYSGF
AVSLLFQSLADTLYDSDNNRDKSEEQIFQDLRKVFSDSSDKSEGVLGFKSKIEATN

SEQ ID NO: 3 *B. turicatae* BpcA/fHbp

MSETSLLNIETNLLNTLDDNQKQALITFKDLLQDKKHLSILEKQQKSILEDLKANQKN
YNLQDKLKKTLNSEYDKNQLNKLFDELGNIKTKQFLQQLHIILQSIKDGKPTNFASSN
FNNLNQTLEQKKEQALKYIKDKLYTDYYLYINGIQDANYFFERIMSLLE

SEQ ID NO: 4 *B. turicatae* P41/FlaB

MRNNSINAANLSKTQEKLSSGHRINRASDDAAGMGVAGKINAQIRGLSQASRNTSK
AINFIQTTEGNLNEVEKVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQ
AQYNQMHMLSNKSAAQNIKTAEELGMQPAKINTPASLAGSQASWTLRVHVGANQD
EAIAVNIYAANVANLFAGEGAQVSPAQEGAQQEGVQAAPAPAAAPAQGGVNSPVN
VTTTIDANMSLSKIENAIRMVSDQRANLGAFQNRLESIKASTEYAIENLKSSYAQIQD
ATMTDEIVASTTNSILTQSAMAMIAQANQVPQYVLSLLR

SEQ ID NO: 5 *B. parkeri* BipA-1H

MDMGSTRDWLTNDDGFVRGTKGFDDSPFRRPDRVDKEVSAGGREIEKAFSRNLGV
AGGQRKGTDDVKNGIAGARESGGVLKEAENAGQRDVDDSGEGIKNDVIQNLGSVG
VQVAVGSENNGDDSGQEAEKGSQNLGDTGTQRVVSTSDLNSHLGVESKGGMSTNK
EGISTNHVTENRNSINSITSTSSGLSTALQIAGTSTRASGYEGEVTTNAQDRSFIDTKTQ
DSKKQYSDFSDQDIRDKILGNVVGGVV

SEQ ID NO: 6 *B. parkeri* BipA-2H

MGNVMSGIDNVIQGAGTFATAAMQGVGTVIDVLQDVGTFVISDIQNMGARMFFGT
GESSSVASEDSESVMSLSSKASSEAKDTTVGLSSDVTSKGNNVAVGLSSDEIQIIGRLE
KYLKSAIKINGRSDSDQSKLESGHKKFFQWLKTSDTNASKRKELVQNLQRVFNLIKE
KSSDSTELKKWMQSIVDDIENKSTIIDINSDDKLNNDKEVDFLIEKTLGSSDYSGFAVS
LLFQALADTLYDSENSRDKSEEQIFKDLRKVFSDKSEGVLEFKSKIEATN

SEQ ID NO: 7 *B. parkeri* GlpQ

MCQNEKMSMTNKKSPLTIAHRGASGYLPEHTLESKAFAYALGADYLEQDIVLTKDN
VPIIMHDPELDTTTNVAKLFPERARENGRYYSVDFTLDELKSLSLSERFDLETRKPIYP
KRFPLNEYNFKIPTLEEEIQFIQGLNKSTGRNVGIYPEIKKPLWHKQQGKDISKIVIEIL
NKYGYKSKEDKIYLQTFDFDELKRIREELGYQGKLIMLVGENDWNEAPTDYEYIKSQ
EGMTEVAKYADGIGPWIPQIIIDGKITDLTSLAHKYNMEVHAYTFRIDSLPSYVKDAN
ELLDLLFNQAKIDGLFTDFTDTVVKFIKQ

SEQ ID NO: 8 *B. parkeri* BpcA/fHbp

MSETSLLNTLDNNQKQALITFKDLLQDKNHRSILEKQQKSILKDLEKHQENSNLQDK
LKKTLNSEYDKTQLNKLFDELGNIKTKQFLQKLHIMLKSINNGTLTSFSSSNFKDSNQ
TLEQKKEQALQYIKGQLYTDYYLYINGIQDANYFFERIMSVLEI

SEQ ID NO: 9 *B. parkeri* P41/FlaB

MRNNSINAANLRKTQEKLSSGHRINRASDDAAGMGVAGKINAQIRGLSQASRNTSK
AINFIQTTEGNLNEVEKVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQ
AQYNQMHMLSNKSAAQNIKTAEELGMQPAKINTPASLAGAQASWTLRVHVGANQ
DEAIAVNIYASNVANLFAGEGAQVSPAQEGAQQEGVQAAPAPAAAPAQGGVNSPV
NVTTTVDANMSLSKIENAIRMVSDQRANLGAFQNRLESIKASTEYAIENLKSSYAQIK
DATMTDEIVASTTNSILTQSAMAMIAQANQVPQYVLSLLR

SEQ ID NO: 10 *B. duttonii* GlpQ

MENAKINKKSALIIAHRGASGYLPEHTLEAKAYAHALGADYIEQDIVLTKDDIPIVMH
DPELDTTTNVAKLFPGRARENGKYYSVDFTLAEIKSLSLSERFDPETQQPIYPNRFPAT
EYDFKIPTLEEEIKFIQGLNKSTGKNIGIYPEIKKPLWHKQQGKDISKIVIDILNKYGYK
SKEDKIYLQTFDFDEIKRIREELGYQGKLIMLVGENDWEEAPTDYEYIKSEEGMAEV
AKYADGIGPWIPQIIINGQITGLISLAHKYNMQVHPYTFRIDALPSYVKDPNELLELLFI
KAKVDGLFTDFVDISIKFMQ

SEQ ID NO: 11 *B. miyamotoi* GlpQ

MASMTGGQQMGRGSEMGENKKSPLIIAHRGASGYLPEHTLEAKAYAYALGADYLE
QDIVLTKDNIPVIMHDPEIDTTTNVAQLFPNRARENGRYYATDFTLTELKSLNLSERF
DPENKKPIYPNRFPLNEYNFKIPTLEEEIQFIQGLNKSTGKNVGIYPEIKKPFWHKQQG
KDISKIVIEILNKYGYKSKEDKIYLQTFDFDELKRIRKELGYQGKLIMLVGENDWNEA
PTDYEYIKSEEGIAEVAKYSDGIGPWIPQIIIDGKITELTNLAHKYNIEVHPYTFRTDAL
PSYVKNENELLDLLFNKAKVDGIFTDFTDTVMNFIKK

DETAILED DESCRIPTION OF THE INVENTION

The RFB species best known for causing TBRF in the United States of America (USA) are *B. hermsii, B. miyamotoi, B. parkeri*, and *B. turicatae*. However, other RFB species that cause TBRF continue to be identified. For example, a patient infected with *B. johnsonii*-like species, previously found only in bat ticks, was identified in Wisconsin. Moreover, TBRF has also been reported in Central and South America. *B. hispanica*, *B. persica*, and *B. miyamotoi* are important causes of TBRF in Europe and Asia, and *B. hispanica*, *B. crocidurae*, and *B. duttonii* are important causes of TBRF in Africa. Although most RFB species are transmitted by soft ticks of the genus *Ornithodoros, B. miyamotoi* may be transmitted by the same hard ticks of the genus *Ixodes* that transmit Lyme Disease *Borrelia* (LDB) species. Other RFB species, *B. lonestari,* the recently described *B. turcicia,* and *B. tachyglossi,* are members of a rapidly expanding *Borrelia* clade associated with reptile (*B. turcica*) or echidna (*B. tachyglossi*) hosts and are also transmitted by hard ticks. In order to provide timely treatment, clinicians must be able to rapidly and correctly identify whether a patient presenting with symptoms of tick-borne disease have been exposed to TBRF species.

Aspects of the invention provide a method of quickly and accurately detecting *Borrelia* antisera in a sample from a subject suspected of having TBRF. A subject suspected of having TBRF can be identified as having symptoms such as a high fever (*e.g.*, 103° F), headache, and muscle and joint aches. Symptoms typically reoccur, producing a telltale pattern of fever lasting roughly 3 days, followed by approximately 7 days without fever, followed by another 3 days of fever. Without proper antibiotic treatment, this process can repeat several times. Since the symptoms of TBRF can mimic, for example, viral flu-like symptoms, accurate diagnosis of TBRF is important for providing an effective treatment for the subject. The present invention provides a quick and easy diagnostic test for detecting the presence of antibodies specific for causative *Borrelia* species, thereby satisfying the need for such a test.

Because TBRF can be caused by several RFB species, and because geographic ranges of RFB species may overlap, tests for RFB species need to be inclusive—that is, a test needs to be able to detect antibodies to multiple species concurrently. The present invention provides for antigenic amino acid sequences specific for various TBRF *Borrelia* species. The amino acid sequences of the present invention encode antigenic peptides that have high specificity and/or sensitivity for the indicated genus. The inclusion of antigenic peptides that exhibit cross-reactivity across *Borrelia* species boundaries is also important with respect to the development of inclusive serological, or other immunologically-based assays, wherein the goal is to detect infection, not necessarily to identify a particular species responsible for infection. For example, the disclosure includes multi-panel immunoassays wherein, in the context of a single test screen, multiple RFB species are detectable.

The present invention provides novel compositions and methods for diagnosing infection by TBRF *Borrelia* species. The invention is based, in part, on the discovery of species-specific amino acid sequences encoding antigenic peptides (which may also be referred to in the art as peptide antigens or antigens), as described herein.

The present invention, in one aspect, is a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, wherein the one or more labeled and/or tagged and/or bound amino acid sequences have at least 90%, 95%, 98%, 99%, 99.5%, or 100% homology to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11. Sequences with less than 100% homology may be modified with one or more substitutions, deletions, insertions, or other modifications with respect to the amino acid sequences provided herein. Exemplary modifications include, but are not limited to conservative amino acid substitutions, which will produce molecules having functional characteristics similar to those of the molecule from which such modifications are made. Conservative amino acid substitutions are substitutions that do not result in a significant change in the activity or tertiary structure of a selected polypeptide or protein. Such substitutions typically involve replacing a selected amino acid residue with a different residue having similar physico-chemical properties. For example, substitution of Glu for Asp is considered a conservative substitution because both are similarly-sized negatively-charged amino acids. Groupings of amino acids by physico-chemical properties are known to those of skill in the art. The following groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I),

Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). One of ordinary skill in the art can determine if sequences with less than 100% homology can bind naturally- or non-naturally-occurring TBRF-related antibodies, as well as the sensitivity and specificity of the antibody to the modified sequences. One of ordinary skill in the art will be able to identify sequences with significant homology to SEQ ID NOs: 1–11 of the present invention that give acceptable or equivalent responses in the methods of the present invention without undue experimentation, in view of the teachings of this specification.

In some embodiments, the present invention is a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, wherein the one or more labeled and/or tagged and/or bound amino acid sequences comprise amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3. As used herein, "consisting of", when used as a claim transition referring to an amino acid sequence, refers to amino acid sequences having 100% homology to the specified amino acid sequence (i.e., SEQ ID NOs: 1–11).

Aspects of the present invention provide antigen-specific amino acid sequences for TBRF *Borrelia* specific species. These novel amino acid sequences may be used in assays to identify TBRF specific *Borrelia* in samples from subjects suspected of having TBRF. With the amino acid sequences of the present invention, identification of TBRF *Borrelia* in subject samples is performed with greater speed, sensitivity and specificity than other current methods. The amino acid sequences of the present invention may be used in diagnostic and scientific assays. Non-limiting examples of suitable assays include immunoblots, line immunoblots, ELISA (enzyme-linked immunosorbent assay), etc. The amino acid sequences of the present invention may be used for the detection of TBRF *Borrelia* specific T-cells, for example, with the IgXSPOT test (IGeneX, Milpitas, CA).

In some embodiments, the invention is a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, wherein the one or more labeled and/or tagged and/or bound amino acid sequences comprise amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants of those sequences. The expression "variants" encompasses

any modification(s) of a specified amino acid sequence (i.e., SEQ ID NOs. 1–11) which retain(s) the immunological binding profile of Fig. 3. Such modifications may include insertions and deletions (internal or from the N- or C- terminus, or both).

