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(54) **PEPTIDES AND METHODS FOR THE DETECTION OF LYME DISEASE ANTIBODIES**

PEPTIDE UND VERFAHREN ZUM NACHWEIS VON LYME-ERKRANKUNGS-ANTIKÖRPERN

PEPTIDES ET PROCÉDÉS POUR LA DÉTECTION D'ANTICORPS CONTRE LA MALADIE DE LYME

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The file contains technical information submitted after the application was filed and not included in this specification

Description

BACKGROUND OF THE INVENTION

- 5 **[0001]** Lyme disease is a debilitating condition that has become a significant public health concern. The disease is caused by infection with a pathogenic *Borrelia* bacterium (a spirochete) and is transmitted by the bite of various species of *Borrelia*-infected *Ixodes* ticks. Accurate and early detection of Lyme disease is critical to effective treatment. The only clinical symptom sufficient for diagnosis of Lyme disease is the presence of erythema migrans, a rash having a distinctive bulls-eye appearance. However, erythema migrans is only present early during infection and, even then, does not appear in all infected individuals. Other clinical symptoms that have been associated with Lyme disease, such as Bell's palsy, are not specific enough, either alone or in combination, to determine clinical diagnosis in the absence of erythema migrans.
- 10 **[0002]** In the absence of erythema migrans, the basis for diagnosis of Lyme disease is an antibody response to a pathogenic *Borrelia* species, such as *Borrelia burgdorferi*, *Borrelia afzelli*, or *Borrelia garinii*. In North America, the Center for Disease Control (CDC) recommends a two-tier approach for serodiagnosis of Lyme disease consisting of a sensitive first-tier assay, such as ELISA, followed by a western blot if the first tier assay is positive or equivocal. First tier assays have traditionally made use of a whole-cell *Borrelia burgdorferi* antigen or recombinant *Borrelia* proteins. Such assays can be difficult to interpret, though, and are complicated by *Borrelia* antibodies arising from vaccination rather than infection. In addition, whole cell sonicates used in some *Borrelia* assays react with *Treponema* antibodies.
- 15 **[0003]** More recently, the C6 peptide assay, based on the conserved IR6 domain of the variable surface antigen (VlsE) of *Borrelia*, has become widely accepted as a first-tier assay having a high degree of sensitivity for disseminated and late Lyme disease. The C6 peptide assay uses a single 25 amino acid sequence of the *Borrelia burgdorferi* VlsE protein as the test antigen. Although it has been suggested that the C6 peptide assay may be suitable for a single-tier approach to diagnosis of Lyme disease, it is becoming apparent that the C6 assay is not sufficiently sensitive for such purposes because it fails to detect certain strains of infectious *Borrelia*.
- 20 **[0004]** Accordingly, there remains a need in the art for additional assays for detecting *Borrelia* antigens and serodiagnosis of Lyme disease.
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SUMMARY OF THE INVENTION

- 30 **[0005]** The present invention is based, in part, on the discovery that certain sequence variants in the IR6 domain of the *Borrelia* VlsE protein provide for robust detection of an antibody response against a wide range of *Borrelia* species. Accordingly, the invention provides compositions, devices, methods, and kits useful for the detection of antibodies that bind to *Borrelia* antigens and the diagnosis of Lyme disease.
- 35 **[0006]** The invention is as defined in the claims. Described herein are peptides capable of binding to antibodies that recognize *Borrelia* antigens. The peptides may comprise a VlsE IR6 domain and a sequence from at least one (*e.g.*, two, three, etc.) other *Borrelia* antigen. The at least one other *Borrelia* antigen may be a surface antigen (*e.g.*, OspC, p41, or a combination thereof).
- 40 **[0007]** The invention provides mixtures of peptides that have three or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 1,

M-K-X₃-X₄-D-X₆-I-A-A-X₁₀-X₁₁-V-L-X₁₄-G-M-A-K-X₁₉-G-X₂₁-F-A-X₂₄-X₂₅ (SEQ ID

- wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is an amino acid selected from the group consisting of N and D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from the group consisting of I, V, Y and W, and X₂₅ is an amino acid selected from the group consisting of K and R.
- 45 **[0008]** In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is N, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is I, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is E, X₂₄ is an amino acid selected from the group consisting of I, W, and Y, and X₂₅ is K. In other embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from
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the group consisting of I, W, and Y, and X₂₅ is an amino acid selected from the group consisting of K and R.

[0009] In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1 and further comprise an additional N-terminal peptide sequence. The additional N-terminal peptide sequence can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids and can be either a native or non-native sequence. In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1 and further comprise an additional C-terminal sequence. The additional C-terminal peptide sequence can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids and can be either a native or non-native sequence. In certain embodiments, the non-native sequence comprises a non-VlsE *Borrelia* antigen (e.g., a *Borrelia* OspC or p41 antigen).

[0010] Peptides may comprise a sequence of (SEQ ID NO:2, n₁-n₂-n₃-n₄-n₅-n₆-n₇-Q-D-n₁₀-M-K-X₁₃-X₁₄-D-X₁₆-I-A-A-X₂₀-X₂₁-V-L-X₂₄-G-M-A-K-X₂₉-G-X₃₁-F-A-X₃₄-X₃₅-D-N-E-c₃₉-D-c₄₁-A-E-c₄₄-G (SEQ ID NO:2) wherein n₁ is an amino acid selected from the group consisting of N and D, n₂ is an amino acid selected from the group consisting of N and D, n₃ is an amino acid selected from the group consisting of A and V, n₄ is an amino acid selected from the group consisting of A and V, n₅ is an amino acid selected from the group consisting of A and V, n₆ is an amino acid selected from the group consisting of F and Y, n₇ is an amino acid selected from the group consisting of S and T, n₁₀ is an amino acid selected from the group consisting of D, E, N, and Q, X₁₃ is an amino acid selected from the group consisting of R and Q, X₁₄ is an amino acid selected from the group consisting of N and D, X₁₆ is an amino acid selected from the group consisting of N and E, X₂₀ is an amino acid selected from the group consisting of V, F and L, X₂₁ is an amino acid selected from the group consisting of I and M, X₂₄ is an amino acid selected from the group consisting of K, Q and H, X₂₉ is an amino acid selected from the group consisting of N, Q and E, X₃₁ is an amino acid selected from the group consisting of E and Q, X₃₄ is an amino acid selected from the group consisting of I, V, Y and W, X₃₅ is an amino acid selected from the group consisting of K and R, c₃₉ is an amino acid selected from the group consisting of H and D, c₄₁ is an amino acid selected from the group consisting of K and R, and c₄₄ is an amino acid selected from the group consisting of K and R. In certain related peptides, n₁₀ is an amino acid selected from the group consisting of D, N, and Q, X₁₄ is N, X₂₁ is I, X₃₁ is E, X₃₄ is an amino acid selected from the group consisting of I, W, and Y, and X₃₅ is K. In other related peptides, n₁₀ is an amino acid selected from the group consisting of D, N, and Q, X₁₄ is D, and X₃₄ is an amino acid selected from the group consisting of I, W, and Y.

[0011] In certain embodiments, peptides of the invention comprise at least, 30, 35, 40, 45, or more amino acids. Peptides of the invention are isolated (e.g., synthetic and/or purified) peptides. In certain embodiments, peptides of the invention are conjugated to a ligand. For example, in certain embodiments, the peptides are biotinylated. In other embodiments, the peptides are conjugated to avidin, streptavidin, or neutravidin. In other embodiments, the peptides are conjugated to a carrier protein (e.g., serum albumin or an immunoglobulin Fc domain). In still other embodiments, the peptides are conjugated to a dendrimer and/or part of a multiple antigenic peptides system (MAPS).

[0012] In certain embodiments, peptides of the invention are attached to or immobilized on a solid support. In certain embodiments, the solid support is a bead (e.g., a colloidal particle, nanoparticle, latex bead, etc.), a flow path in a lateral flow immunoassay device (e.g., a porous membrane), a flow path in an analytical rotor, or a tube or well (e.g., in a plate suitable for an ELISA assay).

[0013] Also described herein are nucleic acids comprising a sequence encoding a peptide of the invention, as well as vectors comprising such nucleic acids, and host cells comprising such vectors. A vector may be a shuttle vector. Alternatively, the vector may be an expression vector (e.g., a bacterial or eukaryotic expression vector). The host cell may be a bacterial cell. Alternatively, the host cell may be a eukaryotic cell.

[0014] Also disclosed are devices. The devices may be useful for performing an immunoassay. For example, the device may be a lateral flow immunoassay device. Alternatively, the device may be an analytical rotor. Alternatively, the device may be a tube or a well, e.g., in a plate suitable for an ELISA assay. Alternatively, the device may be an electrochemical, optical, or opto-electronic sensor.

[0015] The device may comprise a mixture of different peptides of the invention. For example, in certain embodiments, the device comprises three, four or more different peptides of the invention. Each peptide in the mixture may comprise a sequence of SEQ ID NO: 1 or SEQ ID NO:2. In certain embodiments, the peptides are attached to or immobilized upon the device.

[0016] In another aspect, the invention provides methods of detecting in a sample an antibody to an epitope of a *Borrelia* antigen comprising contacting a sample with the mixture of isolated peptides of the invention, and detecting formation of an antibody-peptide complex comprising one or more of said peptides, wherein formation of said complex is indicative of the presence of an antibody to an epitope of a *Borrelia* antigen in said sample. In certain embodiments, the *Borrelia* antigen is from an infectious *Borrelia* species, such as *Borrelia burgdorferi*, *Borrelia afzelli*, or *Borrelia garinii*. In certain embodiments, the methods comprise contacting the sample with a mixture of three, four or more different peptides of the invention.

[0017] In certain embodiments, the mixture of peptides is attached to or immobilized upon a solid support. In certain embodiments, the solid support is a bead (e.g., a colloidal particle, nanoparticle, latex bead, etc.), a flow path in a lateral flow immunoassay device (e.g., a porous membrane), a flow path in an analytical rotor, or a tube or a well (e.g., in a

plate suitable for an ELISA assay). In certain embodiments, the solid support comprises metal, glass, a cellulose-based material (e.g., nitrocellulose), or a polymer (e.g., polystyrene, polyethylene, polypropylene, polyester, nylon, polysulfone, etc.). In certain embodiments, the peptide or mixture of different peptides is attached to a dendrimer and/or incorporated into a MAPS system.