Nucleic acid sequences, including polynucleotides and oligonucleotides, encoding the amino acid sequences of the present invention, and portions thereof, may be expressed in cultured cells to provide isolatable quantities of peptides displaying biological (*e.g.*, immunological) properties of the antigenic peptide encoded by the amino acid sequences of the present invention. Because of redundancy of the genetic code, multiple nucleic acid sequences may be suitable for the production of the peptide sequences of the present invention. One of ordinary skill in the art will be able to determine one or more nucleic acid sequences for production of the amino acid sequences of the present invention. A nucleic acid sequence encoding an amino acid sequence of the present invention may be labeled by any suitable label known to one of ordinary skill in the art.

In this regard, nucleic acid sequences suitable for the production of the amino acid sequences of the present invention may be substantially homologous to naturally occurring sequences. Substantial homology of a nucleic acid sequence as used herein means that: (a) there is greater than 65%, 75%, 85%, 95%, 98%, or 99% homology with the naturally occurring sequence, or (b) the homologous nucleic acid sequence will hybridize to the compared sequence or its complementary strand under stringent conditions of the temperature and salt concentration. These stringent conditions will generally be a temperature greater than about 22°C, usually greater than about 30°C and more usually greater than about 45°C, and a salt concentration generally less than about 1 M, usually less than about 500 mM, and preferably less than about 200 mM. The combination of temperature and salt concentration is more important in defining stringency than either the temperature or the salt concentration alone. Other conditions which affect stringency include GC content of the compared sequence, extent of complementarity of the sequences, and length of the sequences involved in the hybridization, as well as the composition of buffer solution(s) used in the hybridization mixture. These and other factors affecting stringency are well described in the scientific and patent literature. One of ordinary skill in the art will be able to determine suitable conditions for determining the homology of the nucleic acid sequences encoding the antigenic peptides of the present invention.

Homologous nucleic acid sequences may be determined based on the nature of a nucleotide substitution in the nucleic acid sequence. For example, synonymous nucleotide substitutions, that is, nucleotide changes within a nucleic acid sequence that do not alter the

encoded amino acid sequence, will be better tolerated and, therefore, may be more numerous in a particular nucleic acid sequence than non-synonymous nucleotide substitutions. One of ordinary skill in the art will be able to determine the suitable number and location of substitutions that may be allowed in a nucleic acid sequence that encodes an amino acid sequence of the present invention without adversely affecting the antigenicity of the encoded antigenic peptide, without undue experimentation.

Labels and Tags

One or more amino acid sequences of the invention may be labeled and/or tagged and/or bound. As used herein, a "label" or "tag" is a detectable moiety that may be attached to an amino acid sequence of the invention. A label or tag may be covalently or non-covalently attached to an amino acid sequence of the invention. Non-limiting examples of such "tags" are natural and synthetic (*i.e.*, non-naturally occurring) nucleic acid and amino acid sequences (*e.g.*, poly-AAA tags), antibodies and detectable moieties such as labels (discussed elsewhere herein). Thus, the definitions of the phrases "labeled" and "tagged" may have overlap in that a tag may also, in some instances, function as a label. Furthermore, tags useful with the present invention may be linked to a label.

The amino acid sequences of the present invention, or any tags attached to an amino acid sequence of the present invention, may be labeled with any suitable label known to one of ordinary skill in the art. Such labels may include, but are not limited to, biotin/streptavidin, enzyme conjugates (*e.g.*, horseradish peroxidase (HRP), alkaline phosphatase (AP), glucose oxidase, and β-galactosidase), fluorescent moieties (*e.g.*, FITC, fluorescein, rhodamine, etc.), biological fluorophores (*e.g.*, green fluorescent protein (GFP), R-phycoerythrin) or other luminescent proteins, etc. Any suitable label known to one of ordinary skill in the art may be used with the present invention.

In some embodiments, amino acid sequences of the invention may be "bound." A "bound" amino acid sequence is an amino acid sequence that has been immobilized in order to permit the use of the amino acid sequence in a biological test such as, for example, an immunoassay. In the context of the present invention, a "bound" amino acid sequence is an amino acid sequence attached (*e.g.*, covalently or non-covalently bound, etc.) directly or indirectly to a non-natural surface or substance. Additionally or alternatively, "bound" amino acid sequences of the present invention may be attached, directly or indirectly, to a natural surface or substance, either of which is not naturally associated with the amino acid sequence. Non-limiting examples of substances to which the amino acid sequences of the present

invention may be bound are nitrocellulose, nylon, polyvinylidene difluoride (PVDF) plastics, metals, magnetic beads and agarose (*e.g.*, beads). Linking agents known to those of ordinary skill in the art may be used to aid or enhance binding of the amino acid sequences of the present invention to a surface or substance.

Production of amino acid sequences

In some embodiments, amino acid sequences of the invention may be non-natural, synthetic sequences, such as sequences produced by recombinant technology or sequences synthesized by protein synthesizing apparatuses. As such, the amino acid sequences of the present invention may be produced by recombinant technology, as is described and enabled in the literature and in commonly referred to manuals such as, *e.g.*, Short Protocols in Molecular Biology, Second Edition, F.M. Ausubel, Ed., all John Wiley & Sons, N.Y., edition as of 2008; and, Sambrook, et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001, and as is well known to one of ordinary skill in the art. In one embodiment, the amino acid sequences of the present invention are made recombinantly in *E. coli*.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. In addition to the nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3, the vectors of the present invention also include a heterologous nucleic acid sequence. As used herein, heterologous refers to a nucleic acid sequence that does not naturally occur in the organism from which the Markush group sequences are derived. The term "vector" may also refer to a virus or organism that is capable of transporting the nucleic acid molecule. One type of vector is a plasmid, a small, circular, double-stranded, extrachromosomal DNA molecule that is physically separate from and can self-replicate independently from chromosomal DNA. Some useful vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of nucleic acids to which they are operatively linked are referred to herein as "expression vectors." Other useful vectors, include, but are not limited to bacterial plasmids and bacterial artificial chromosomes (BACs), cosmids, and viruses such as lentiviruses, retroviruses, adenoviruses, and phages.

Vectors useful in methods of the invention may include additional sequences including, but not limited to, one or more signal sequences and/or promoter sequences, or a combination thereof. Promoters that may be used in methods and vectors of the invention include, but are not limited to, cell-specific promoters or general promoters. Non-limiting examples of promoters that can be used in vectors of the invention are: ubiquitous promoters, such as, but not limited to: CMV, CAG, CBA, and EF1a promoters. Methods to select and use suitable promoters are well known in the art.

Vectors useful in methods of the invention may be used to express a fusion protein comprising sequences of the invention in a cell. Expression vectors and methods of their preparation and use are well known in the art. In some embodiments of the invention, a nucleic acid sequence of an expression vector encodes a fusion protein comprising an amino acid sequence of the invention. It is well known in the art how to prepare and utilize fusion proteins that comprise a polypeptide sequence. In some embodiments, a fusion protein comprising an amino acid sequence of the invention may also include an epitope tag that may be used for purification of the fusion protein or in a method of the invention. Non-limiting examples of epitope tags are a FLAG tag, a fluorescent tag (including but not limited to green fluorescent protein (GFP)), a GST tag, a hemagglutinin (HA), a poly-histidine (poly-His) tag, a Myc tag, an MBP tag, or a V5 tag. In some embodiments, a fusion protein comprising an amino acid sequence of the invention may also include a detectable label, as described elsewhere herein.

Subjects and cells

As used herein, a subject may be an animal, such as a mammal or a non-mammal. Non-limiting examples of mammalian subjects include primates (including but not limited to humans), rodents (including but not limited to mice, rats, squirrels, chipmunks, prairie dogs), lagomorphs, deer, canids (including but not limited to dogs, foxes, coyotes, and wolves), felids (including but not limited to domestic cats, bobcats, cougars, and other wild cats), bears, horses, cows, sheep, goats, and pigs. Non-limiting examples of non-mammalian subjects include birds, amphibians, lizards, insects, and arthropods. As used herein, a cell may be a bacterial cell, including but not limited to *E. coli*, or an animal cell, either mammalian or non-mammalian.

Assays and methods of detection

Amino acid sequences of the invention may be tagged with an antibody with specificity for any of said amino acid sequences. Specificity for said amino acid sequence, *i.e.*, antibody specificity, is the property of antibodies which enables them to react preferentially with some antigenic determinants and not with others. Specificity is dependent on chemical composition, physical forces and molecular structure at the binding site. Sensitivity is how strongly the antibody binds to the antigenic determinant. One of ordinary skill in the art can determine specificity and sensitivity of an antibody for a particular amino acid sequence using standard affinity assays, such as immunoblotting, Ouchterlony assays, titer assays, etc. In another aspect, the present invention provides a method of quickly and accurately detecting TBRF *Borrelia* antisera in a sample from a subject suspected of having TBRF. The method of the present invention for detecting TBRF *Borrelia* antisera in a sample from a subject suspected of having TBRF, may comprise, for example, providing a biological sample (including but not limited to blood, saliva) obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences of the present invention and detecting a positive reaction which indicates the presence of Lyme disease antisera in the sample. The antisera may be detected by, for example, immunoblotting, ELISPOT, ELISA, Western blotting, lateral flow assay, or any other appropriate immunoassay known to one of ordinary skill in the art. These techniques are known to one of ordinary skill in the art and procedures can be found in common technical references. While similar, each of these techniques has its advantages and disadvantages. Other suitable techniques may be known to those of skill in the art and are incorporated herein.

To assess the impact of testing limitations and to determine levels of exposure to TBRF Borrelia, a modified Western blot procedure, the line immunoblot, was developed and employed in aspects of the invention described herein. A line immunoblot uses recombinant antigens from common strains and species of the TBRF *Borrelia* complex for serological identification and diagnosis of TBRF in serum from patients with a suspected tick-borne disease. As described elsewhere herein, the serotype makeup of TBRF *Borrelia* exposure may be more complex than has been previously acknowledged, and infection with more than one TBRF *Borrelia* species is possible.

Western blotting can involve separating proteins by electrophoresis and then transferring to nitrocellulose or other solid media (*e.g.*, polyvinylidene fluoride or PVDF-membrane and nylon membrane), and is described in more detail below. Immunoblotting can also involve applying proteins to a solid media manually or by machine. Preferably, the

proteins are applied in straight lines or spots and dried, binding them to the solid support medium, *e.g.*, nitrocellulose. The proteins used in an immunoblot can be isolated from biological samples or produced by recombinant technology, as is well known by those of ordinary skill in the art. The bound proteins are then exposed to a sample or samples suspected of having antibodies specific for the target proteins. With this procedure, a known antibody can be used to determine if a protein is present in a sample, such as when the proteins of lysed cells are separated by electrophoresis and transferred to the solid medium. Western blotting allows for the identification of proteins by size as well as by specificity for a specific antibody.

Similarly, with a procedure called immunoblotting, known proteins can be bound to the solid medium and samples, such as samples from subjects suspected of having an infection, can be tested for the presence of specific antibodies in the sample by contacting the bound protein with the sample. An antibody that binds the target protein is usually referred to as the primary antibody. A secondary antibody, specific for conserved regions of the primary antibody (for example, a rabbit- anti-human IgG antibody may be used to detect primary human antibodies) is used to detect any bound primary antibodies. The secondary antibody is usually labeled with a detectable moiety for visualization. Non-limiting examples of suitable labels include, for example, chromophores such as biotin, radioactive moieties and enzymes such as alkaline phosphatase, etc. The use of these and other materials for the visualization of antibodies are well known to one of ordinary skill in the art.

The Enzyme-Linked ImmunoSpot (ELISPOT) method can detect human T-cells that respond to Lyme-specific antigens *in vitro*. In an ELISPOT assay, the surfaces of PVDF membrane in a 96- well microtiter plate are coated with capture antibody that binds, for example, anti-Interferon gamma (IFNγ) or other cytokine-specific antibody. During the cell incubation and stimulation step, the T-cells isolated from patient whole blood are seeded into the wells of the plate along with aforementioned sequence(s), and form substantially a monolayer on the membrane surface of the well. Upon stimulation of any antigen-specific cells with one or more of the sequences of the present invention they are activated and they release the IFNγ, which is captured directly on the membrane surface by the immobilized antibody. The IFNγ is thus "captured" in the area directly surrounding the secreting cell, before it has a chance to diffuse into the culture media, or to be degraded by proteases and bound by receptors on bystander cells. Subsequent detection steps visualize the immobilized IFNγ as an ImmunoSpot; essentially the secretory footprint of the activated cell.

For a specific example of an ELISPOT test, each well of the plate is coated with a purified cytokine-specific antibody specific for the test or cell being detected. T-cells are isolated from a subject (for example, a subject suspected of having Lyme Disease or TBRF) and cultured in each well and stimulated with recombinant antigens of one or more sequences of the present invention. Lyme-positive patient cells secrete cytokine in response to stimuli, which is captured by the antibody coated in the well and further detected by ELISA.

ELISA assays may also be used to detect antigens. ELISA assays permit quantification of a specific protein in a mix of proteins (for example, a lysate) or may be used to determine if a peptide is present in a sample. Likewise, ELISA assays may be used to determine if a specific antibody is present by using a specific antigen as a target. As used with the present invention, target amino acid sequence(s) are attached to a surface. Then, if present in the sample being tested, the reactive antibody can bind to the antigen. A secondary antibody linked to an enzyme is added, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

Lateral flow assays, also referred to by a variety of other names that include but are not limited to lateral flow tests, lateral flow devices, lateral flow immunoassays, lateral flow immunochromatographic assays, and rapid tests, are simple, versatile, paper-based platforms for detecting and/or quantifying the presence of one or more analytes, such as an antigen, in a mixture, such as a liquid sample. Lateral flow assays may be qualitative or quantitative. In a lateral flow assay, a sample containing one or more analytes of interest is applied to an adsorbent sample pad and is drawn via capillary action through various zones of polymeric test strips to which are attached molecules that can interact with the analyte(s). The sample migrates to the conjugate release pad, which contains molecules that specifically bind to the analyte(s) of interest and are conjugated to fluorescent, colored, or otherwise detectable particles. Finally, the sample, including the bound analyte(s) migrates into the detection zone. Within the porous membrane of the detection zone are biological components such as antibodies or antigens, that are immobilized in lines and that will react with the detectable particles. Lateral flow assays typically have a control line for confirming sample flow through the strip and one or more test lines for detecting the presence of the analyte(s) of interest. The results may be read by eye or with a machine capable of reading and interpreting the results. A lateral flow assay may be designed as a direct or “sandwich” assay, in which the presence of a colored line at the test line position indicates a positive test,

or as a competitive assay, in which the absence of a colored line indicates a positive test. Direct and competitive assays may be multiplexed.

In one aspect of the method of the present invention, a positive result is indicated when two or more of the labeled and/or bound amino acid sequences of the present invention are mixed with the biological sample and when at least two amino acid sequences are detected. In another aspect of the invention, a positive result is indicated when at least one of the labeled and/or bound amino acid sequences of the present invention are mixed with the biological sample and when at least one amino acid sequence is detected.

In the method of the present invention, any primary antibody bound to a peptide encoded by an amino acid sequence of the present invention may be detected with anti-human antibodies, such as IgG or IgM, used as the secondary antibody conjugated to a detectable moiety. As described elsewhere herein, the detectable moiety may be selected from the group consisting of chromophores, radioactivity moieties and enzymes or other detectable moiety known to one of ordinary skill in the art. In one embodiment, the detectable moiety comprises alkaline phosphatase. In another embodiment the detectable moiety comprises biotin.

In another aspect of the invention, a method is provided for detecting and distinguishing various species of *Borrelia* in a sample. The sample may be from a subject suspected of having Lyme disease. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. Amino acids may be labeled to confirm their presence if positive results are not obtained in the assay.

In some embodiments of the invention, a sample may be considered positive in a multi-species panel assay for TBRF *Borrelia* if at least one amino acid sequence is detected. In some embodiments of the invention, a sample may be considered positive in a multi-species panel assay for TBRF *Borrelia* if at least two amino acid sequences are detected. In some embodiments of the invention, a sample may be considered positive for a specific species of TBRF *Borrelia* if at least two amino acid sequences identified with a species are detected.

Equivalents

Although several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other

means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto; the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one." The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified, unless clearly indicated to the contrary.

All references, patents and patent applications and publications that are cited or referred to in this application are incorporated by reference in their entirety herein.

EXAMPLES

Example 1. TBRF ImmunoBlot antigen inclusivity study

TBRF ImmunoBlot IgG and IgM tests, qualitative immunoblot assays, were designed and performed to detect IgG and IgM antibodies directed against TBRF *Borrelia* species in

serum samples suspected of having Relapsing Fever or Lyme-like symptoms. Recombinant *TBRF Borrelia* antigens were sprayed as straight lines onto nitrocellulose strips, which were then used in the TBRF ImmunoBlot Test. Experiments were undertaken to determine the specificity of the TBRF ImmunoBlot Test.

Methods

Antigen preparation

Recombinant target proteins were obtained by cloning hybrid gene constructs or portions of genes into pET vectors, expressing the gene products in *Escherichia coli* (GenScript, Piscataway, NJ), then isolating the proteins to > 90% purity, as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99; Shah et al., *Healthcare* (2019) 7:121]. Recombinant proteins from several TBRF *Borrelia* species (*B. hermsii*, *B. miyamotoi*, *B. turicatae* and *B. turcica*) were derived for the detection of four target antigens: BipA, GlpQ, BpcA/fHbp and P41/FlaB, as previously described [Shah et al., *Healthcare* (2019) 7:121].

Preparation of antigen strips

Antigen strips for TBRF immunoblots were prepared as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99; Shah et al., *Healthcare* (2019) 7:121]. Purified proteins and control proteins were diluted (7–19 ng protein/line) and sprayed in straight lines on nitrocellulose sheets (Cytiva, Marlborough, MA) using a BioDot liquid dispenser (BioDot, Irvine, CA). The sheets were then blocked with 5% non-fat dry milk and sliced into 3 mm wide strips.

Detection of antibody reactivity

Serological immunoblot testing was performed at IGeneX Reference Laboratory, a high-complexity testing facility with Clinical Laboratory Improvement Amendments (CLIA) certification.

Reactivity between TBRF *Borrelia*-specific antibodies from test sera and TBRF *Borrelia* antigens on immunoblots was detected as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99; Shah et al., *Healthcare* (2019) 7:121]. Strips were labeled, soaked in diluent (100 mM Tris, 0.9% NaCl, 0.1% Tween-20, and 1% non-fat dry milk) for 5 minutes (min) in a trough; then, a 10 µL aliquot of either test serum or control serum was added to the strip. Strips with sera were incubated at room temperature for one hour, washed three times with wash buffer (KPL, Gaithersburg, MD) at room temperature, and the

final wash solution was then aspirated. To detect IgG and IgM reactivity, strips were incubated with alkaline phosphatase-conjugated goat anti-human IgG at 1:10,000 dilution or IgM at 1:6,000 dilution, respectively (KPL, Gaithersburg, MD), for one hour, and then washed three times. To visualize bands of antibody/antigen reactivity, the strips were reacted with a chromogenic substrate, 5-bromo-4-chloro-3-indolylphosphatenitro-blue tetrazolium (BCIP/NBT, KPL, Gaithersburg, MD), and the reaction was terminated by washing with distilled water after the calibration control produced a visible band at 39 kDa. Bands demonstrating an intensity lower than that of the calibration control were reported as negative. Human sera from patients with confirmed *Borrelia* infection were used as positive controls and sera from uninfected persons were used as negative controls. All immunoblot testing of patient samples was performed with simultaneous testing of positive and negative control serum samples.

Scoring of Immunoblots

For TBRF *Borrelia* ImmunoBlots, detection of either IgG or IgM antibodies against P41/FlaB (41kDa), as well as any two out of the four antigens BipA (75kDa), GlpQ, BpcA/fHbp (21-23kDa) gave the best specificity for TBRF species.

Detection of either IgG or IgM antibodies to two or more proteins within each antigen type was regarded as a positive reaction for that antigen type. Applying the same criteria for reactivity of either IgM or IgG antibodies to the four TBRF *Borrelia* antigens led to optimum sensitivity of detection, as previously described [Shah et al., *Healthcare* (2019) 7:121].

Results

As shown in Fig. 1, antibodies to TBRF *Borrelia* species *B. turicica*, *B. miyamotoi*, *B. hermsii*, *B. parkeri*, and *B. turicatae* species were detected by the TBRF ImmunoBlot strip prepared from recombinant antigens from all five species, indicating that TBRF ImmunoBlots detected TBRF *Borrelia* group antibodies.

Example 2. TBRF ImmunoBlot Clinical Study of Patients with Suspected Tick-borne Disease

A study of patients who met the case definition of Chronic Lyme Disease (CLD) was performed to assess the impact of testing limitations and to determine levels of exposure to TBRF *Borrelia*. The results also revealed that all had exposure to *B. burgdorferi* species.

Methods

Methods used were as described in Example 1 and elsewhere herein.

Antigen preparation and preparation of antigen strips

Additional recombinant proteins from several TBRF *Borrelia* species (*B. hermsii*, *B. miyamotoi*, *B. turicatae*, *B. parkeri*, and *B. duttonii*) were derived as described elsewhere herein for the detection of target antigens BipA, GlpQ, BpcA/fHbp, P41/FlaB, and Vlp7. These recombinant proteins, comprising the amino acid sequences of SEQ ID NOs: 1-11, were included on TBRF ImmunoBlot strips.

Clinical testing

A total of 265 patient serum samples were tested by TBRF ImmunoBlots IgM and IgG, including 90 patients with CLD symptoms and 212 patient serum samples that were negative for TBRF.

Chronic Lyme Disease patient cohort (n=90)

A patient cohort of 90 patients with CLD was recruited from a medical practice located in San Francisco, CA, specializing in the diagnosis and treatment of tick-borne diseases. The Western Institutional Review Board (WIRB), Puyallup, WA approved the anonymous retrospective data collection protocol and consent form. Patients of either sex qualified for the study provided they were at least 18 years of age, had a medical history of musculoskeletal, neuropsychiatric and/or cardiac symptoms consistent with LD, and gave written informed consent for data collection. Subjects were included in the study if they met the case definition of untreated or previously treated CLD with symptoms lasting more than six months, as described in detail elsewhere [Cameron et al., *Expert Rev Anti Infect Ther*. (2014) 12:1103–1135; Stricker et al., *Am J Infect Dis* (2018) 14:1-44]. Patients were not required to have had a documented tick bite or erythema migrans rash for participation in the study because serological testing was used to detect exposure rather than active infection. De-identified patient samples were coded according to the patient's place of residence. Blood was drawn and serum was separated at independent laboratories including BioReference®, LabCorp®, and AnyLabTestNow®, and serum samples were transported to the reference laboratory for immunoblot testing.

Control Patients (n=175)

A total of 175 human sera expected to be negative for *B. burgdorferi* species and TBRF *Borrelia* species were obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA), College of American Pathologists, New York State Department of Health, New York Biologics (Southampton, NY, USA) and IGeneX Reference Laboratory (Milpitas, CA, USA). The IGeneX samples were leftover sera received for routine testing for tick-borne diseases that would otherwise have been discarded. ImmunoBlot IgM and IgG testing of patient sera and control sera was performed by laboratory personnel in a blinded fashion in the same manner as testing of clinical samples from TBRF patients. Results are shown in Tables 1-2 and Figs. 2-3.

Results

Immunoblot reactivity for TBRF *Borrelia* in representative patient serum samples is shown in Fig. 2, demonstrating that TBRF ImmunoBlots successfully detect TBRF *Borrelia* group antibodies in serum samples.

The results obtained with the 175 control sera that were expected to be negative for *B. burgdorferi* species and RFB yielded a false positive rate of 2.3% (4/175 samples) for the *B. burgdorferi* species immunoblot and 2.9% (5/175 samples) for the RFB immunoblot (Table 1). False positive tests for RFB were seen with an allergy patient serum (one control), multiple sclerosis (one control), viral infection (one control), and syphilis (two controls).

Using the TBRF ImmunoBlot test described in Example 1 and including SEQ ID NOs: 1–11, the study of patients who met the CLD case definition revealed that all had exposure to *B. burgdorferi* species, and 62% had exposure to TBRF species. In the CLD cohort (Table 2), ImmunoBlot testing revealed that out of the 90 subjects with suspected LD, a total of 48 patients (53%) were seropositive for TBRF alone, and 8 patients (9%) had mixed infection with TBRF *Borrelia* species and *B. burgdorferi* species. Fig. 2 shows TBRF ImmunoBlots of representative patient serum samples. Positive immunoblots were further characterized for the presence of antibodies to one or more RFB species, and instances of infection with more than one RFB species were identified (Table 2). Immunoblot testing of control sera demonstrated excellent specificity of 97.1% for the TBRF assay (Table 2). As shown in Fig. 3, various combinations of SEQ ID NOs: 1–11 were identified in patient serum samples, demonstrating that antigens from multiple species are required for an inclusive test.