5 **[0018]** In certain embodiments, the detecting step comprises performing an ELISA assay. In other embodiments, the detecting step comprises performing a lateral flow immunoassay. In other embodiments, the detecting step comprises performing an agglutination assay. In other embodiments, the detecting step comprises spinning the sample in an analytical rotor. In still other embodiments, the detecting step comprises analyzing the sample with an electrochemical sensor, an optical sensor, or an opto-electronic sensor.

10 **[0019]** In certain embodiments, the sample is a bodily fluid, such as blood, serum, plasma, cerebral spinal fluid, urine, mucus, or saliva. In other embodiments, the sample is a tissue (e.g., a tissue homogenate) or a cell lysate. In certain embodiments, the sample is from a wild animal (e.g., a deer or rodent, such as a mouse, chipmunk, squirrel, etc.). In other embodiments, the sample is from a lab animal (e.g., a mouse, rat, guinea pig, rabbit, monkey, primate, etc.). In other embodiments, the sample is from a domesticated or feral animal (e.g., a dog, a cat, a horse). In still other embodiments, the sample is from a human.

15 **[0020]** In another aspect, the invention provides methods of diagnosing Lyme disease in a subject comprising contacting a sample from the subject with the mixture of isolated peptides of the invention, and detecting formation of an antibody-peptide complex comprising one or more of said peptides, wherein formation of said complex is indicative of the subject having Lyme disease. In certain embodiments, the methods comprise contacting the sample with a mixture of three, four or more different peptides of the invention.

20 **[0021]** In certain embodiments, the peptide or mixture of different peptides is attached to or immobilized upon a solid support. For example, in certain embodiments, the solid support is a bead (e.g., a colloidal particle, nanoparticle, latex bead, etc.), a flow path in a lateral flow immunoassay device (e.g., a porous membrane), a flow path in an analytical rotor, or a tube or a well (e.g., in a plate suitable for an ELISA assay). In certain embodiments, the solid support comprises metal, glass, a cellulose-based material (e.g., nitrocellulose), or a polymer (e.g., polystyrene, polyethylene, polypropylene, polyester, nylon, polysulfone, etc.). In certain embodiments, the peptide or mixture of different peptides is attached to a dendrimer and/or incorporated into a MAPS system.

25 **[0022]** In certain embodiments, the detecting step comprises performing an ELISA assay. In other embodiments, the detecting step comprises performing a lateral flow immunoassay. In other embodiments, the detecting step comprises performing an agglutination assay. In other embodiments, the detecting step comprises spinning the sample in an analytical rotor. In still other embodiments, the detecting step comprises analyzing the sample with an electrochemical sensor, optical sensor, or opto-electronic sensor.

30 **[0023]** In certain embodiments, the sample is a bodily fluid, such as blood, serum, plasma, cerebral spinal fluid, urine, or saliva. In other embodiments, the sample is a tissue (e.g., a tissue homogenate) or a cell lysate. In certain embodiments, the subject is a wild animal (e.g., a deer or rodent, such as a mouse, chipmunk, squirrel, etc.). In other embodiments, the subject is a lab animal (e.g., a mouse, rat, guinea pig, rabbit, monkey, primate, etc.). In other embodiments, the subject is a domesticated or feral animal (e.g., a dog, a cat, a horse). In still other embodiments, the subject is a human.

35 **[0024]** In another aspect, the invention provides kits comprising the mixture of isolated peptides of the invention and a labeling reagent capable of binding to an antibody that recognizes and epitope of the isolated peptides. In certain embodiments, the kits comprise three, four or more different peptides. The peptides can comprise a sequence of SEQ ID NO:1 as defined in the claims. In certain embodiments, the peptides are attached to or immobilized on a solid support. For example, in certain embodiments, the solid support is a bead (e.g., a colloidal particle, nanoparticle, latex bead, etc.), a flow path in a lateral flow immunoassay device, a flow path in an analytical rotor, or a tube or a well (e.g., in a plate). In certain embodiments, the peptide or mixture of different peptides is attached to a dendrimer and/or incorporated into a MAPS system.

40 **[0025]** In certain embodiments, the kits further comprise a population of beads or a plate (e.g., a plate suitable for an ELISA assay). In other embodiments, the kits further comprise a device, such as a lateral flow immunoassay device, an analytical rotor, an electrochemical sensor, an optical sensor, or an opto-electronic sensor. In certain embodiments, the population of beads, the plate, or the device is useful for performing an immunoassay. For example, in certain embodiments, the population of beads, the plate, or the device is useful for detecting formation of an antibody-peptide complex comprising an antibody from a sample and a peptide of the invention. In certain embodiments, a peptide or a mixture of differed peptides of the invention is attached to or immobilized on the beads, the plate, or the device.

45 **[0026]** In certain embodiments, the kits further comprise an instruction. For example, in certain embodiments, the kits comprise an instruction indicating how to use a peptide of the invention to detect an antibody to a *Borrelia* antigen or to diagnose Lyme disease. In certain embodiments, the kits comprise an instruction indicating how to use a population of beads, a plate, or a device (e.g., comprising a peptide or a mixture of different peptides of the invention) to detect an antibody to a *Borrelia* antigen or to diagnose Lyme disease.

50 **[0027]** Additional aspects and embodiments of the invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028]

5 **Figure 1** is a diagram of an indirect sandwich assay which can be used to detect antibodies to *Borrelia* antigens. In this embodiment, anti-human IgG/IgM or anti-dog IgG/IgM antibodies are immobilized to a suitable substrate (e.g., nitrocellulose membrane) at a test site. Antibodies in a test sample are bound by the immobilized antibodies. Test sample antibodies to appropriate *Borrelia* antigens will then bind to peptides of the invention. When the peptides of the invention are conjugated to biotin, colloidal gold-labeled streptavidin can be used to detect the presence of the peptides at the test site. It can be appreciated that the indirect sandwich assay can be operated in the reverse—that is the peptides of the invention can be immobilized to a substrate to capture anti-*Borellia* antibodies in a test sample and anti-human IgG/IgM or anti-dog IgG/IgM antibodies conjugated to a label (e.g. colloidal gold) can be used to detect the presence of the antibodies bound to the immobilized peptides at the test site.

15 **Figure 2** is a diagram of a lateral flow immunoassay device based on the indirect sandwich assay of Fig. 1. In this embodiment of a lateral flow immunoassay device, sample is applied at a sample loading pad and then flows through the conjugate pad to the test membrane. Peptide-biotin-streptavidin-gold complexes are solubilized as the sample passes through the conjugate pad and complexes between peptides of the invention and appropriate anti-*Borrelia* antigen antibodies are then formed. The test site comprises sample appropriate anti-IgG or anti-IgM antibodies, which bind to all antibodies in the sample. Protein L, for example, can be used in place of anti-IgG or anti-IgM antibodies. If sufficient antibodies in the sample have bound to peptides of the invention, a positive signal will appear at the test site. In another embodiment of a lateral flow immunoassay device, peptides of the invention are immobilized at the test site (T) and sample appropriate anti-IgG or anti-IgM antibodies (e.g. anti-human or anti-canine) conjugated to a detectable label (e.g. colloidal gold particles) are present in the conjugate pad. Sample passing through the conjugate pad solubilizes the labeled antibodies and any anti-*Borrelia* antigen antibodies present in the test sample bind to the labeled antibodies and such antibody complexes are captured by the immobilized *Borellia* peptides of the invention at the test site, thereby producing a positive signal. In either embodiment, the device can further comprise a control site (C) at which binding partners that recognize the labeled peptide or labeled antibody in the conjugate pad is immobilized.

30 **Figure 3** is a diagram of a double antigen sandwich assay which can be used to detect antibodies to *Borrelia* antigens. In this embodiment, peptides of the invention are immobilized to a suitable substrate (e.g., nitrocellulose membrane, well of an ELISA plate) at a test site. Antibodies in a test sample are bound by the immobilized peptides of the invention. Test sample antibodies to appropriate *Borrelia* antigens will then bind to a second set of peptides of the invention that are conjugated to a detector molecule (e.g., colloidal gold, horse radish peroxidase (HRP), alkaline phosphatase (ALP)), which detects the presence of the antibodies bound to the first set of peptides immobilized at the test site.

DETAILED DESCRIPTION

40 [0029] As used herein, the following terms shall have the following meanings:

[0030] The term "antigen," as used herein, refers to a molecule capable of being recognized by an antibody. An antigen can be, for example, a peptide or a modified form thereof. An antigen can comprise one or more epitopes.

45 [0031] The term "epitope," as used herein, is a portion of an antigen that is specifically recognized by an antibody. An epitope, for example, can comprise or consist of a portion of a peptide (e.g., a peptide of the invention). An epitope can be a linear epitope, sequential epitope, or a conformational epitope.

[0032] The terms "nucleic acid," "oligonucleotide" and "polynucleotide" are used interchangeably herein and encompass DNA, RNA, cDNA, whether single stranded or double stranded, as well as chemical modifications thereof.

50 [0033] Single letter amino acid abbreviations used herein have their standard meaning in the art, and all peptide sequences described herein are written according to convention, with the N-terminal end to the left and the C-terminal end to the right.

[0034] Additional terms shall be defined, as required, in the detailed description that follows.

Compositions and Devices

55 [0035] The present invention is based, in part, on the discovery that certain sequence variants in the IR6 domain of the *Borrelia* VlsE protein provide for robust detection of an antibody response against a wide range of *Borrelia* species. Accordingly, in one aspect, the invention provides peptides capable of binding to antibodies that recognize *Borrelia*

antigens.

[0036] , peptides may comprise a VlsE IR6 domain, or a fragment thereof, and a sequence (e.g., a sequence comprising an epitope) from at least one (e.g., two, three, etc.) other *Borrelia* antigen. The at least one other *Borrelia* antigen may be a surface antigen or an antigen selected from the group consisting of OspA, OspB, OspC, p41, and combinations thereof. Thus, for example, peptides may comprise (i) a VlsE IR6 domain, or a fragment thereof, and (ii) a sequence comprising an epitope of an OspA protein, a sequence comprising an epitope of an OspB protein, a sequence comprising an epitope of an OspC protein, a sequence comprising an epitope of a p41 protein, or a combination of such sequences. Peptides may comprise (i) a VlsE IR6 domain, or a fragment thereof, (ii) a sequence comprising an epitope of an OspC protein, and (iii) a sequence comprising an epitope of a p41 protein.

[0037] Peptides in the mixtures of the invention comprise or consist of a sequence of SEQ ID NO: 1, M-K-X₃-X₄-D-X₆-1-A-A-X₁₀-X₁₁-V-L-X₁₄-G-M-A-K-X₁₉-G-X₂₁-F-A-X₂₄-X₂₅ (SEQ ID NO: 1) wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is an amino acid selected from the group consisting of N and D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from the group consisting of I, V, Y and W, and X₂₅ is an amino acid selected from the group consisting of K and R.

[0038] In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 1, wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is N, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is I, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is E, X₂₄ is an amino acid selected from the group consisting of I, W, and Y, and X₂₅ is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 1, wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from the group consisting of I, W, and Y, and X₂₅ is K. In still other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 1, wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from the group consisting of I, W, and Y, and X₂₅ is K or R.

[0039] In certain embodiments, a peptide of the invention comprises or consists of the sequence M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 3); M-K-R-D-D-N-1-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 4); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 5); M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 6); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-V-K (SEQ ID NO: 7); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-V-K (SEQ ID NO: 8); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-V-K (SEQ ID NO: 9); M-K-R-D-D-N-1-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-V-K (SEQ ID NO: 10); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-W-K (SEQ ID NO: 11); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-W-K (SEQ ID NO: 12); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-W-K (SEQ ID NO: 13); M-K-R-D-D-N-I-A-A-Y-M-V-L-K-G-M-A-K-N-G-E-F-A-W-K (SEQ ID NO: 14); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-Y-K (SEQ ID NO: 15); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-Y-K (SEQ ID NO: 16); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-Y-K (SEQ ID NO: 17); M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-E-F-A-Y-K (SEQ ID NO: 18); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-I-K (SEQ ID NO: 19); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-I-K (SEQ ID NO: 20); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-I-K (SEQ ID NO: 21); M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-I-K (SEQ ID NO: 22); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-V-K (SEQ ID NO: 23); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-V-K (SEQ ID NO: 24); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-V-K (SEQ ID NO: 25); M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-V-K (SEQ ID NO: 26); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-W-K (SEQ ID NO: 27); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-W-K (SEQ ID NO: 28); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-W-K (SEQ ID NO: 29); M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-W-K (SEQ ID NO: 30); M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-Y-K (SEQ ID NO: 31); M-K-R-D-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-Q-F-A-Y-K (SEQ ID NO: 32); M-K-R-N-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-Y-K (SEQ ID NO: 33); or M-K-R-D-D-N-I-A-A-V-M-V-L-K-G-M-A-K-N-G-Q-F-A-Y-K (SEQ ID NO: 34).

[0040] In other embodiments, a peptide of the invention comprises or consists of the sequence M-K-R-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 35); M-K-Q-N-D-N-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID

NO: 36); M-K-R-N-D-E-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 37); M-K-Q-N-D-E-I-A-A-V-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 38); M-K-R-N-D-N-I-A-A-F-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 39); M-K-Q-N-D-N-I-A-A-F-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 40); M-K-R-N-D-E-I-A-A-F-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 41); M-K-Q-N-D-E4-A-A-F-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 42); M-K-R-N-D-N-I-A-A-L-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 43); M-K-Q-N-D-N-1-A-A-L-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 44); M-K-R-N-D-E-I-A-A-L-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 45); M-K-Q-N-D-E-I-A-A-L-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 46); M-K-R-N-D-N-I-A-A-V-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 47); M-K-Q-N-D-N-I-A-A-V-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 48); M-K-R-N-D-E-I-A-A-V-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 49); M-K-Q-N-D-E-I-A-A-V-I-Y-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 50); M-K-R-N-D-N-I-A-A-F-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 51); M-K-Q-N-D-N-I-A-A-F-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 52); M-K-R-N-D-E-I-A-A-F-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 53); M-K-Q-N-D-E-I-A-A-F-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 54); M-K-R-N-D-N-I-A-A-L-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 55); M-K-Q-N-D-N-I-A-A-L-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 56); M-K-R-N-D-E-I-A-A-L-I-V-L-Q-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 57); M-K-Q-N-D-E-I-A-A-L-I-V-L-K-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 58); M-K-R-N-D-N-I-A-A-V-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 59); M-K-Q-N-D-N-I-A-A-V-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 60); M-K-R-N-D-E-I-A-A-V-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 61); M-K-Q-N-D-E-I-A-A-V-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 62); M-K-R-N-D-N-I-A-A-F-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 63); M-K-Q-N-D-N-I-A-A-F-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 64); M-K-R-N-D-E-I-A-A-F-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 65); M-K-Q-N-D-E-I-A-A-F-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 66); M-K-R-N-D-N-I-A-A-L-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 67); M-K-Q-N-D-N-I-A-A-L-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 68); M-K-R-N-D-E-I-A-A-L-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 69); or M-K-Q-N-D-E-I-A-A-L-I-V-L-H-G-M-A-K-N-G-E-F-A-I-K (SEQ ID NO: 70).

[0041] In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1 and an additional N-terminal peptide sequence (*e.g.*, an N-terminal extension). The additional N-terminal peptide sequence can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or more amino acids. In certain embodiments, the N-terminal peptide sequence has a length of about 5 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 40, or about 40 to about 50 amino acids. The additional N-terminal peptide sequence can be a native sequence. As used herein, a "native" sequence is a peptide sequence from a naturally-occurring *Borrelia* VlsE sequence, or a variant thereof. In certain embodiments, the peptide sequence is a fragment of a naturally-occurring *Borrelia* VlsE sequence. The peptide sequence can be, *e.g.*, from a conserved or non-conserved region of VlsE. The peptide sequence can comprise, *e.g.*, an epitope, such as an immunodominant epitope or any other epitope recognizable by a host (*e.g.*, human, dog, etc.) immune system. VlsE proteins and peptides thereof have been described, *e.g.*, in U.S. Patent Nos. 6,475,492, 6,660,274, 6,719,983, and 6,740,744, U.S. Patent Application 2009/0162875, and European Patent Nos. 0894143, 1012181, 1171605, and 1589109.

[0042] Variant polypeptides are at least about 80, 85, 90, 95, 98, or 99% identical to a peptide shown in (SEQ ID NO: 1-70). Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. *See, e.g.*, Lesk, Ed., Computational Molecular Biology, Oxford University Press, New York, (1988); Smith, Ed., Biocomputing: Informatics And Genome Projects, Academic Press, New York, (1993); Griffin & Griffin, Eds., Computer Analysis Of Sequence Data, Part I, Humana Press, New Jersey, (1994); von Heinje, Sequence Analysis In Molecular Biology, Academic Press, (1987); and Gribskov & Devereux, Eds., Sequence Analysis Primer, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux et al., Nuc. Acids Res. 12:387 (1984)), BLASTP, BLASTN, FASTA (Atschul et al., J Molec. Biol. 215:403 (1990)), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711) which uses the local homology algorithm of Smith and Waterman (Adv. App. Math., 2:482-489 (1981)). For example, the computer program ALIGN which employs the FASTA algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

[0043] When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

[0044] Variants of the peptide sequences can be readily selected by one of skill in the art, based in part on known properties of the sequence. For example, a variant peptide can include amino acid substitutions (*e.g.*, conservative amino acid substitutions) and/or deletions (*e.g.*, small, single amino acid deletions, or deletions encompassing 2, 3, 4, 5, 10, 15, 20, or more contiguous amino acids). Thus, in certain embodiments, a variant of a native peptide sequence is one that differs from a naturally-occurring sequence by (i) one or more (*e.g.*, 2, 3, 4, 5, 6, or more) conservative amino acid substitutions, (ii) deletion of 1 or more (*e.g.*, 2, 3, 4, 5, 6, or more) amino acids, or (iii) a combination thereof. Deleted amino acids can be contiguous or non-contiguous. Conservative amino acid substitutions are those that take place within

a family of amino acids that are related in their side chains and chemical properties. These include, e.g., (1) acidic amino acids: aspartate, glutamate; (2) basic amino acids: lysine, arginine, histidine; (3) nonpolar amino acids: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; (4) uncharged polar amino acids: glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine; (5) aliphatic amino acids: glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally grouped separately as aliphatic-hydroxyl; (6) aromatic amino acids: phenylalanine, tyrosine, tryptophan; (7) amide amino acids: asparagine, glutamine; and (9) sulfur-containing amino acids: cysteine and methionine. See, e.g., Biochemistry, 2nd ed., Ed. by L. Stryer, W H Freeman and Co.: 1981. Methods for confirming that variant peptides are suitable are conventional and routine.

[0045] Variants of the peptide sequences encompass variations on previously defined peptide sequences. For example, a previously described peptide sequence comprising a known epitope may be lengthened or shortened, at one or both ends (e.g., by about 1-3 amino acids), and/or one, two, three, four or more amino acids may be substituted by conservative amino acids, etc. Furthermore, if a region of a protein has been identified as containing an epitope of interest, an investigator can "shift" the region of interest (e.g., by about 5 amino acids in either direction) from the endpoints of the original rough region to optimize the activity.

[0046] In certain embodiments, the additional N-terminal peptide sequence can comprise or consist of another IR6 domain peptide. In other embodiments, the native sequence is a VlsE sequence that is naturally adjacent to the N-terminal end of a VlsE IR6 domain.

[0047] In certain embodiments, the additional N-terminal peptide sequence is a non-native sequence. As used herein, a "non-native" sequence is any protein sequence, whether from a *Borrelia* protein or otherwise, other than a native VlsE peptide sequence. In certain embodiments, the additional N-terminal peptide sequence comprises an epitope of a *Borrelia* antigen, such as OspA, OspB, DbpA, flagella-associated proteins FlaA (p37) and FlaB (p41), OspC (25 kd), BBK32, BmpA (p39), p21, p39, p66 or p83. Polypeptides or peptides derived from other microorganisms can also be used.