These results illustrated how detection of antibodies to TBRF Borrelia species identified patients with Chronic Lyme Disease symptoms that would otherwise be missed by

only testing for *Borrelia burgdorferi* antigens. These results also confirmed that the serotype makeup of TBRF *Borrelia* exposure is complex, thereby showing the power and importance of inclusivity in TBRF testing, that is, simultaneous testing for antigens from multiple TBRF species.

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Table 1. Reference human sera for determining specificity of *B. Burgdorferi* species and RFB species immunoblots

Source	Characteristic	Total No. of Sera	BbsI Immunoblots (+)	RFB Immunoblots (+)
CDC Reference Set (n = 25)	Fibromyalgia	2	0	0
	Healthy endemic	7	1	0
	Healthy non-endemic	6	0	0
	Mononucleosis	2	0	0
	Multiple sclerosis	2	0	1
	Rheumatoid arthritis	2	0	0
	Severe Periodontitis	2	0	0
	Syphilis	2	0	1
CAP and NYSHD (n = 42) Autoimmunity and Allergy	**Autoimmune**			
	ANA (+)	3	0	0
	ANA (−)	2	0	0
	DNA (+)	1	0	0
	Rheumatoid factor (+)	9	0	0
	Rheumatoid factor (−)	8	0	0
	Allergy (n = 19)			
	IgG (+)	13	0	1
	Spec. IgE (+)	4	0	0
	Spec. IgE (−)	2	0	0
NYB (n = 21) Viruses and RPR (+)	Epstein–Barr virus (EBV)	4	1	0
	Human immunodeficiency virus (HIV)	4	0	0
	Cytomegalovirus (CMV)	5	0	1
	Hepatitis C virus (HCV)	0	0	0
	RPR (+)	8	2	1
IGeneX (n = 87)	*Bartonella henselae* infection	7	0	0
	Human granulocytic anaplasmosis	16	0	0
	Babesia microti infection	14	0	0
	Babesia duncani infection	41	0	0
	Human monocytic ehrlichiosis	5	0	0
	Negative controls	4	0	0
False Positives		**175**	4	5
Specificity			97.7%	97.1%

ANA—anti-nuclear antibodies; CAP—College of American Pathologists; CDC—Center for Disease Control, NYB—New York Biologics, Southampton, NY; NYSH—New York State Department of Health; RF—rheumatoid factor; RPR—rapid plasma regain test for syphilis.

Table 2. Summary of seroreactivity for subjects in Group 1 (*B. Burgdorferi* species), Group 2 (RFB species), or both

Immuno Blot	Total
Group 1 Bbsl Positive	**42**
Group 2 RFB Positive	**56**
Dual Group 1 and 2 Positive	**8**
Group 2. RFB Positive Samples	**48 (53%)**
RFB alone	25
B. hermsii alone	7
B. miyamotoi alone	4
B. turicatae alone	8
B. turcica alone	2
B. hermsii + B. turcica	1
B. hermsii + B. miyamotoi	1
Dual Group 1 and 2	**8 (9%)**
Bb + RFB	2
Bb + B. hermsii	1
B. californiensis + RFB	2
B. spielmanii + RFB	1
B. afzelii/garinii + RFB	1
B. afzelii/garinii + B. turicatae	1
Total cases	**56**

Discussion for Examples 1-2

TBRF are found worldwide and are a significant cause of morbidity and mortality, particularly with respect to unexplained fever cases. Advances in molecular testing have led to widespread identification of *Borrelia* species in human specimens, thus challenging prevailing thought regarding geographic distribution and prevalence of RFB infection. Although RFB is a growing concern in the Western United States, Central America, and South America, TBRF is not reportable nationally in the United States, and there is no standard case definition. In 2011, TBRF was reportable in 12 Western states, yielding 504 cases, with 70% of the cases reported in three states: California (33%), Washington (25%), and Colorado (11%). Disease-causing species in the United States include *B. miyamotoi*, *B. hermsii*, *B. lonestari*, *B. parkeri*, *B. turicatae*, and *B. mazzotii*. Most cases in the USA are caused by *B. hermsii*, transmitted by *Ornithodoros hermsi* ticks, but *B. miyamotoi*, *B.*

hermsii, and *B. parkeri* human infections were reported in California, and *B. coriaceae* was detected in ticks, although human infection was not confirmed [Fesler et al. Healthcare 8(2): 97-112 (2020)].

The genetic diversity of *Borrelia* spirochetes, as well as the symptoms of infection that are as diverse as the organisms causing them, makes it challenging to diagnose *Borrelia*-associated disease. In summary, exposure to TBRF *Borrelia* is a cause for concern, and TBRF *Borrelia* may explain Lyme Disease symptoms in patients who are seronegative for *B. burgodorferi sensu lato* species. As shown herein, some patients may demonstrate dual exposure to both *B. burgodorferi* and TBRF species, further complicating diagnosis and treatment. Immunoblot testing for RFB species using methods and compositions of the invention allows the detection of a diverse group of TBRF *Borrelia* serotypes and provides a greater understanding of human exposure to pathogenic *Borrelia*.

Equivalents

Although several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto; the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one." The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified, unless clearly indicated to the contrary.

All references, patents and patent applications and publications that are cited or referred to in this application are incorporated by reference in their entirety herein.

What is claimed is:

CLAIMS

1. A composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, wherein the one or more labeled and/or tagged and/or bound amino acid sequences comprise amino acid sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3.

2. The composition of Claim 1, wherein said amino acid sequences are bound to a substance selected from the group consisting of nitrocellulose, nylon, polyvinylidene difluoride (PVDF), magnetic beads, and agarose.

3. The composition of Claim 1, wherein each of said one or more amino acid sequences are tagged with an antibody with specificity for said amino acid sequence.

4. A method for detecting antibodies resulting from infection by one or more members of Relapsing Fever (TBRF) Borrelia genus, wherein the genus comprises *B. hermsii*, *B. turicatae*, *B. miyamotoi*, *B. duttonii*, and *B. parkeri*, if present in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences of Claim 1; and

detecting a positive immunobinding reaction which indicates the presence of TBRF specific antibodies in the sample.

5. The method of Claim 4, wherein two or more of the labeled and/or tagged and/or bound amino acid sequences are mixed with the biological sample and a sample is considered positive for TBRF if at least two amino acid sequences are detected.

6. The method of Claim 4, wherein the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG or anti-human IgM antibody linked to a detectable moiety.

7. The method of Claim 6, wherein said detectable moiety is selected from the group consisting of chromophores, radioactive moieties and enzymes.

8. The method of claim 6, wherein said detectable moiety comprises alkaline phosphatase.

9. The method of Claim 6, wherein said detectable moiety comprises biotin.

10. A vector comprising a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, and variants thereof which retain the immunological binding profile of Fig. 3, and a heterologous nucleic acid sequence.

11. The vector of claim 10, wherein the nucleic acid sequence is operatively linked to a promoter sequence.

12. The vector of claim 10 or claim 11, wherein the vector is a prokaryotic vector.

13. A cell comprising the vector of any one of claims 10-12.

14. The cell of claim 13, wherein the cell is a bacterial cell.

Fig. 1

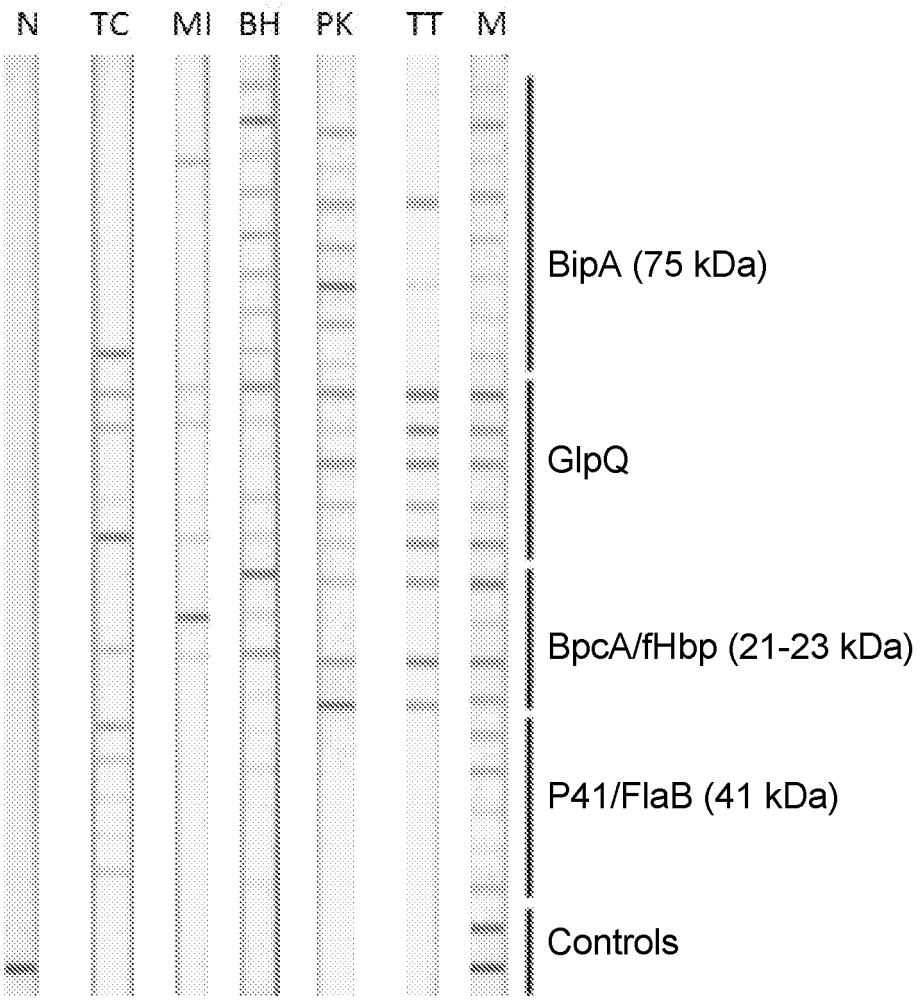

N
TC
MI
BH
PK
TT
M
BipA (75 kDa)
GlpQ
BpcA/fHbp (21-23 kDa)
P41/FlaB (41 kDa)
Controls

Fig. 2

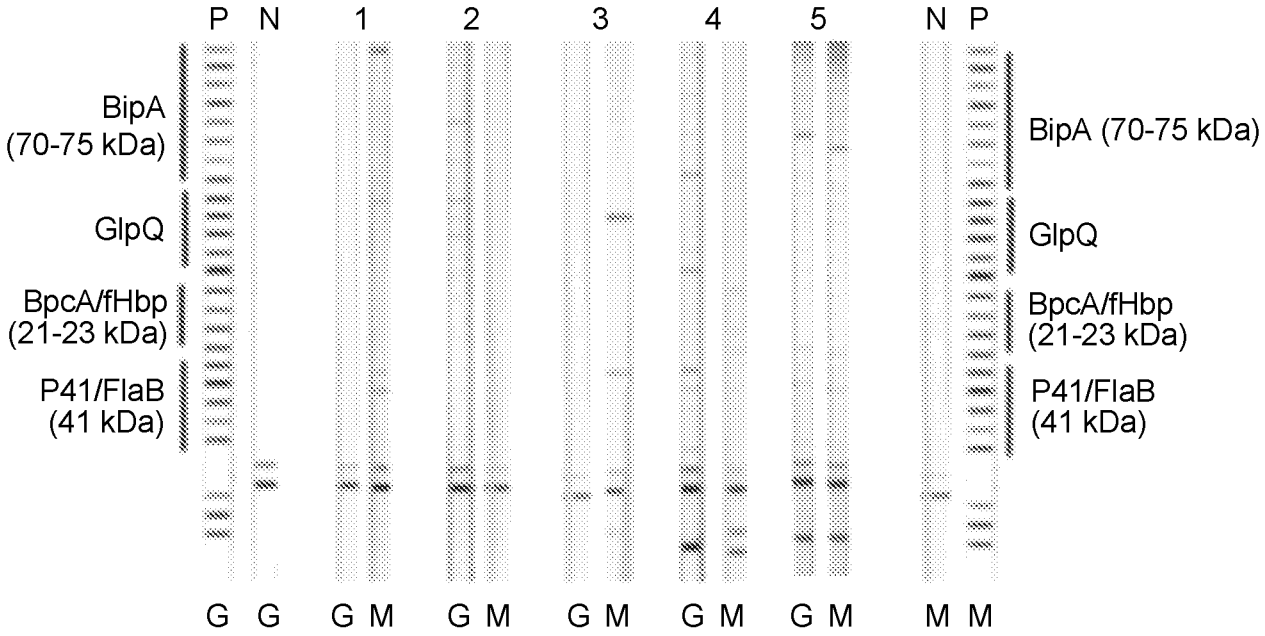

P N 1 2 3 4 5 N P
BipA (70-75 kDa)
GlpQ
BpcA/fHbp (21-23 kDa)
P41/FlaB (41 kDa)
BipA (70-75 kDa)
GlpQ
BpcA/fHbp (21-23 kDa)
P41/FlaB (41 kDa)
G G G M G M G M G M G M M M

Fig. 3

TBRF IgM and IgG Blots - Performance on Patient Serum Samples												
TBRF ImmunoBlot IgM												Result
Antigens	Bip A (70-75kDa)			GlpQ			BpcA/fHbp		P41/FlaB		Vlp7	
SEQ ID NO	2	5	6	11	7	10	3	8	4	9	1	
Sample #	Tt-2	Pk-1	Pk-2	Mia	Pk	Du	Tt	Pk	Tt	Pk	Bh	
1												Neg
2	Pos	Pos	Pos			Pos						Pos
3				I								Neg
4											Pos	Neg
5	Pos	Pos	Pos	Pos	Pos		Pos		Pos	I	Pos	Pos
6						I						Neg
7						Pos		Pos		Pos		Pos
8												Neg
9												Neg
10												Neg
TBRF ImmunoBlot IgG												Result
Antigens	Bip A (70-75kDa)			GlpQ			BpcA/fHbp		P41/FlaB		Vlp7	
SEQ ID NO	2	5	6	11	7	10	3	8	4	9	1	
Sample#	Tt-2	Pk-1	Pk-2	Mia	Pk	Du	Tt	Pk	Tt	Pk	Bh	
1									I	I		Neg
2												Neg
3		Pos										Neg
4				I							I	Neg
5	I					Pos	Pos		I	I		Pos
6												Neg
7					Pos							Neg
8	Pos						Pos					Pos
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10			Pos					I				Neg

SEQUENCE LISTING

<110> ID-FISH TECHNOLOGY, INC.
Shah, Jyotsna
Liu, Song

<120> SPECIES-SPECIFIC ANTIGEN SEQUENCES FOR TICK-BORNE RELAPSING FEVER (TBRF) AND METHODS OF USE

<130> 0153-2015WO01

<140> 17/248,236
<141> 15 January 2021

<160> 20

<170> PatentIn version 3.5

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Ile Arg Asn Ser Val Ala Ser Glu Leu Gln Lys Pro Glu Asn Ile Gly
20 25 30

Gln Arg Gly Lys Ser Val Gly Lys Glu Val Gly Lys Asp Ala Ala Ala
35 40 45

Ser Gly Glu Gly Ala Val Val Ala Val Gly Ser Lys Gln Asn Thr Leu
50 55 60

Gln Asn Ser Glu Asn Ser Ser Gln Glu Gly Ala Gly Ser Ala Leu Gln
65 70 75 80

Lys Pro Gly Asp Ser Pro Gln Lys Gly Val Ala Ser Gln Glu Gly Thr
85 90 95

Asn Gly Ala Leu Gln Gly Val Val Ala Ala Gly Gly Val Ser Val Gly

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            100                 105                 110

Gly Ser Gly Val Gly Ala Ala Ala Ser Asp Gly Asn Ser Ser Ser Ser
        115                 120                 125

Gln Glu Ala Glu Ser Val Asp Leu Lys Asn Val Leu Ala Asp Ser His
    130                 135                 140

Gly Val Gly Ala Ser Asn Leu Asn Ile Lys Ala Glu Gly Asp Ile Ser
145                 150                 155                 160

Thr Gly His Gly Thr Glu Gly Val Ile Ala Ser Gly Asp Leu Thr Asn
                165                 170                 175

Thr Ile Ile Thr Ser Gly Val Thr Ala Ala Ala Ser Pro Ala Ile Val
            180                 185                 190

Ser Gly Asp Glu Arg Gly Val Ala Ala Ile Lys Asp Ser Ile Ala Val
        195                 200                 205

Val Leu Glu Thr Gln Lys Glu Ala Gln Lys Glu Thr Glu Lys Glu Thr
    210                 215                 220

Glu Asn Val Lys Phe Ala Val Leu Gly Thr Val Lys Lys Ile Val Asp
225                 230                 235                 240

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            20                  25                  30

Leu Gln Asp Val Gly Asn Phe Val Met Asp Ala Thr Leu Ser Met Gly
        35                  40                  45

Asp Met Phe Ala Gly Ile Lys Ser Asp Ser Asp Val Ser Ser Ala Asn
    50                  55                  60

Ser Gly Met Thr Val Asn Ser Gly Met Thr Val Ser Leu Ser Ser Asn
65                  70                  75                  80

Glu Thr Gln Val Ile Gly His Leu Glu Glu Tyr Leu Lys Ser Ala Ile
                85                  90                  95

Lys Val Asn Gly Asn Glu Ser Ser Arg Gln Ser Lys Leu Glu Asn Gly
            100                 105                 110

Arg Gln Lys Phe Phe Ala Trp Leu Arg Glu Lys Asp Thr Asp Phe Ser
        115                 120                 125

Lys Arg Lys Glu Leu Val Gln Ala Met Gln Arg Val Tyr Asn Phe Ile
    130                 135                 140

Lys Glu Lys Ser Ser Asn Ser Arg Glu Leu Gln Thr Trp Val Leu Gly
145                 150                 155                 160

Val Val Gly Asp Asp Asp Thr Val Val Asp Val Asp Arg Asp Asp Glu
                165                 170                 175

Leu Asn Ser Asp Val Glu Ile Asp Phe Leu Ile Lys Lys Thr Leu Ser
            180                 185                 190

Ser Arg Asp Tyr Ser Gly Phe Ala Val Ser Leu Leu Phe Gln Ala Leu
        195                 200                 205
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Ala Asp Thr Leu Tyr Asp Ala Glu Asn Asp Arg Asp Lys Pro Glu Glu
    210                 215                 220

Gln Ile Phe Lys Asp Leu Lys Ala Val Phe Ser Asp Asn Ala Gly Glu
225                 230                 235                 240

Asp Lys Gly Phe Gly Glu Phe Lys Ser Ile Ile Glu Asp Lys Ser Gln
                245                 250                 255

Ala
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1               5                   10                  15

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            20                  25                  30

Val Asp Gly Asn Ile Asp Gln Asn Pro Gly Asn Val Asp Gly His Ile
        35                  40                  45

Val Leu Val Asp Ala Gln Gly Val Ser Val Pro Thr Val Val Glu His
    50                  55                  60

Ser Ala Val Ser Ser Ser Val Glu Arg Arg Val Val Thr Val Gly Gln
65                  70                  75                  80

Asn Val Glu Ser Arg Arg Leu Thr Lys Val Glu Arg Leu Glu Lys Tyr
                85                  90                  95

Leu Glu Ser Ala Ile Lys Ser Gly Gly Lys Leu Asn Glu Gln Gln Arg
```

```
            100                 105                 110

Arg Leu Lys Asn Gly Lys Gln Met Leu Phe Thr Trp Leu Asn Glu Asp
        115                 120                 125

Ala Asn Ala Ser Lys Arg Ala Glu Leu Glu Gln Asp Met Gln Lys Leu
    130                 135                 140

Tyr Gly Leu Ile Lys Glu Ser Ile Thr Asp Ser Ser Ser Gln Phe Ser
145                 150                 155                 160

Ser Arg Asp Gly Glu Tyr Ser Asp Glu Ala Ile Asp Gly Leu Leu Asn
                165                 170                 175

Ser Leu Phe Ser Ser Ser Phe Asp Asn Thr Phe Ala Leu Asp Leu Phe
            180                 185                 190

Phe Gln Ala Leu Leu Asn Thr Leu Cys Asp Phe Lys Lys Asp Asn Cys
        195                 200                 205

Lys Asp Glu Glu Val Ile Phe Ala Asp Ile Arg Lys Val Phe Ser Asp
    210                 215                 220

Glu Ser Asp Thr Lys Gly His Phe Gly Tyr Leu Lys Ser Lys Leu Lys
225                 230                 235                 240

Asp Glu Leu Val Ile Ser Asp Glu Gly Asp Tyr Gly Glu Asp Glu Asp
                245                 250                 255

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            260
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            20                  25                  30

Asp Gly Thr Pro Glu Gln Ile Val Ala Ser Val Gly Leu Glu Gly Glu
        35                  40                  45

Glu Ser Lys Lys Phe Glu His Leu Lys Gly Ser Leu Gly Asp Ala Ile
    50                  55                  60

Lys Val Asn Gly Lys Gly Glu Glu Lys Arg Lys Glu Asn Glu Glu Arg
65                  70                  75                  80

Gln Lys Glu Phe Phe Asp Trp Leu Asp Lys Asn Asp Pro Asp Leu Ser
                85                  90                  95

Lys Arg Lys Glu Leu Ala Glu Leu Met Lys Lys Val Tyr Gly Leu Leu
            100                 105                 110

Lys Glu His Ala Gln Asn Ser Glu Gln Ile Lys Ser Phe Val Glu Gly
        115                 120                 125

Thr Pro Lys Asp Glu Asn Val Lys Lys Ile Gly Val Thr Ser Ala Arg
    130                 135                 140

Asp Ile Lys Thr Asp Glu Gln Val Glu Ala Leu Ile Lys Ala Val Leu
145                 150                 155                 160

Gly His Ser Glu Glu Ser Gly Thr Asn Leu Ser Leu Phe Phe Gln Lys
                165                 170                 175

Leu Gly Asp Ala Phe Gly Thr Glu Asp Gly Glu Ser Gln Lys Ser Asn
            180                 185                 190

Glu Lys Ile Leu Glu Glu Leu Lys Arg Val Cys Glu Ser Ser Asp Glu
        195                 200                 205
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            20                  25                  30


Thr Gly Leu Ser Lys Leu Asp Lys Lys Glu Lys Lys Val Ser Ser Leu
        35                  40                  45


Lys Glu Thr Leu Glu Asn Ser Ser Asn Val Leu Tyr Glu Ser Ser Ser
    50                  55                  60


Pro Thr Lys Thr Arg Gln Glu Glu Phe Phe Lys Trp Leu Glu Glu Asn
65                  70                  75                  80


Asp Ser Asp Tyr Ser Lys Arg Lys Gln Leu Glu Glu Ser Met Asp Lys
                85                  90                  95


Val Phe Ser Leu Ile Lys Asp Ser Ala Ser Ser Ser Thr Glu Ile Lys
            100                 105                 110


Glu Ile Ile Ala Lys Gly Gln Ser Asp Ala Gly Ile Ile Lys Ala Gly
        115                 120                 125


Ile Lys Thr Ala Asp Asp Ile Lys Thr Asp Glu Gln Val Asp Ala Leu
    130                 135                 140


Val Lys Phe Val Thr Gly Thr Gly Asp Asp Leu Asp Leu Glu Ser Gly
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145 150 155 160

Ser Ser Ser Ile Lys Ala Phe Phe Gly Thr Leu Ala Glu Val Phe Asp
165 170 175

Asp Asp Leu Asn Asp Val Met Thr Asp Lys Lys Gly Gln Lys Arg Gly
180 185 190

His Asp Lys Val Phe Glu Asp Leu Lys Lys Val Phe Ser Glu Asp Ser
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20 25 30

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35 40 45

Asn Leu Arg Val Ala Gly Gly Gln Arg Gln Gly Ile Ala Asp Asp Gly
50 55 60

Ile Gly Val Ala Gly Val Arg Glu Ala Gly Gly Val Leu Lys Asp Ala
65 70 75 80

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Gly Asn Ala Val Gln Arg Asp Ile Asn Gly Ser Gly Glu Gly Ile Lys
                85                  90                  95