[0048] A peptide of the invention comprising an additional N-terminal peptide sequence can be designed for diagnosing *Borrelia* infections early after infection (e.g., within one to two weeks after the onset of infection). Among the pathogenic *Borrelia* proteins whose expression has been recognized in early stages of infection (e.g., to which IgM antibody appears early after infection) are OspC, BBK32, the flagella-associated protein FlaB (p41), and, to a lesser extent, BmpA (p39), and the flagella-associated protein FlaA (p37). Polypeptides or peptides which derive from those polypeptides are suitable for assays for early infection. For example, some suitable linear epitopes which can be used for the diagnosis of early infection include peptides in OspC: PVVAESPCKP (SEQ ID NO: 71), ILMTLFLFISCNNS (SEQ ID NO: 72), and one or more epitopes contained between amino acids 161 and 210, as reported by Jobe et al. (2003) Clin Diagn Lab Immunol 10, 573-8). The OspC peptides described in U.S. Pat. No. 6,716,574 can also be used. Other suitable regions, which have been shown not to contain major cross-reactive epitopes, have been identified in FlaB (p41), such as residues 120 to 235. See, e.g., Crother et al. ((2003) Infect. Immun. 71, 3419-3428; Wang et al. (1999)) Clin Microbial Rev 12, 633-653; and U.S. Patent Nos. 5,618,533, 5,643,733, 5,643,751, 5,932,220, and 6,617,441. Other peptides bearing either linear or conformational epitopes are known in the art. Methods for identifying additional non-native epitope sequences, particularly from variable regions of, e.g., OspC, BBK32 or DbpA, are discussed in, e.g., US 2009/0162875.

[0049] In certain embodiments, the additional N-terminal peptide sequence is from OspC. For example, in certain embodiments, the additional N-terminal peptide sequence is a sequence of SEQ ID NO: 73, n_1 - n_2 -S-P- n_5 - n_6 -P (SEQ ID NO: 73) or a fragment thereof (e.g., a C-terminal fragment), wherein n_1 is an amino acid selected from the group consisting of A and V, n_2 is an amino acid selected from the group consisting of E and D, n_5 is an amino acid selected from the group consisting of K and R, and n_6 is an amino acid selected from the group consisting of K and R.

[0050] In certain embodiments, the additional N-terminal peptide sequence is a combination of sequences. For example, the additional N-terminal peptide sequence can comprise a native, a non-native sequence, or any combination of such sequences (e.g., two or more native sequences, two or more non-native sequence, a native and non-native sequence, etc.).

[0051] In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1 and further comprise an additional C-terminal sequence. The additional C-terminal peptide sequence can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, or more amino acids. The additional C-terminal peptide sequence can be a native sequence. For example, in certain embodiments, the additional C-terminal peptide sequence can comprise or consist of another IR6 domain peptide. In other embodiments, the native sequence is a VlsE sequence that is naturally adjacent to the C-terminal end of a VlsE IR6 domain.

[0052] In certain embodiments, the additional C-terminal peptide sequence is a non-native sequence. In certain embodiments, the additional C-terminal peptide sequence comprises an epitope of a *Borrelia* antigen, such as OspA, OspB, DbpA, flagella-associated proteins FlaA (p37) and FlaB (p41), OspC (25 kd), BBK32, BmpA (p39), p21, p39, p66 or p83, as discussed above. Polypeptides or peptides derived from other microorganisms can also be used.

[0053] In certain embodiments, the additional C-terminal peptide sequence is from FlaB (p41). For example, in certain embodiments, the additional C-terminal peptide sequence is a sequence of SEQ ID NO: 74, V-c₂-E-G-c₅-Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 74) or a fragment thereof (e.g., an N-terminal fragment), wherein c₂ is an amino acid selected

from the group consisting of Q and R, and c_5 is an amino acid selected from the group consisting of V and A. In other embodiments, the additional C-terminal peptide sequence is a sequence of SEQ ID NO: 76, A-V- c_3 -E-G- c_6 -Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 76) or a fragment thereof (e.g., an N-terminal fragment), wherein c_3 is an amino acid selected from the group consisting of Q and R, and c_6 is an amino acid selected from the group consisting of V and A.

[0054] In certain embodiments, the additional C-terminal peptide sequence is a combination of sequences. For example, the additional C-terminal peptide sequence can comprise a native, a non-native sequence, or any combination of such sequences (e.g., two or more native sequences, two or more non-native sequence, a native and non-native sequence, etc.).

[0055] In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1 and further comprise an additional N-terminal peptide sequence and an additional C-terminal peptide sequence. The additional N-terminal and C-terminal peptide sequences can be as described above. Peptides do not consist of a full-length VIsE protein. However, peptides can comprise a full-length VIsE protein. In other embodiments, peptides do not comprise a full-length VIsE protein.

[0056] In addition to the sequences described above, the additional N-terminal and C-terminal sequences can comprise or consist of a flexible sequence, designed to better present the peptides of the invention for detection in an immunoassay (e.g., ELISA assay, lateral flow immunoassay, agglutination assay, etc.). Such flexible sequences can be readily identified by persons skilled in the art.

[0057] Peptides may comprise or consist of a sequence of SEQ ID NO:2, n_1 - n_2 - n_3 - n_4 - n_5 - n_6 - n_7 -Q-D- n_{10} -M-K- X_{13} - X_{14} -D- X_{16} -I-A-A- X_{20} - X_{21} -V-L- X_{24} -G-M-A-K- X_{29} -G- X_{31} -F-A- X_{34} - X_{35} -D-N-E- c_{39} -D- c_{41} -A-E- c_{44} -G (SEQ ID NO:2) wherein n_1 is an amino acid selected from the group consisting of N and D, n_2 is an amino acid selected from the group consisting of N and D, n_3 is an amino acid selected from the group consisting of A and V, n_4 is an amino acid selected from the group consisting of A and V, n_5 is an amino acid selected from the group consisting of A and V, n_6 is an amino acid selected from the group consisting of F and Y, n_7 is an amino acid selected from the group consisting of S and T, n_{10} is an amino acid selected from the group consisting of D, E, Q, and N, X_{13} is an amino acid selected from the group consisting of R and Q, X_{14} is an amino acid selected from the group consisting of N and D, X_{16} is an amino acid selected from the group consisting of N and E, X_{20} is an amino acid selected from the group consisting of V, F and L, X_{21} is an amino acid selected from the group consisting of I and M, X_{24} is an amino acid selected from the group consisting of K, Q and H, X_{29} is an amino acid selected from the group consisting of N, Q and E, X_{31} is an amino acid selected from the group consisting of E and Q, X_{34} is an amino acid selected from the group consisting of I, V, Y and W, X_{35} is an amino acid selected from the group consisting of K and R, c_{39} is an amino acid selected from the group consisting of H and D, c_{41} is an amino acid selected from the group consisting of K and R, and c_{44} is an amino acid selected from the group consisting of K and R. In certain related peptides, n_{10} is an amino acid selected from the group consisting of D, Q, and N, X_{14} is N, X_{21} is I, X_{31} is E, X_{34} is an amino acid selected from the group consisting of I, W, and Y, and X_{35} is K. In other related peptides, n_{10} is an amino acid selected from the group consisting of Q and N, X_{14} is N, X_{21} is I, X_{31} is E, X_{34} is an amino acid selected from the group consisting of I, W, and Y, and X_{35} is K. In other related peptides, n_{10} is an amino acid selected from the group consisting of D, Q, and N, X_{14} is D, and X_{34} is an amino acid selected from the group consisting of I, W, and Y. In still other related peptides, n_{10} is an amino acid selected from the group consisting of Q and N, X_{14} is D, and X_{34} is an amino acid selected from the group consisting of I, W, and Y.

[0058] Peptides may comprise a sequence defined by SEQ ID NO: 2 and further comprise an additional N-terminal peptide sequence, an additional C-terminal peptide sequence, or a combination thereof. The additional N-terminal and C-terminal peptide sequences can be as described above.

[0059] In certain embodiments, peptides of the invention comprise or consist of 25 or more (e.g., 26, 27, 28, 29, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 30 or more (e.g., 31, 32, 33, 34, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 35 or more (e.g., 36, 37, 38, 39, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 40 or more (e.g., 41, 42, 43, 44, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 45 or more (e.g., 46, 47, 48, 49, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 50 or more (e.g., 51, 52, 53, 54, or more) amino acid residues. In certain embodiments, peptides of the invention comprise or consist of 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more amino acid residues.

[0060] In certain embodiments, peptides of the invention comprise an epitope of a peptide sequence described herein. For example, in certain embodiments, peptides of the invention comprise an epitope of a sequence selected from the group consisting of SEQ ID NO: 1-70.

[0061] Peptides may comprise a fragment of a peptide sequence described herein. For example, peptides may comprise a fragment of a sequence selected from the group consisting of SEQ ID NO:1-70. The fragment can be, e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44 amino acids in length. The fragment can be contiguous or can include one or more deletions (e.g., a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acid residues). The fragment may comprise a

sequence set forth in U.S. Patent Nos. 6,475,492, 6,660,274, 6,719,983, or 6,740,744, U.S. Patent Application 2009/0162875, or European Patent Nos. 0894143, 1012181, 1171605, or 1589109. The fragment may not consist of a sequence set forth in one or more of U.S. Patent Nos. 6,475,492, 6,660,274, 6,719,983, and 6,740,744, U.S. Patent Application 2009/0162875, and European Patent Nos. 0894143, 1012181, 1171605, and 1589109. Peptides that comprise a fragment of a peptide sequence described herein can further comprise an additional N-terminal peptide sequence, an additional C-terminal peptide sequence, or a combination thereof. The additional N-terminal and C-terminal peptide sequences can be as described above.

[0062] Peptides of the invention comprising an additional N-terminal or C-terminal peptide sequence can further comprise a linker connecting the peptide with the additional N-terminal or C-terminal peptide sequence. The linker can be, e.g., a peptide spacer. Such spacer can consist of, for example, between about one and five (e.g., about three) amino acid residues, preferably uncharged amino acids, e.g., aliphatic residues such as glycine or alanine. In one embodiment, the spacer is a triplet glycine spacer. In another embodiment, the spacer is a triplet alanine spacer. In yet another embodiment, the spacer comprises both glycine and alanine residues. Alternatively, the linker can be a chemical (i.e., non-peptide) linker.

[0063] In certain embodiments, peptides of the invention are produced by synthetic chemistry (i.e., a "synthetic peptide"). In other embodiments, peptides of the invention are produced biologically (i.e., by cellular machinery, such as a ribosome). Peptides of the invention are isolated. As used herein, an "isolated" peptide is a peptide that has been produced either synthetically or biologically and then purified, at least partially, from the chemicals and/or cellular machinery used to produce the peptide. An isolated peptide may be substantially purified. The term "substantially purified," as used herein, refers to a molecule, such as a peptide, that is substantially free of cellular material (proteins, lipids, carbohydrates, nucleic acids, etc.), culture medium, chemical precursors, chemicals used in synthesis of the peptide, or combinations thereof. A peptide that is substantially purified has less than about 40%, 30%, 25%, 20%, 15%, 10%, 5%, 2%, 1% or less of the cellular material, culture medium, other polypeptides, chemical precursors, and/or chemicals used in synthesis of the peptide. Accordingly, a substantially pure molecule, such as a peptide, can be at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, by dry weight, the molecule of interest. An isolated peptide of the invention can be in water, a buffer, or in a dry form awaiting reconstitution, e.g., as part of a kit. An isolated peptide of the present invention can be in the form of a pharmaceutically acceptable salt. Suitable acids and bases that are capable of forming salts with the peptides of the present invention are well known to those of skill in the art, and include inorganic and organic acids and bases.

[0064] In certain embodiments, peptides of the invention are affinity purified. For example, in certain embodiments, the peptides of the invention are purified by means of their ability to bind to anti-*Borrelia* antibodies (e.g., antibodies to VlsE proteins and, optionally, other *Borrelia* antigens) by contacting such antibodies with the peptides of the invention such that peptide-antibody complexes are able to form, washing the peptide-antibody complexes to remove impurities, and then eluting the peptides from the antibodies. The antibodies can be, e.g., attached to a solid support. Methods of affinity purification are well-known and routine to those skilled in the art.

[0065] In certain embodiments, peptides of the invention are modified. The peptides of the invention may be modified by a variety of techniques, such as by denaturation with heat and/or a detergent (e.g., SDS). Alternatively, peptides of the invention may be modified by association with one or more further moieties. The association can be covalent or non-covalent, and can be, for example, via a terminal amino acid linker, such as lysine or cysteine, a chemical coupling agent, or a peptide bond. The additional moiety can be, for example, a ligand, a ligand receptor, a fusion partner, a detectable label, an enzyme, or a substrate that immobilizes the peptide.

[0066] Peptides of the invention can be conjugated to a ligand, such as biotin (e.g., via a cysteine or lysine residue), a lipid molecule (e.g., via a cysteine residue), or a carrier protein (e.g., serum albumin, immunoglobulin Fc domain via e.g., a cysteine or lysine residue). Attachment to ligands, such as biotin, can be useful for associating the peptide with ligand receptors, such as avidin, streptavidin, polymeric streptavidin (see e.g., US 2010/0081125 and US 2010/0267166), or neutravidin. Avidin, streptavidin, polymeric streptavidin, neutravidin, in turn, can be linked to a signaling moiety (e.g., a moiety that can be visualized, such as colloidal gold, a fluorescent moiety, or an enzyme (horseradish peroxidase or alkaline phosphatase) or a solid substrate (e.g., an Immobilon or nitrocellulose membrane). Alternatively, the peptides of the invention can be fused or linked to a ligand receptor, such as avidin, streptavidin, polymeric streptavidin, or neutravidin, thereby facilitating the association of the peptides with the corresponding ligand, such as biotin and any moiety (e.g., signaling moiety) or solid substrate attached thereto. Examples of other ligand-receptor pairs are well-known in the art and can similarly be used.

[0067] Peptides of the invention can be fused to a fusion partner (e.g., a peptide or other moiety) that can be used to improve purification, to enhance expression of the peptide in a host cell, to aid in detection, to stabilize the peptide, etc. Examples of suitable compounds for fusion partners include carrier proteins (e.g., serum albumin, immunoglobulin Fc domain), beta-galactosidase, glutathione-S-transferase, a histidine tag, etc. The fusion can be achieved by means of, e.g., a peptide bond. For example, peptides of the invention and fusion partners can be fusion proteins and can be directly fused in-frame or can comprise a peptide linker, as discussed above in the context of additional N-terminal and

C-terminal peptide sequences.

[0068] In addition, peptides of the invention may be modified to include any of a variety of known chemical groups or molecules. Such modifications include, but are not limited to, glycosylation, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment to polyethylene glycol (*e.g.*, PEGylation), covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, ubiquitination, modifications with fatty acids, transfer-RNA mediated addition of amino acids to proteins such as arginylation, etc. Analogues of an amino acid (including unnatural amino acids) and peptides with substituted linkages are also included. Peptides of the invention that consist of any of the sequences discussed herein may be modified by any of the discussed modifications. Such peptides still "consist of" the amino acids.

[0069] Modifications as set forth above are well-known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in many basic texts, such as *Proteins-Structure and Molecular Properties*, 2nd ed., T. E. Creighton, W.H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as by Wold, F., *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York 1-12 (1983); Seifter et al. (1990) *Meth. Enzymol.* 182:626-646 and Rattan et al. (1992) *Ann. N.Y. Acad. Sci.* 663:48-62.

[0070] In certain embodiments, peptides of the invention are attached to or immobilized on a substrate, such as a solid or semi-solid support. The attachment can be covalent or non-covalent, and can be facilitated by a moiety associated with the peptide that enables covalent or non-covalent binding, such as a moiety that has a high affinity to a component attached to the carrier, support or surface. For example, the peptide can be associated with a ligand, such as biotin, and the component associated with the surface can be a corresponding ligand receptor, such as avidin. The peptide can be attached to or immobilized on the substrate either prior to or after the addition of a sample containing antibody during an immunoassay.

[0071] In certain embodiments, the substrate is a bead, such as a colloidal particle (*e.g.*, a colloidal nanoparticle made from gold, silver, platinum, copper, metal composites, other soft metals, core-shell structure particles, or hollow gold nanospheres) or other type of particle (*e.g.*, a magnetic bead or a particle or nanoparticle comprising silica, latex, polystyrene, polycarbonate, polyacrylate, or PVDF). Such particles can comprise a label (*e.g.*, a colorimetric, chemiluminescent, or fluorescent label) and can be useful for visualizing the location of the peptides during immunoassays. In certain embodiments, a terminal cysteine of a peptide of the invention is used to bind the peptide directly to the nanoparticles made from gold, silver, platinum, copper, metal composites, other soft metals, etc.

[0072] In certain embodiments, the substrate is a dot blot or a flow path in a lateral flow immunoassay device. For example, the peptides can be attached or immobilized on a porous membrane, such as a PVDF membrane (*e.g.*, an Immobilon™ membrane), a nitrocellulose membrane, polyethylene membrane, nylon membrane, or a similar type of membrane.

[0073] In certain embodiments, the substrate is a flow path in an analytical rotor. In other embodiments, the substrate is a tube or a well, such as a well in a plate (*e.g.*, a microtiter plate) suitable for use in an ELISA assay. Such substrates can comprise glass, cellulose-based materials, thermoplastic polymers, such as polyethylene, polypropylene, or polyester, sintered structures composed of particulate materials (*e.g.*, glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone, or the like. A substrate can be sintered, fine particles of polyethylene, commonly known as porous polyethylene, for example, 0.2-15 micron porous polyethylene from Chromex Corporation (Albuquerque, NM). All of these substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

[0074] Also described herein are devices. The devices may be useful for performing an immunoassay. For example, the device may be a lateral flow immunoassay device. Alternatively, the device may be an analytical rotor. Alternatively, the device may be a dot blot. Alternatively, the device may be a tube or a well, *e.g.*, in a plate suitable for an ELISA assay. Alternatively, the device may be an electrochemical sensor, an optical sensor, or an opto-electronic sensor.

[0075] The device may comprise a peptide described herein. Alternatively, the device comprises a mixture of different peptides. For example, the device may comprise two, three, four, or more different peptides. The peptide or each peptide in the mixture may comprise a sequence of SEQ ID NO:1 or SEQ ID NO:2. The peptides may be attached to or immobilized upon the device.

[0076] Compositions comprising peptides described herein may comprise a mixture of 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, or more peptides (*e.g.*, all possible peptides defined

by SEQ ID NO:1 or SEQ ID NO:2). The peptides may be modified (e.g., by association with one or more further moieties), as described herein.

[0077] The compositions may comprise peptides of the invention and one or more additional peptides, such as a *Borrelia* peptide or antigen, a peptide or antigen from one or more infectious *Borrelia* species, or a peptide or antigen from one or more causative agents of Lyme disease. The *Borrelia* peptide or antigen can be any *Borrelia* peptide or antigen described herein (e.g., an OspA, OspB, DbpA, flagella-associated proteins FlaA (p37) and FlaB (p41), OspC (25 kd), BBK32, BmpA (p39), p21, p39, p66, p83, or VlsE protein), or any fragment or epitope thereof. Some suitable *Borrelia* peptides have been described, e.g., in U.S. Patent Application 2009/0162875. The combination may comprise a cocktail (a simple mixture) of individual peptides or polypeptide, it may be in the form of a fusion peptide or polypeptide (e.g., a multimeric peptide), or the peptides may be linked by a dendrimer (e.g., as in a MAPS structure) optionally through a linking residue (e.g. lysine residue). A peptide of the invention may be fused at its N-terminus or C-terminus to another suitable peptide. Two or more copies of a peptide of the invention may be joined to one another, alone or in combination with one or more additional peptides. Combinations of fused and unfused peptides or polypeptides can be used. In one embodiment, the additional peptide(s) contain B-cell and/or T-cell epitopes from a *Borrelia* peptide or antigen, a peptide or antigen from an infectious *Borrelia* species, or a peptide or antigen from a causative agent of Lyme disease.