Asn Asp Val Ile Gln Asn Pro Glu Gly Val Gly Val Gln Val Ala Val
            100                 105                 110

Gly Ser Ala Asp Thr Gly Ala Asp Ser Gly Gln Glu Ala Gly Lys Val
        115                 120                 125

Phe Gln Asn Leu Gly Asp Thr Gly Thr Gln Ser Ile Gln Arg Val Val
    130                 135                 140

Ser Ser Ser Asp Leu Asn Ser Asp Leu Gly Val Gly Ser Lys Asp Gly
145                 150                 155                 160

Ile Ser Thr Asn Gly Met Ser Thr Asn His Val Thr Glu Asn Glu Asn
                165                 170                 175

Ser Ile Asn Ser Ile Thr Ser Thr Ser Ser Gly Leu Asn Thr Ala Leu
            180                 185                 190

Gln Met Ala Gly Thr Ser Thr Arg Thr Ser Gly Tyr Glu Gly Glu Ile
        195                 200                 205

Thr Thr Asn Thr Gln Asp Arg Thr Phe Val Glu Thr Gly Thr Gln Asp
    210                 215                 220

Ser Lys Ala Gln Tyr Ser Asp Phe Ser Asp Gln Asp Ile Arg Asp Lys
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Val Leu Gly Ser Val Val Gly Gly Val Val
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            20                  25                  30

Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ala Lys Ala Tyr Ala Tyr
        35                  40                  45

Ala Leu Gly Ala Asp Tyr Leu Glu Gln Asp Ile Val Leu Thr Lys Asp
    50                  55                  60

Asn Ile Pro Val Ile Met His Asp Pro Glu Leu Asp Thr Thr Thr Asn
65                  70                  75                  80

Val Ala Lys Leu Phe Pro Glu Arg Ala Arg Glu Asn Gly Arg Tyr Tyr
                85                  90                  95

Ser Val Asp Phe Thr Leu Asp Glu Leu Lys Ser Leu Ser Leu Ser Glu
            100                 105                 110

Arg Phe Asp Pro Ala Ser Arg Asn Pro Ile Tyr Pro Asn Arg Phe Pro
        115                 120                 125

Leu Asn Glu His Asp Phe Lys Ile Pro Thr Leu Glu Glu Glu Ile Gln
    130                 135                 140

Phe Ile Gln Gly Leu Asn Lys Ser Thr Gly Arg Asn Val Gly Ile Tyr
145                 150                 155                 160

Pro Glu Ile Lys Lys Pro Leu Trp His Lys Gln Gln Gly Lys Asp Ile
                165                 170                 175

Ser Lys Ile Val Ile Glu Ile Leu Asn Lys Tyr Gly Tyr Lys Ser Lys
            180                 185                 190

Glu Asp Lys Ile Tyr Leu Gln Thr Phe Asp Phe Asp Glu Leu Lys Arg
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```
        195                 200                 205

Ile Arg Glu Glu Leu Gly Tyr Gln Gly Lys Leu Ile Met Leu Val Gly
    210                 215                 220

Glu Asn Asp Trp Asp Glu Ala Pro Thr Asp Tyr Glu Tyr Ile Lys Ser
225                 230                 235                 240

Glu Glu Gly Met Ala Glu Val Ala Lys Tyr Ser Asp Gly Ile Gly Pro
                245                 250                 255

Trp Ile Pro Gln Ile Ile Ile Glu Gly Lys Ile Thr Asp Leu Thr Ser
            260                 265                 270

Leu Ala His Lys His Asn Met Glu Val His Pro Tyr Thr Phe Arg Ile
        275                 280                 285

Asp Ala Leu Pro Ser Tyr Val Lys Asp Ala Asp Glu Leu Leu Asp Leu
    290                 295                 300

Leu Phe Asn Lys Ala Lys Val Asp Gly Ile Phe Thr Asp Phe Thr Asp
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Thr Val Val Asn Phe Ile Thr Lys Ile Lys Pro Lys Gly Glu
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        35                  40                  45

Leu Gly Ala Asp Tyr Leu Glu Gln Asp Ile Val Leu Thr Lys Asp Asn
    50                  55                  60

Ile Pro Val Ile Met His Asp Pro Glu Ile Asp Thr Thr Thr Asn Val
65                  70                  75                  80

Ala Gln Leu Phe Pro Asn Arg Ala Arg Glu Asn Gly Arg Tyr Tyr Ala
                85                  90                  95

Thr Asp Phe Thr Leu Thr Glu Leu Lys Ser Leu Asn Leu Ser Glu Arg
            100                 105                 110

Phe Asp Pro Glu Asn Lys Lys Pro Ile Tyr Pro Asn Arg Phe Pro Leu
        115                 120                 125

Asn Glu Tyr Asn Phe Lys Ile Pro Thr Leu Glu Glu Glu Ile Gln Phe
    130                 135                 140

Ile Gln Gly Leu Asn Lys Ser Thr Gly Lys Asn Val Gly Ile Tyr Pro
145                 150                 155                 160

Glu Ile Lys Lys Pro Phe Trp His Lys Gln Gln Gly Lys Asp Ile Ser
                165                 170                 175

Lys Ile Val Ile Glu Ile Leu Asn Lys Tyr Gly Tyr Lys Ser Lys Glu
            180                 185                 190

Asp Lys Ile Tyr Leu Gln Thr Phe Asp Phe Asp Glu Leu Lys Arg Ile
        195                 200                 205

Arg Lys Glu Leu Gly Tyr Gln Gly Lys Leu Ile Met Leu Val Gly Glu
    210                 215                 220

Asn Asp Trp Asn Glu Ala Pro Thr Asp Tyr Glu Tyr Ile Lys Ser Glu
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Gly Tyr Leu Pro Glu His Thr Leu Glu Ala Lys Ala Phe Ala Tyr Ala
        35                  40                  45


Leu Gly Ala His Tyr Leu Glu Gln Asp Ile Val Leu Thr Lys Asp Asp
    50                  55                  60


Ile Pro Ile Ile Met His Asp Pro Glu Ile Asp Thr Thr Thr Asn Val
65                  70                  75                  80


Ala Glu Ile Phe Pro Glu Arg Ala Arg Lys Asp Gly Arg Tyr Tyr Ser
                85                  90                  95


Val Asp Phe Thr Leu Arg Glu Leu Lys Ser Leu Lys Leu Ser Glu Arg
            100                 105                 110


Phe Asp Pro Lys Thr Gly Lys Pro Ile Tyr Pro Asn Arg Phe Pro Leu
        115                 120                 125


Asn Glu Tyr Asn Phe Lys Ile Pro Thr Leu Glu Glu Glu Ile Gln Phe
    130                 135                 140


Ile Gln Gly Leu Asn Lys Ser Thr Gly Arg Asn Val Gly Ile Tyr Pro
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145                 150                 155                 160

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                165                 170                 175

Lys Ile Val Ile Glu Met Leu Asn Lys Tyr Gly Tyr Lys Ser Lys Glu
            180                 185                 190

Asp Lys Ile Tyr Leu Gln Ile Phe Asp Phe Asp Glu Leu Lys Arg Ile
        195                 200                 205

Arg Glu Glu Leu Gly Tyr Lys Gly Lys Leu Val Met Leu Ile Gly Glu
    210                 215                 220

Asn Asp Trp Asn Glu Ala Pro Thr Asp Tyr Glu Tyr Ile Lys Ser Glu
225                 230                 235                 240

Glu Gly Ile Ala Glu Val Ala Lys Tyr Ser Asp Gly Ile Gly Pro Trp
                245                 250                 255

Ile Pro Gln Val Ile Ile Asp Gly Lys Val Thr Gly Leu Thr Ser Leu
            260                 265                 270

Ala His Lys His Lys Met Glu Val His Pro Tyr Thr Met Arg Ile Asp
        275                 280                 285

Ala Leu Pro Ser Tyr Val Lys Asp Ala Asn Glu Leu Leu Asn Leu Leu
    290                 295                 300

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Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ala Lys Ala Phe Ala
        35                  40                  45


Tyr Ala Leu Gly Ala Asp Tyr Leu Glu Gln Asp Ile Val Leu Thr Lys
    50                  55                  60


Asp Asn Val Pro Ile Ile Met His Asp Pro Glu Leu Asp Thr Thr Thr
65                  70                  75                  80


Asn Val Ala Lys Leu Phe Pro Glu Arg Ala Arg Glu Asn Gly Arg Tyr
                85                  90                  95


Tyr Ser Val Asp Phe Thr Leu Asp Glu Leu Lys Ser Leu Ser Leu Ser
            100                 105                 110


Glu Arg Phe Asp Leu Glu Thr Arg Lys Pro Ile Tyr Pro Asn Arg Phe
        115                 120                 125


Pro Leu Asn Glu Tyr Asn Val Lys Ile Pro Thr Leu Glu Glu Glu Ile
    130                 135                 140


Gln Phe Ile Gln Gly Leu Asn Lys Ser Thr Gly Arg Asn Val Gly Ile
145                 150                 155                 160


Tyr Pro Glu Ile Lys Lys Pro Leu Trp His Lys Gln Gln Gly Lys Asp
                165                 170                 175


Ile Ser Lys Ile Val Ile Glu Ile Leu Asn Lys Tyr Gly Tyr Lys Ser
            180                 185                 190
```

```
Lys Glu Asp Lys Ile Tyr Leu Gln Thr Phe Asp Phe Asp Glu Leu Lys
        195                 200                 205


Arg Ile Arg Glu Glu Leu Gly Tyr Gln Gly Lys Leu Ile Met Leu Val
    210                 215                 220


Gly Glu Asn Asp Trp Asp Glu Ala Pro Thr Asp Tyr Glu Tyr Ile Lys
225                 230                 235                 240


Ser Gln Glu Gly Met Thr Glu Val Ala Lys Tyr Ala Asp Gly Ile Gly
                245                 250                 255


Pro Trp Ile Pro Gln Ile Ile Ile Asp Gly Lys Ile Thr Asp Leu Thr
            260                 265                 270


Ser Leu Ala His Lys Tyr Asn Met Glu Val His Ala Tyr Thr Phe Arg
        275                 280                 285


Ile Asp Ser Leu Pro Ser Tyr Val Lys Asp Ala Asn Glu Leu Leu Asp
    290                 295                 300


Leu Leu Phe Asn Lys Ala Lys Ile Asp Gly Leu Phe Thr Asp Phe Thr
305                 310                 315                 320


Asp Thr Val Val Lys Phe Val Lys Gln
                325


<210>  11
<211>  171
<212>  PRT
<213>  Borrelia hermsii

<400>  11

Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Gly Ser Ser Asp
1               5                   10                  15


Ala Asn Leu Leu Lys Thr Leu Asp Asn Asn Gln Lys Gln Ala Leu Ile
```

```
            20                  25                  30

Tyr Phe Lys Asp Thr Leu Gln Asp Lys Lys Tyr Leu Asn Asp Leu Thr
        35                  40                  45

Ala Ser Gln Lys Asn Phe Leu Asp Asp Leu Glu Lys Asn Lys Lys Asp
    50                  55                  60

Pro Gly Leu Gln Asp Lys Leu Lys Lys Thr Leu Ser Ser Glu Tyr Asp
65                  70                  75                  80

Gly Ser Gln Phe Asn Lys Leu Leu Asn Glu Leu Gly Asn Ala Lys Val
                85                  90                  95

Lys Gln Phe Leu Gln Gln Leu His Ile Met Leu Gln Ser Ile Lys Asp
            100                 105                 110

Gly Thr Leu Thr Ser Phe Ser Tyr Ala Asn Phe Lys Asp Leu Gln Thr
        115                 120                 125

Leu Glu Gln Lys Lys Glu Arg Ala Leu Gln Tyr Ile Asn Gly Arg Leu
    130                 135                 140

Tyr Val Glu Tyr Tyr Phe Tyr Ile Asn Gly Ile Ser Asn Ala Asp Asn
145                 150                 155                 160

Phe Phe Glu Ser Val Met Gln Leu Leu Glu Thr
                165                 170


<210>  12
<211>  169
<212>  PRT
<213>  Borrelia miyamotoi

<400>  12

Met Leu Asp His Asn Leu Gln Pro Asn Lys Ile Asn Asn Ile Ile Ser
1               5                   10                  15
```

```
Ser Leu Asp Ser Asn Gln Lys Gln Ala Leu Ile Phe Phe Lys Asn Leu
            20                  25                  30