[0078] Also described herein are nucleic acids comprising a sequence encoding a peptide of the invention. Nucleic acids as described herein contain less than an entire microbial genome and can be single- or double-stranded. A nucleic acid can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The nucleic acids can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the nucleic acids can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% purified. The nucleic acids encode the peptides described herein. The nucleic acids may encode a peptide having the sequence of SEQ ID NO: 1-70 or combinations thereof. Nucleic acids can comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and staphylococcal protein A.

[0079] Nucleic acids can be isolated. An "isolated" nucleic acid is one that is not immediately contiguous with one or both of the 5' and 3' flanking genomic sequences that it is naturally associated with. An isolated nucleic acid can be, e.g., a recombinant DNA molecule of any length, provided that the nucleic acid sequences naturally found immediately flanking the recombinant DNA molecule in a naturally-occurring genome is removed or absent. Isolated nucleic acids also include non-naturally occurring nucleic acid molecules. Nucleic acids can also comprise fragments that encode immunogenic peptides. Nucleic acids can encode full-length polypeptides, peptide fragments, and variant or fusion peptides.

[0080] Nucleic acids can be isolated, at least in part, from nucleic acid sequences present in, for example, a biological sample, such as blood, serum, saliva, or tissue from an infected individual. Nucleic acids can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify nucleic acids, at least in part, from either genomic DNA or cDNA encoding the polypeptides.

[0081] Nucleic acids can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences that do not occur in nature. If desired, nucleic acids can be cloned into an expression vector comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the invention in host cells. An expression vector can be, for example, a plasmid, such as pBR322, pUC, or ColE1, or an adenovirus vector, such as an adenovirus Type 2 vector or Type 5 vector. Optionally, other vectors can be used, including but not limited to Sindbis virus, simian virus 40, alphavirus vectors, poxvirus vectors, and cytomegalovirus and retroviral vectors, such as murine sarcoma virus, mouse mammary tumor virus, Moloney murine leukemia virus, and Rous sarcoma virus. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda cos sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

[0082] Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. See, e.g., U.S. Pat. No. 4,366,246. A nucleic acid is operably linked when it is positioned adjacent to or close to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

[0083] Thus, for example, a peptide of the invention can be produced recombinantly following conventional genetic engineering techniques. To produce a recombinant peptide of the invention, a nucleic acid encoding the peptide is inserted into a suitable expression system. Generally, a recombinant molecule or vector is constructed in which the polynucleotide sequence encoding the selected peptide is operably linked to an expression control sequence permitting expression of the peptide. Numerous types of appropriate expression vectors are known in the art, including, e.g., vectors containing bacterial, viral, yeast, fungal, insect or mammalian expression systems. Methods for obtaining and using such expression vectors are well-known. For guidance in this and other molecular biology techniques used for compo-

sitions or methods described herein, *see, e.g.*, Sambrook et al., *Molecular Cloning, A Laboratory Manual*, current edition, Cold Spring Harbor Laboratory, New York; Miller et al., *Genetic Engineering*, 8:277-298 (Plenum Press, current edition), Wu et al., *Methods in Gene Biotechnology* (CRC Press, New York, N.Y., current edition), *Recombinant Gene Expression Protocols*, in *Methods in Molecular Biology*, Vol. 62, (Tuan, ed., Humana Press, Totowa, N.J., current edition), and *Current Protocols in Molecular Biology*, (Ausabel et al, Eds.) John Wiley & Sons, NY (current edition), and references cited therein.

[0084] Also described herein are vectors comprising nucleic acids, and host cells comprising such vectors. A vector may be a shuttle vector. Alternatively, the vector may be an expression vector (*e.g.*, a bacterial or eukaryotic expression vector). A host cell may be a bacterial cell. Alternatively, a host cell may be a eukaryotic cell.

[0085] Suitable host cells or cell lines for transfection by this method include bacterial cells. For example, various strains of *E. coli* (*e.g.*, HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, *Streptomyces*, and other bacilli and the like can also be employed in this method. Alternatively, a peptide can be expressed in yeast, insect, mammalian, or other cell types, using conventional procedures.

[0086] Also described herein is a method for producing a recombinant peptide or polypeptide, which involves transfecting or transforming, *e.g.*, by conventional means such as electroporation, a host cell with at least one expression vector containing a polynucleotide under the control of an expression control sequence (*e.g.*, a transcriptional regulatory sequence). The transfected or transformed host cell is then cultured under conditions that allow expression of the peptide or polypeptide. The expressed peptide or polypeptide is recovered, isolated, and optionally purified from the cell (or from the culture medium, if expressed extracellularly) by appropriate means known to one of skill in the art, including liquid chromatography such as normal or reversed phase, using HPLC, FPLC and the like, affinity chromatography, such as with inorganic ligands or monoclonal antibodies, size exclusion chromatography, immobilized metal chelate chromatography, gel electrophoresis, and the like. One of skill in the art may select the most appropriate isolation and purification techniques without departing from the scope of this invention. One skilled in the art can determine the purity of the peptide or polypeptide by using standard methods including, *e.g.*, polyacrylamide gel electrophoresis (*e.g.*, SDS-PAGE), capillary electrophoresis, column chromatography (*e.g.*, high performance liquid chromatography (HPLC)), or amino-terminal amino acid analysis.

Methods

[0087] In another aspect, the invention provides methods of detecting in a sample an antibody to an epitope of a *Borrelia* antigen, the method comprising contacting a sample with the mixture of isolated peptides of the invention, and detecting formation of an antibody-peptide complex comprising one or more of said peptides, wherein formation of said complex is indicative of the presence of an antibody to an epitope of a *Borrelia* antigen in said sample. In certain embodiments, the *Borrelia* antigen is from an infectious *Borrelia* species. In certain embodiments, the *Borrelia* antigen is from a pathogenic *Borrelia* species, such as *Borrelia burgdorferi sensu stricto*, *Borrelia afzelli*, or *Borrelia garinii*. Other species of *Borrelia* which have been implicated in Lyme disease, such as *B. lusitaniae* and *B. valaisianae*, can also be detected using the methods of the invention, provided they induce antibodies which can react specifically with a peptide of the invention. Thus, it is to be understood that the term "pathogenic *Borrelia*," as used herein, refers to any such *Borrelia* species that causes Lyme disease.

[0088] In certain embodiments, the methods comprise contacting the sample with a mixture of three, four or more (*e.g.*, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, or more) different peptides of the invention. In certain embodiments, the methods comprise contacting the sample with a mixture of one or more peptides of the invention and one or more other peptides (*e.g.*, a *Borrelia* peptide, or antigenic fragment or epitope thereof, such as an OspA, OspB, DbpA, flagella-associated proteins FlaA (p37) and FlaB (p41), OspC (25 kd), BBK32, BmpA (p39), p21, p39, p66, p83, or VlsE protein).

[0089] In certain embodiments, the mixture of peptides is attached to or immobilized upon a solid support. In certain embodiments, the solid support is a bead (*e.g.*, a colloidal particle, nanoparticle, latex bead, etc.), a flow path in a lateral flow immunoassay device (*e.g.*, a porous membrane), a flow path in an analytical rotor, a tube or a well (*e.g.*, in a plate suitable for an ELISA assay), or a sensor (*e.g.*, an electrochemical, optical, or opto-electronic sensor).

[0090] In certain embodiments, the detecting step comprises performing an ELISA assay. In other embodiments, the detecting step comprises performing a lateral flow immunoassay. In other embodiments, the detecting step comprises performing an agglutination assay. In other embodiments, the detecting step comprises spinning the sample in an analytical rotor. In still other embodiments, the detecting step comprises analyzing the sample with an electrochemical, optical, or opto-electronic sensor.

[0091] There are a number of different conventional assays for detecting formation of an antibody-peptide complex comprising a peptide of the invention. For example, the detecting step can comprise performing an ELISA assay, performing a lateral flow immunoassay, performing an agglutination assay, analyzing the sample in an analytical rotor, or analyzing the sample with an electrochemical, optical, or opto-electronic sensor. These different assays are described

above and/or are well-known to those skilled in the art.

[0092] In one embodiment, the methods involve detecting the presence of naturally occurring antibodies against a *Borrelia* antigen (e.g., the antigen of a pathogenic *Borrelia*, such as *B. Burgdorferi*) which are produced by the infected subject's immune system in its biological fluids or tissues, and which are capable of binding specifically to a peptide of the invention or combinations of a peptide of the invention and, optionally, one or more suitable additional antigenic polypeptides or peptides.

[0093] Suitable immunoassay methods typically include: receiving or obtaining (e.g., from a patient) a sample of body fluid or tissue likely to contain antibodies; contacting (e.g., incubating or reacting) a sample to be assayed with a mixture of peptides of the invention, under conditions effective for the formation of a specific peptide-antibody complex (e.g., for specific binding of the peptide to the antibody); and assaying the contacted (reacted) sample for the presence of an antibody-peptide reaction (e.g., determining the amount of an antibody-peptide complex). The presence of an elevated amount of the antibody-peptide complex indicates that the subject was exposed to and infected with an infectious *Borrelia* species. A peptide, including a modified form thereof, which "binds specifically" to (e.g., "is specific for" or binds "preferentially" to) an antibody against a *Borrelia* antigen interacts with the antibody, or forms or undergoes a physical association with it, in an amount and for a sufficient time to allow detection of the antibody. By "specifically" or "preferentially," it is meant that the peptide has a higher affinity (e.g., a higher degree of selectivity) for such an antibody than for other antibodies in a sample. For example, the peptide can have an affinity for the antibody of at least about 1.5-fold, 2-fold, 2.5-fold, 3-fold, or higher than for other antibodies in the sample. Such affinity or degree of specificity can be determined by a variety of routine procedures, including, e.g., competitive binding studies. In an ELISA assay, a positive response is defined as a value 2 or 3 standard deviations greater than the mean value of a group of healthy controls. In some embodiments, a second tier assay is required to provide an unequivocal serodiagnosis of Lyme disease.

[0094] Phrases such as "sample containing an antibody" or "detecting an antibody in a sample" are not meant to exclude samples or determinations (e.g., detection attempts) where no antibody is contained or detected. In a general sense, this invention involves assays to determine whether an antibody produced in response to infection with an infectious *Borrelia* is present in a sample, irrespective of whether or not it is detected.