Val Lys Asn Lys Gln Tyr Ser Lys Asp Leu Glu Gln Ala Ser Lys Ser
        35                  40                  45

Tyr Leu Glu Asn Leu Lys Glu Lys Asn Asn Gln Asn Leu Asn Leu Gln
    50                  55                  60

Asn Lys Leu Asn Gln Gly Leu Asn Cys Asp Tyr Asp Asp Ser Lys Ile
65                  70                  75                  80

Glu Lys Leu Phe Asp Gln Leu Gly Asn Asp Lys Met Lys Lys Phe Leu
                85                  90                  95

Gln Gln Leu His Leu Met Leu Lys Ser Ile Asn Asp Gly Thr Leu Ile
            100                 105                 110

Ser Phe Ser Ser Ser Asn Phe Arg Asp Thr Thr Thr Leu Ser Gln Lys
        115                 120                 125

Lys Glu Lys Ala Leu Glu Tyr Ile Lys Ser Gln Leu Tyr Ile Glu Phe
    130                 135                 140

Tyr Phe His Ser Asn Asp Ile Ser Asp Thr Glu Phe Phe Phe Gln Arg
145                 150                 155                 160

Thr Ile Ala Leu Leu Glu Thr Gln Asn
                165
```

<210> 13
<211> 322
<212> PRT
<213> Borrelia hermsii

<400> 13

```
Met Arg Asn Asn Ser Ile Asn Ala Thr Asn Leu Ser Lys Thr Gln Glu
1               5                   10                  15
```

```
Lys Leu Ser Ser Gly His Arg Ile Asn Arg Ala Ser Asp Asp Ala Ala
            20                  25                  30

Gly Met Gly Val Ala Gly Lys Ile Asn Ala Gln Ile Arg Gly Leu Ser
        35                  40                  45

Gln Ala Ser Arg Asn Thr Ser Lys Ala Ile Asn Phe Ile Gln Thr Thr
    50                  55                  60

Glu Gly Asn Leu Asn Glu Val Glu Arg Val Leu Val Arg Met Lys Glu
65                  70                  75                  80

Leu Ala Val Gln Ser Gly Asn Gly Thr Tyr Ser Asp Ala Asp Arg Gly
                85                  90                  95

Ser Ile Gln Ile Glu Ile Glu Gln Leu Thr Asp Glu Ile Asn Arg Ile
            100                 105                 110

Ala Asp Gln Ala Gln Tyr Asn Gln Met His Met Leu Ser Asn Lys Ser
        115                 120                 125

Ala Ala Gln Asn Val Lys Thr Ala Glu Glu Leu Gly Met Gln Pro Ala
    130                 135                 140

Lys Ile Asn Thr Pro Ala Ser Leu Ala Gly Ser Gln Ala Ser Trp Thr
145                 150                 155                 160

Leu Arg Val His Val Gly Ala Asn Gln Asp Glu Ala Ile Ala Val Asn
                165                 170                 175

Ile Tyr Ala Ser Asn Val Ala Asn Leu Phe Ala Gly Glu Gly Ala Gln
            180                 185                 190

Ala Ala Pro Val Gln Glu Ile Gly Gln Gln Glu Glu Gly Gln Ala Ala
        195                 200                 205

Pro Ala Pro Ala Ala Ala Pro Ala Gln Gly Gly Val Asn Ser Pro Ile
```

```
    210                 215                 220

Asn Val Thr Thr Ala Val Asp Ala Asn Met Ser Leu Ala Lys Ile Glu
225                 230                 235                 240

Gly Ala Ile Arg Met Val Ser Asp Gln Arg Ala Asn Leu Gly Ala Phe
                245                 250                 255

Gln Asn Arg Leu Glu Ser Ile Lys Asp Ser Thr Glu Tyr Ala Ile Glu
            260                 265                 270

Asn Leu Lys Ala Ser Tyr Ala Gln Ile Lys Asp Ala Thr Met Thr Asp
        275                 280                 285

Glu Val Val Ala Ser Thr Thr His Ser Ile Leu Thr Gln Ser Ala Met
    290                 295                 300

Ala Met Ile Ala Gln Ala Asn Gln Val Pro Gln Tyr Val Leu Ser Leu
305                 310                 315                 320

Leu Arg
```

<210> 14
<211> 322
<212> PRT
<213> Borrelia miyamotoi

<400> 14

```
Met Arg Asn Asn Gly Ile Asn Ala Ala Asn Leu Ser Lys Thr Gln Glu
1               5                   10                  15

Lys Leu Ser Ser Gly Tyr Arg Ile Asn Arg Ala Ser Asp Asp Ala Ala
            20                  25                  30

Gly Met Gly Val Ala Gly Lys Leu Asn Ser Gln Ile Arg Gly Leu Ser
        35                  40                  45
```

```
Gln Ala Ser Arg Asn Thr Ser Lys Ala Ile Asn Phe Ile Gln Thr Thr
    50                  55                  60

Glu Gly Asn Leu Asn Glu Val Glu Lys Val Leu Val Arg Met Lys Glu
65                  70                  75                  80

Leu Ala Val Gln Ser Gly Asn Gly Thr Tyr Ser Asp Ser Asp Arg Gly
                85                  90                  95

Ser Ile Gln Ile Glu Ile Glu Gln Leu Thr Asp Glu Ile Asn Arg Ile
            100                 105                 110

Ala Asp Gln Ala Gln Tyr Asn Gln Met His Met Leu Ser Asn Lys Ser
        115                 120                 125

Ala Ala Gln Asn Val Lys Thr Ala Glu Glu Leu Gly Met Gln Pro Ala
    130                 135                 140

Lys Ile Asn Thr Pro Ala Ser Leu Ala Gly Ser Gln Ala Ser Trp Thr
145                 150                 155                 160

Leu Arg Val His Val Gly Ala Asn Gln Asp Glu Ala Ile Ala Val Asn
                165                 170                 175

Ile Tyr Ala Ala Asn Val Ala Asn Leu Phe Asn Gly Glu Gly Ala Gln
            180                 185                 190

Ala Ala Pro Ala Gln Glu Gly Ala Gln Gln Glu Gly Val Gln Ala Val
        195                 200                 205

Pro Ala Pro Ala Ala Ala Pro Val Gln Gly Gly Val Asn Ser Pro Ile
    210                 215                 220

Asn Val Thr Thr Ala Ile Asp Ala Asn Met Ser Leu Ser Lys Ile Glu
225                 230                 235                 240

Asp Ala Ile Arg Met Val Thr Asp Gln Arg Ala Asn Leu Gly Ala Phe
                245                 250                 255
```

```
Gln Asn Arg Leu Glu Ser Val Lys Ala Ser Thr Asp Tyr Ala Ile Glu
            260                 265                 270

Asn Leu Lys Ala Ser Tyr Ala Gln Val Lys Asp Ala Ile Met Thr Asp
        275                 280                 285

Glu Ile Val Ala Ser Thr Thr Asn Ser Ile Leu Thr Gln Ser Ala Met
    290                 295                 300

Ala Met Ile Ala Gln Ala Asn Gln Val Pro Gln Tyr Val Leu Ser Leu
305                 310                 315                 320

Leu Arg
```

<210> 15
<211> 324
<212> PRT
<213> Borrelia turcica

<400> 15

```
Met Arg Asn Asn Gly Ile Asn Ala Ser Asn Leu Ser Lys Thr Gln Glu
1               5                   10                  15

Lys Leu Ser Ser Gly Tyr Arg Ile Asn Arg Ala Ser Asp Asp Ala Ala
            20                  25                  30

Gly Met Gly Val Ala Gly Lys Ile Asn Ala Gln Ile Arg Gly Leu Ser
        35                  40                  45

Gln Ala Ser Arg Asn Thr Ser Lys Ala Ile Asn Phe Ile Gln Thr Thr
    50                  55                  60

Glu Gly Asn Leu Asn Glu Val Glu Lys Val Leu Val Arg Met Lys Glu
65                  70                  75                  80

Leu Ala Val Gln Ser Gly Asn Gly Thr Tyr Ser Asp Ala Asp Arg Gly
```

```
                85                  90                  95

Ser Ile Gln Ile Glu Ile Glu Gln Leu Thr Asp Glu Ile Asn Arg Ile
            100                 105                 110

Ala Asp Gln Ser Gln Tyr Asn Gln Met His Met Leu Ser Asn Lys Ser
        115                 120                 125

Ala Ala Gln Asn Val Lys Thr Ala Glu Glu Leu Gly Met Gln Pro Ala
    130                 135                 140

Lys Ile Asn Thr Pro Ala Ser Leu Ser Gly Ala Gln Ala Ser Trp Thr
145                 150                 155                 160

Leu Arg Val His Val Gly Ala Asn Gln Asp Glu Ala Ile Ala Val Asn
                165                 170                 175

Ile Tyr Ala Ala Asn Val Pro Asn Leu Phe Ala Gly Glu Gly Ala Gln
            180                 185                 190

Thr Ala Ala Ala Ala Pro Ala Gln Ala Gly Thr Gln Gln Glu Gly Ala
        195                 200                 205

Gln Glu Pro Ala Ala Ala Ala Ala Pro Ala Gln Gly Gly Val Asn Ser
    210                 215                 220

Pro Val Asn Val Thr Thr Thr Val Asp Ala Asn Met Ser Leu Ala Lys
225                 230                 235                 240

Ile Glu Asn Ala Ile Arg Met Ile Ser Asp Gln Arg Ala Asn Leu Gly
                245                 250                 255

Ala Phe Gln Asn Arg Leu Glu Ser Ile Lys Asn Ser Thr Glu Tyr Ser
            260                 265                 270

Ile Glu Asn Leu Lys Ala Ser Tyr Ala Gln Ile Lys Asp Ala Thr Met
        275                 280                 285
```

```
Thr Asp Glu Ile Val Ser Ser Thr Thr Asn Ser Ile Leu Thr Gln Ser
    290                 295                 300

Ala Met Ala Met Ile Ala Gln Ala Asn Gln Val Pro Gln Tyr Val Leu
305                 310                 315                 320

Ser Leu Leu Arg
```

<210> 16
<211> 320
<212> PRT
<213> Borrelia hermsii

<400> 16

```
Met Ser Lys Ala Gly Ser Gly Leu Thr Lys Ile Ala Ile Leu Val Asp
1               5                   10                  15

Gly Thr Phe Asp Asp Glu Ser Phe Asn Gly Ser Ala Trp Lys Gly Ala
            20                  25                  30

Lys Lys Val Glu Lys Glu Phe Gly Leu Glu Ile Met Met Lys Glu Ser
        35                  40                  45

Asn Ala Asn Ser Tyr Leu Ala Asp Leu Glu Ser Leu Lys Asn Asn Gly
    50                  55                  60

Ser Asn Phe Leu Trp Leu Ile Gly Tyr Lys Phe Ser Asp Phe Ala Ile
65                  70                  75                  80

Ile Ala Ala Leu Glu Asn Pro Glu Ser Lys Tyr Val Ile Ile Asp Pro
                85                  90                  95

Val Tyr Glu Ser Asp Leu Val Ile Pro Glu Asn Leu Ser Ala Ile Thr
            100                 105                 110

Phe Arg Thr Glu Glu Gly Ala Phe Leu Val Gly Tyr Ile Ala Ala Lys
        115                 120                 125
```

```
Met Ser Lys Thr Gly Lys Ile Gly Phe Leu Gly Gly Phe Asp Asp Val
    130                 135                 140

Val Val Asn Thr Phe Arg Tyr Gly Tyr Glu Ala Gly Ala Ile Tyr Ala
145                 150                 155                 160

Asn Lys His Ile Asn Ile Asp Asn Lys Tyr Ile Gly Asn Phe Val Asn
                165                 170                 175

Thr Glu Thr Gly Lys Asn Met Ala Asn Ala Met Tyr Ala Glu Gly Val
            180                 185                 190

Asp Ile Ile Tyr His Val Ala Gly Leu Ala Gly Leu Gly Val Ile Glu
        195                 200                 205

Ser Ala Arg Asp Leu Gly Asp Gly His Tyr Val Ile Gly Val Asp Gln
    210                 215                 220

Asp Gln Ser His Leu Ala Pro Asp Asn Val Ile Thr Ser Ser Ile Lys
225                 230                 235                 240