[0095] Conditions for reacting peptides and antibodies so that they react specifically are well-known to those of skill in the art. See, e.g., Current Protocols in Immunology (Coligan et al., editors, John Wiley & Sons, Inc).

[0096] The methods comprise receiving or obtaining a sample of body fluid or tissue likely to contain antibodies from a subject. The antibodies can be, e.g., of IgG, IgE, IgD, IgM, or IgA type. Generally, IgM and/or IgA antibodies are detected, e.g., for detection at early stages of infection. Although, in the case of a *Borrelia* infection, IgM antibodies can persist for a long time. IgG antibodies can be detected when some of the additional peptides discussed above are used in the method (e.g., peptides for the detection of flagellum proteins). The sample is preferably easy to obtain and may be serum or plasma derived from a venous blood sample or even from a finger prick. Tissue from other body parts or other bodily fluids, such as cerebro-spinal fluid (CSF), saliva, gastric secretions, mucus, urine, etc., are known to contain antibodies and may be used as a source of the sample.

[0097] Once the peptide antigen and sample antibody are permitted to react in a suitable medium, an assay is performed to determine the presence or absence of an antibody-peptide reaction. Among the many types of suitable assays, which will be evident to a skilled worker, are immunoprecipitation and agglutination assays.

[0098] The assay may comprise: immobilizing the antibody(s) in the sample; adding a mixture of peptides of the invention; and detecting the degree of antibody bound to a peptide, e.g., by the peptide being labeled or by adding a labeled substance, such as a labeled binding partner (e.g., streptavidin-colloidal gold complex) or a labeled antibody which specifically recognizes the peptide. See, e.g., Figure 1. Alternately, the assay may comprise: immobilizing a mixture of peptides of the invention; adding the sample containing antibodies; and detecting the amount of antibody bound to peptide, e.g., by adding another mixture of peptides of the invention conjugated, directly or indirectly, to a label (e.g., colloidal gold complex, fluorescent label, enzyme (e.g., horseradish peroxidase or alkaline phosphatase)) or by adding a labeled substance, such as a binding partner or a labeled antibody which specifically recognizes the sample antibodies (e.g., anti-human IgG antibodies, anti-human IgM antibodies, anti-dog IgG antibodies, anti-dog IgM antibodies, protein A, protein G, protein L, or combinations thereof, etc.). See, e.g., Figure 3. Alternatively, the assay may comprise: reacting the mixture of peptides and the sample containing antibodies without any of the reactants being immobilized, and then detecting the amount of complexes of antibody and peptide, e.g., by the peptide being labeled or by adding a labeled substance, such as a labeled binding partner (e.g., streptavidin-colloidal gold complex) or a labeled antibody which specifically recognizes the peptide.

[0099] Immobilization of a peptide of the invention can be either covalent or non-covalent, and the non-covalent immobilization can be non-specific (e.g., non-specific binding to a polystyrene surface in, e.g., a microtiter well). Specific or semi-specific binding to a solid or semi-solid carrier, support or surface, can be achieved by the peptide having, associated with it, a moiety which enables its covalent or non-covalent binding to the solid or semi-solid carrier, support or surface. For example, the moiety can have affinity to a component attached to the carrier, support or surface. In this case, the moiety may be, e.g., a biotin or biotinyl group or an analogue thereof bound to an amino acid group of the

peptide, such as 6-aminohexanoic acid, and the component is then avidin, streptavidin, neutravidin, or an analogue thereof. An alternative is a situation in which the moiety has the amino acid sequence His-His-His-His-His (SEQ ID NO:75) and the carrier comprises a Nitrilotriacetic Acid (NTA) derivative charged with Ni⁺⁺ or Co⁺⁺ ions. Suitable carriers, supports, and surfaces include, but are not limited to, beads (e.g., magnetic beads, colloidal particles or nanoparticles, such as colloidal gold, or nanoparticles comprising silica, latex, polystyrene, polycarbonate, or PDVF), latex of copolymers such as styrene-divinyl benzene, hydroxylated styrene-divinyl benzene, polystyrene, carboxylated polystyrene, beads of carbon black, non-activated or polystyrene or polyvinyl chloride activated glass, epoxy-activated porous magnetic glass, gelatin or polysaccharide particles or other protein particles, red blood cells, mono- or polyclonal antibodies or Fab fragments of such antibodies.

[0100] The protocols for immunoassays using antigens for detection of specific antibodies are well known in art. For example, a conventional sandwich assay can be used, or a conventional competitive assay format can be used. For a discussion of some suitable types of assays, see Current Protocols in Immunology (*supra*). In certain embodiments, a peptide of the invention is immobilized on a solid or semi-solid surface or carrier by means of covalent or non-covalent binding, either prior to or after the addition of the sample containing antibody.

[0101] Devices for performing specific binding assays, especially immunoassays, are known and can be readily adapted for use in the present methods. Solid phase assays, in general, are easier to perform than heterogeneous assay methods which require a separation step, such as precipitation, centrifugation, filtration, chromatography, or magnetism, because separation of reagents is faster and simpler. Solid-phase assay devices include microtiter plates, flow-through assay devices (e.g., lateral flow immunoassay devices), dipsticks, and immunocapillary or immunochromatographic immunoassay devices.

[0102] In embodiments of the invention, the solid or semi-solid surface or carrier is the floor or wall in a microtiter well, a filter surface or membrane (e.g., a nitrocellulose membrane or a PVDF (polyvinylidene fluoride) membrane, such as an Immobilon™ membrane), a hollow fiber, a beaded chromatographic medium (e.g., an agarose or polyacrylamide gel), a magnetic bead, a fibrous cellulose matrix, an HPLC matrix, an FPLC matrix, a substance having molecules of such a size that the molecules with the peptide bound thereto, when dissolved or dispersed in a liquid phase, can be retained by means of a filter, a substance capable of forming micelles or participating in the formation of micelles allowing a liquid phase to be changed or exchanged without entraining the micelles, a water-soluble polymer, or any other suitable carrier, support or surface.

[0103] In some embodiments of the invention, the peptide is provided with a suitable label which enables detection. Conventional labels may be used which are capable, alone or in concert with other compositions or compounds, of providing a detectable signal. Suitable detection methods include, e.g., detection of an agent which is tagged, directly or indirectly, with a fluorescent label by immunofluorescence microscopy, including confocal microscopy, or by flow cytometry (FACS), detection of a radioactively labeled agent by autoradiography, electron microscopy, immunostaining, subcellular fractionation, or the like. In one embodiment, a radioactive element (e.g., a radioactive amino acid) is incorporated directly into a peptide chain; in another embodiment, a fluorescent label is associated with a peptide via biotin/avidin interaction, association with a fluorescein conjugated antibody, or the like. In one embodiment, a detectable specific binding partner for the antibody is added to the mixture. For example, the binding partner can be a detectable secondary antibody or other binding agent (e.g., protein A, protein G, protein L) which binds to the first antibody. This secondary antibody or other binding agent can be labeled, e.g., with a radioactive, enzymatic, fluorescent, luminescent, or other detectable label, such as an avidin/biotin system. In another embodiment, the binding partner is a peptide of the invention, which can be conjugated directly or indirectly (e.g. via biotin/avidin interaction) to an enzyme, such as horseradish peroxidase or alkaline phosphatase. In such embodiments, the detectable signal is produced by adding a substrate of the enzyme that produces a detectable signal, such as a chromogenic, fluorogenic, or chemiluminescent substrate.

[0104] A "detection system" for detecting bound peptide, as used herein, may comprise a detectable binding partner, such as an antibody specific for the peptide. In one embodiment, the binding partner is labeled directly. In another embodiment, the binding partner is attached to a signal generating reagent, such as an enzyme that, in the presence of a suitable substrate, can produce a detectable signal. A surface for immobilizing the peptide may optionally accompany the detection system.

[0105] In embodiments of the invention, the detection procedure comprises visibly inspecting the antibody-peptide complex for a color change, or inspecting the antibody-peptide complex for a physical-chemical change. Physical-chemical changes may occur with oxidation reactions or other chemical reactions. They may be detected by eye, using a spectrophotometer, or the like.

[0106] A particularly useful assay format is a lateral flow immunoassay format. Antibodies to human or animal (e.g., dog, mouse, deer, etc.) immunoglobulins, or staph A or G protein antibodies, can be labeled with a signal generator or reporter (e.g., colloidal gold) that is dried and placed on a glass fiber pad (sample application pad or conjugate pad). The diagnostic peptide is immobilized on membrane, such as nitrocellulose or a PVDF (polyvinylidene fluoride) membrane (e.g., an Immobilon™ membrane). When a solution of sample (blood, serum, etc.) is applied to the sample application

pad (or flows through the conjugate pad), it dissolves the labeled reporter, which then binds to all antibodies in the sample. The resulting complexes are then transported into the next membrane (PVDF or nitrocellulose containing the diagnostic peptide) by capillary action. If antibodies against the diagnostic peptide are present, they bind to the diagnostic peptide striped on the membrane, thereby generating a signal (e.g., a band that can be seen or visualized). An additional

antibody specific to the labeled antibody or a second labeled antibody can be used to produce a control signal.

[0107] An alternative format for the lateral flow immunoassay comprises the peptides being conjugated to a ligand (e.g., biotin) and complexed with labeled ligand receptor (e.g., streptavidin-colloidal gold). The labeled peptide complexes can be placed on the sample application pad or conjugate pad. Anti-human IgG/IgM or anti-animal (e.g., dog, mouse, deer) IgG/IgM antibodies or other peptides of the invention are immobilized on a membrane, such as nitrocellulose or PVDF, at a test site (e.g., a test line). When sample is added to the sample application pad, antibodies in the sample react with the labeled peptide complexes such that antibodies that bind to peptides of the invention become indirectly labeled. The antibodies in the sample are then transported into the next membrane (PVDF or nitrocellulose containing the diagnostic peptide) by capillary action and bind to the immobilized anti-human IgG/IgM or anti-animal IgG/IgM antibodies (or protein A, protein G, protein L, or combinations thereof) or immobilized peptides of the invention. If any of the sample antibodies are bound to the labeled peptides of the invention, the label associated with the peptides can be seen or visualized at the test site. One embodiment of this type of lateral flow device is shown in Figure 2. Another embodiment of this type of lateral flow device in which the peptides of the invention are used both as the immobilized capture agent at a test site and as a soluble labeled complex to react with antibodies in a sample is shown in Figure 3. Suitable controls for this assay can include, e.g., a chicken IgY-colloidal gold conjugate located at the sample application pad or conjugate pad, and an anti-chicken IgY antibody immobilized at a control site located proximal to the test site.