Asp Ile Gly Arg Val Leu Asn Ile Met Ile Ser Asn Tyr Leu Lys Thr
                245                 250                 255

Asn Ala Phe Glu Gly Gly Gln Val Leu Ser Tyr Gly Leu Lys Glu Gly
            260                 265                 270

Phe Leu Asp Phe Val Lys Asn Pro Lys Met Ile Ser Phe Glu Leu Glu
        275                 280                 285

Lys Glu Leu Asp Asp Leu Ser Glu Gly Ile Ile Asn Gly Lys Ile Ile
    290                 295                 300

Val Pro Asn Asn Glu Arg Thr Tyr Asn Gln Phe Met Arg Lys Ile Leu
305                 310                 315                 320
```

<210> 17
<211> 202
<212> PRT
<213> Borrelia hermsii

<400> 17

```
Met Phe Leu Phe Ile Ser Cys Asn Asn Gly Gly Pro Glu Leu Lys Gly
1               5                  10                  15


Asn Glu Val Ala Lys Ser Asp Gly Thr Val Leu Asp Leu Ser Lys Ile
            20                  25                  30


Ser Thr Lys Ile Lys Asn Ala Gly Ala Phe Ala Ala Ser Val Gln Glu
        35                  40                  45


Val Ala Thr Leu Val Lys Ser Val Asp Glu Leu Ala Ser Ala Ile Gly
    50                  55                  60


Lys Lys Ile Lys Glu Asp Gly Thr Leu Asp Thr Leu Asn Asn Lys Asn
65                  70                  75                  80


Gly Ser Leu Leu Ala Gly Ala Phe Gln Val Ile Leu Thr Val Glu Ala
                85                  90                  95


Lys Leu Lys Glu Leu Glu Lys Gln Asp Gly Leu Ser Val Glu Leu Arg
            100                 105                 110


Ala Lys Val Thr Ser Ala Lys Ser Ala Ser Ser Gly Leu Val Asn Lys
        115                 120                 125


Leu Lys Gly Gly His Ala Glu Leu Gly Ile Glu Gly Ala Thr Asp Glu
    130                 135                 140


Asn Ala Gln Lys Ala Ile Lys Lys Asp Asn Gly Asp Gln Ser Lys Gly
145                 150                 155                 160


Ala Glu Glu Leu Gly Lys Leu Asn Thr Ala Ile Gly Ala Leu Leu Ser
                165                 170                 175
```

```
Ala Ala Asn Asp Ala Val Glu Ala Ala Ile Lys Glu Leu Thr Ala Ala
            180                 185                 190


Pro Ala Lys Pro Ala Thr Pro Ala Lys Pro
        195                 200


<210>  18
<211>  337
<212>  PRT
<213>  Borrelia coriaceae

<400>  18

Met Lys Ser Met Lys Pro Lys Leu Leu Met Leu Ile Ile Asn Ile Phe
1               5                   10                  15


Leu Ile Ile Ser Cys Gln Asn Glu Lys Val Ser Met Asn Glu Lys Ser
            20                  25                  30


Pro Leu Ile Ile Ala His Arg Gly Ala Ser Gly Tyr Leu Pro Glu His
        35                  40                  45


Thr Leu Glu Ala Lys Ala Tyr Ala Tyr Ala Leu Gly Ala Asp Tyr Leu
    50                  55                  60


Glu Gln Asp Ile Val Leu Thr Lys Asp Asn Val Pro Ile Ile Met His
65                  70                  75                  80


Asp Ser Glu Leu Asp Thr Thr Thr Asn Val Ala Lys Leu Phe Pro Glu
                85                  90                  95


Arg Ala Arg Glu Asn Gly Lys Tyr Tyr Ala Val Asp Phe Thr Leu Asp
            100                 105                 110


Glu Ile Lys Ser Leu Ser Ile Ser Glu Arg Phe Asp Pro Glu Thr Arg
        115                 120                 125


Glu Pro Ile Tyr Pro Asn Arg Phe Pro Leu Asn Glu Tyr Asn Phe Lys
    130                 135                 140
```

```
Ile Pro Thr Leu Glu Glu Glu Ile Gln Phe Ile Gln Gly Leu Asn Lys
145                 150                 155                 160

Ser Thr Gly Lys Asn Val Gly Ile Tyr Pro Glu Ile Lys Lys Pro Phe
                165                 170                 175

Trp His Lys Gln Gln Gly Lys Asp Ile Ser Lys Ile Val Ile Glu Ile
            180                 185                 190

Leu Asn Gln Tyr Gly Tyr Lys Ser Lys Glu Asp Lys Ile Tyr Leu Gln
        195                 200                 205

Thr Phe Asp Phe Asp Glu Leu Lys Arg Ile Arg Glu Glu Leu Gly Tyr
    210                 215                 220

Gln Gly Lys Leu Ile Met Leu Val Gly Glu Asn Asp Trp Asn Glu Ala
225                 230                 235                 240

Pro Thr Asp Tyr Glu Tyr Ile Lys Ser Gln Glu Gly Met Thr Glu Val
                245                 250                 255

Ala Lys Tyr Ala Asp Gly Ile Gly Pro Trp Ile Ser Gln Ile Ile Ile
            260                 265                 270

Asp Gly Gln Val Thr Asp Leu Ile Ser Leu Ala His Lys His Asn Met
        275                 280                 285

Glu Val His Pro Tyr Thr Phe Arg Ile Asp Ala Leu Pro Ser Tyr Val
    290                 295                 300

Lys Asp Ala Asn Glu Leu Leu Asp Leu Leu Phe Asn Lys Ala Gln Val
305                 310                 315                 320

Asp Gly Ile Phe Thr Asp Phe Val Asp Lys Ala Met Glu Phe Val Lys
                325                 330                 335
```

Lys

<210> 19
<211> 562
<212> PRT
<213> Borrelia coriaceae

<400> 19

Met Lys Ser Phe Ser Val Phe Ile Leu Phe Leu Ser Thr Phe Thr Leu
1 5 10 15

Ser Cys Lys Phe Tyr Asp Thr Ala Asn Ala Ser Asp Leu Gln Ala Asp
20 25 30

Gly Asp Lys Phe Ser Gln Gly Phe Ser Ser Phe Asn Asp Ile Leu Pro
35 40 45

Phe Ser Asp Leu Lys Ile Asn Lys Asp Val Ser Thr Gly Ser Leu Lys
50 55 60

Ala Gln Gly Thr Ser Ser Ile Lys Gly Asp Glu Lys Lys Gly Lys Gly
65 70 75 80

Thr Ser Lys Asp Pro Ile Lys Asp Gln Glu Ala Ser Gly Leu Lys Gly
85 90 95

Val Gly Val Ala Gly Ala Gly Ala Lys Ser Phe Gly Asp Asp Gly Lys
100 105 110

Lys Glu Glu Val Val Ser Lys Asp Ser Leu Lys Asn Glu Gly Thr Ser
115 120 125

Gly Thr Ala Glu Val Leu Lys Val Ser Lys Glu Val Glu Val Ala Gly
130 135 140

Val Asp Thr Ala Lys Pro Ala Gly Gly Asn Gly Glu Glu Val Ala Ser
145 150 155 160

```
Ile Ser Glu Asn Tyr Leu Gln Asn Gln Glu Thr Leu Val Ala Gln Gly
                165                 170                 175

Ala Gly Val Gly Ser Val Gly Asp Ala Ile Gly Asp Arg Ser Leu Phe
            180                 185                 190

Phe Lys Asn Thr Asp Ser Asn Asn Ala Glu Gln Val Val Ala Thr Glu
        195                 200                 205

Asp Leu Leu Val Gly Ala Ser Glu Gly Val Asn Thr Ser Asp Leu Gly
    210                 215                 220

Leu Lys Val Ala Ile Pro Thr Asp His Val Arg Gly Asp Val Val Ala
225                 230                 235                 240

Thr Glu Thr Gln Asn Ala Glu Lys Lys Gly Asp Lys Thr Gln Asn Thr
                245                 250                 255

Glu Leu Ala Ser Leu Asp Ile Lys Asp Asn Ile Thr Val Asn Val Val
            260                 265                 270

Asp Gly Thr Lys Ile Asn Ile Asn Lys Asn Ser Ser Asn Thr Asn Glu
        275                 280                 285

Ser Ile Asn Val Thr Lys Asp Gly Val Asn Thr Val Ile Lys Gly Val
    290                 295                 300

Glu Thr Ser Ile Lys Thr Ala Asp Gly Lys Val Val Val Lys Lys Arg
305                 310                 315                 320

Thr Leu Lys Lys Gly Leu Lys Lys Lys Asn Ser Lys Lys Gln Ala Ser
                325                 330                 335

Lys Ser Lys Thr Pro Glu Ala Ala Val Val Gly Asn Lys Lys Asn Val
            340                 345                 350

Asp Thr Asn Met Ser Ser Val Ile Gly Leu Asp Ser Glu Ala Leu Gly
```

```
        355                 360                 365

Lys Asp Lys Asn Ile Asp Leu Asp Ser Lys Ser Asp Glu Thr Tyr Val
    370                 375                 380

Ile Glu Arg Val Glu Lys Leu Ala Lys Tyr Leu Gln Ser Ala Ile Lys
385                 390                 395                 400

Ile Asn Gly Lys Lys Val Glu Glu Gln Asp Lys Leu Glu Ala Gly Arg
                405                 410                 415

Gln Lys Phe Phe Glu Trp Leu Ser Lys Asn Asp Thr Asp Leu Leu Lys
            420                 425                 430

Arg Lys Ala Leu Val Gln Asp Leu Gln Lys Ile Tyr Asp Leu Met Lys
        435                 440                 445

Asp Lys Ile Ala Asp Ser Thr Glu Leu Gln Asp Trp Phe Gln Ile Val
    450                 455                 460

Ser Asp Asp Ile Gly Asp Glu Glu Thr Asn Ile Ile Asp Val Glu Ser
465                 470                 475                 480

Tyr Tyr Glu Leu Ser Ser Asp Thr Glu Ile Asp Phe Leu Leu Glu Arg
                485                 490                 495

Thr Leu Glu Asp Glu Asn Tyr Ser Gly Phe Ser Ile Ser Leu Phe Met
            500                 505                 510

Gln Ala Leu Ala Asp Thr Leu Tyr Asp Ile Gln Asn Asp Ser His Lys
        515                 520                 525

Ser Gly Glu Glu Ile Leu Gln Glu Leu Lys Arg Val Phe Asp Asp Thr
    530                 535                 540

Phe Tyr Lys Ile Arg Gly Phe Glu Glu Phe Lys Ser Gln Ile Ala Ala
545                 550                 555                 560
```

Glu Asp

<210> 20
<211> 193
<212> PRT
<213> Borrelia coriaceae

<400> 20

Met Lys Leu Thr Lys Lys Tyr Leu Leu Ala Val Leu Leu Leu Ser Leu
1 5 10 15

Ile Asn Cys Asp Leu Leu Ser Lys Asn Lys Ile Leu Thr Ser His Leu
20 25 30

Leu Asn Thr Leu Asp Asn Asn Lys Lys Glu Ala Leu Val Thr Phe Lys
35 40 45

Asn Leu Leu Gln Asp Lys Ser His Leu Glu Tyr Leu Lys Ser Glu Gln
50 55 60

Ala Lys Met Leu Thr Asn Phe Thr Glu Asp Asp Gly Ile Glu Gln Pro
65 70 75 80

His Leu Gln Glu Lys Leu Lys Gly Thr Leu Ser Ser Glu Tyr Asn Glu
85 90 95

Asn Gln Leu Asn Gln Leu Phe Ser Glu Leu Gly Tyr Glu Lys Thr Lys
100 105 110

Gln Phe Leu Asp Asn Leu His Lys Met Leu Gln Ala Ile Lys Asp Gly
115 120 125

Thr Leu Arg Ala Phe His Asp Ser Ser Ser Phe Lys Asp Tyr Asn Thr
130 135 140

Thr Leu Glu Ala Lys Lys Ala Glu Ala Leu Ser Ser Val Lys Lys Glu
145 150 155 160

```
Leu Tyr Val Gln Tyr Tyr Phe Tyr Ile Asn Asp Leu Gln Thr Ala Asp
                165                 170                 175


Asp Phe Phe Val Leu Thr Arg Asn His Leu Met Ile Phe Lys Asn Asn
            180                 185                 190


Leu
```