[0108] Another assay for the screening of blood products or other physiological or biological fluids is an enzyme linked immunosorbent assay, i.e., an ELISA. Typically in an ELISA, isolated peptides or compositions of the invention are adsorbed to the surface of a microtiter well directly or through a capture matrix (e.g., an antibody). Residual, non-specific protein-binding sites on the surface are then blocked with an appropriate agent, such as bovine serum albumin (BSA), heat-inactivated normal goat serum (NGS), or BLOTTO (a buffered solution of nonfat dry milk which also contains a preservative, salts, and an antifoaming agent). The well is then incubated with a biological sample suspected of containing specific anti-*Borrelia* (e.g., *B. burgdorferi*) antibody. The sample can be applied neat, or more often it can be diluted, usually in a buffered solution which contains a small amount (0.1-5.0% by weight) of protein, such as BSA, NGS, or BLOTTO. After incubating for a sufficient length of time to allow specific binding to occur, the well is washed to remove unbound protein and then incubated with an optimal concentration of an appropriate anti-immunoglobulin antibody (e.g., for human subjects, an anti-human immunoglobulin (α HuIg) from another animal, such as dog, mouse, cow, etc.) or another peptide of the invention that is conjugated to an enzyme or other label by standard procedures and is dissolved in blocking buffer. The label can be chosen from a variety of enzymes, including horseradish peroxidase (HRP), beta-galactosidase, alkaline phosphatase, glucose oxidase, etc. Sufficient time is allowed for specific binding to occur again, then the well is washed again to remove unbound conjugate, and a suitable substrate for the enzyme is added. Color is allowed to develop and the optical density of the contents of the well is determined visually or instrumentally (measured at an appropriate wave length). The cutoff OD value may be defined as the mean OD+3 standard deviations (SDs) of at least 50 serum samples collected from individuals from an area where Lyme disease is not endemic, or by other such conventional definitions. In the case of a very specific assay, OD+2 SD can be used as a cutoff value.

[0109] In one embodiment of an ELISA, peptides of the invention are immobilized on a surface, such as a ninety-six-well ELISA plate or equivalent solid phase that is coated with streptavidin or an equivalent biotin-binding compound, such as avidin or neutravidin, at an optimal concentration in an alkaline coating buffer and incubated at 4° C overnight. After a suitable number of washes with standard washing buffers, an optimal concentration of a biotinylated form of a peptide or composition of the invention, dissolved in a conventional blocking buffer, is applied to each well. A sample is then added, and the assay proceeds as above. Conditions for performing ELISA assays are well-known in the art.

[0110] In another embodiment, the methods comprise an agglutination assay. For example, in certain embodiments, colloidal particles (e.g., colloidal gold, etc.) or latex beads are conjugated to peptides of the invention. Subsequently, the biological fluid is incubated with the bead/peptide conjugate, thereby forming a reaction mixture. The reaction mixture is then analyzed to determine the presence of the antibodies. In certain embodiments, the agglutination assays comprise the use of a second population of particles, such as colloidal particles (e.g., colloidal gold, etc.) or latex beads, conjugated to (1) antibodies specific to the peptides of the invention, in the case of a competition assay, or (2) antibodies capable of detecting sample antibodies (e.g., anti-human IgG or IgM antibodies, anti-dog IgG or IgM antibodies, etc.), in the case of a sandwich assay. Suitable agglutination methods can comprise centrifugation as a means of assessing the extent of agglutination.

[0111] In still other embodiment, peptides of the invention are electro- or dot-blotted onto nitrocellulose paper. Subsequently, a sample, such as a biological fluid (e.g., serum or plasma) is incubated with the blotted antigen, and antibody in the biological fluid is allowed to bind to the antigen(s). The bound antibody can then be detected, e.g., by standard immunoenzymatic methods or by visualization using colloidal nanoparticles couples to secondary antibodies or other

antibody binding agents, such as protein A, protein G, protein L, or combinations thereof.

[0112] It should be understood by one of skill in the art that any number of conventional protein assay formats, particularly immunoassay formats, may be designed to utilize the isolated peptides of this invention for the detection of *Borelia* antibodies and infection by pathogenic *Borrelia* (e.g., *B. burgdorferi*) in a subject. This invention is thus not limited by the selection of the particular assay format, and is believed to encompass assay formats that are known to those of skill in the art.

[0113] In certain embodiments, the sample used in the methods is a bodily fluid, such as blood, serum, cerebral spinal fluid, urine, or saliva. In other embodiments, the sample is a tissue (e.g., a tissue homogenate) or a cell lysate. In certain embodiments, the sample is from a wild animal (e.g., a deer or rodent, such as a mouse, chipmunk, squirrel, etc.). In other embodiments, the sample is from a lab animal (e.g., a mouse, rat, guinea pig, rabbit, monkey, primate, etc.). In other embodiments, the sample is from a domesticated or feral animal (e.g., a dog, a cat, a horse). In still other embodiments, the sample is from a human.

[0114] Much of the preceding discussion is directed to the detection of antibodies against pathogenic *Borrelia*. However, it is to be understood that the discussion also applies to the detection of primed T-cells, either *in vitro* or *in vivo*.

[0115] It is expected that a cell-mediated immune response (e.g., a T-helper response) is generated, since IgG is produced. It is therefore expected that it will be possible to determine the immunological reactivity between primed T-cells and a peptide of the invention. *In vitro* this can be done by incubating T-cells isolated from the subject with a peptide of the invention and measuring the immunoreactivity, e.g., by measuring subsequent T-cell proliferation or by measuring release of cytokines from the T-cells, such as IFN- γ . These methods are well-known in the art.

[0116] Any of a variety of conventional assays can be used to carry out methods *in vivo*. For example, one can perform an assay in the form of a skin test, e.g., by intradermally injecting, in the subject, a peptide of the invention. A positive skin reaction at the location of injection indicates that the subject has been exposed to and infected with a pathogenic *Borrelia* capable of causing Lyme disease, and a negative skin response at the location of injection indicates that the subject has not been so exposed/infected. This or other *in vivo* tests rely on the detection of a T-cell response in the subject.

[0117] In another aspect, the invention provides methods of diagnosing Lyme disease in a subject. The subject can be a subject suspected of having antibody against a causative agent of Lyme disease. The diagnostic method is useful for diagnosing subjects exhibiting the clinical symptoms of Lyme disease.

[0118] The diagnostic methods comprise contacting a sample from the subject with the mixture of isolated peptides of the invention, and detecting formation of an antibody-peptide complex comprising one or more of said peptides, wherein formation of said complex is indicative of the subject having Lyme disease. In certain embodiments, the methods comprise contacting the sample with a mixture of three, four or more (e.g., 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, or more) different peptides of the invention. In certain embodiments, the methods comprise contacting the sample with a mixture of peptides of the invention and one or more other peptides (e.g., a *Borrelia* peptide, or antigenic fragment or epitope thereof, such as an OspA, OspB, DbpA, flagella-associated proteins FlaA (p37) and FlaB (p41), OspC (25 kd), BBK32, BmpA (p39), p21, p39, p66, p83, or VlsE protein).

[0119] In certain embodiments, the mixture of different peptides is attached to or immobilized upon a substrate (e.g., a solid or semi-solid support). For example, in certain embodiments, the substrate is a bead (e.g., a colloidal or other type of particle or nanoparticle), a flow path in a lateral flow immunoassay device (e.g., a porous membrane), a flow path in an analytical rotor, or a tube or a well (e.g., in a plate suitable for an ELISA assay).

[0120] There are a number of different conventional assays for detecting formation of an antibody-peptide complex comprising a peptide of the invention. For example, the detecting step can comprise performing an ELISA assay, performing a lateral flow immunoassay, performing an agglutination assay, analyzing the sample in an analytical rotor, or analyzing the sample with an electrochemical, optical, or opto-electronic sensor. These different assays are described above and/or are well-known to those skilled in the art.

[0121] In certain embodiments, the sample is a bodily fluid, such as blood, serum, cerebral spinal fluid, urine, or saliva. In other embodiments, the sample is a tissue (e.g., a tissue homogenate) or a cell lysate. In certain embodiments, the subject is a wild animal (e.g., a deer or rodent, such as a mouse, chipmunk, squirrel, etc.). In other embodiments, the subject is a lab animal (e.g., a mouse, rat, guinea pig, rabbit, monkey, primate, etc.). In other embodiments, the subject is a domesticated or feral animal (e.g., a dog, a cat, a horse). In still other embodiments, the subject is a human.

Kits

[0122] In yet another aspect, the invention provides kits comprising the mixture of peptides of the invention and a labeling reagent capable of binding to an antibody that recognized an epitope of the peptides. In certain embodiments, the kits comprise three, four or more different peptides of the invention. In certain embodiments, the peptides are attached to or immobilized on a solid support. For example, in certain embodiments, the solid support is a bead (e.g., a colloidal particle or a nanoparticle), a flow path in a lateral flow immunoassay device, a flow path in an analytical rotor, or a tube or a well (e.g., in a plate).

[0123] Reagents for particular types of assays can also be provided in kits of the invention. Thus, the kits can include a population of beads (e.g., suitable for an agglutination assay or a lateral flow assay), or a plate (e.g., a plate suitable for an ELISA assay). In other embodiments, the kits comprise a device, such as a lateral flow immunoassay device, an analytical rotor, or an electrochemical, optical, or opto-electronic sensor. The population of beads, the plate, and the devices are useful for performing an immunoassay. For example, they can be useful for detecting formation of an antibody-peptide complex comprising an antibody from a sample and a peptide of the invention. In certain embodiments, a mixture of different peptides of the invention is attached to or immobilized on the beads, the plate, or the device.

[0124] In addition, the kits can include various diluents and buffers, labeled conjugates or other agents for the detection of specifically bound antigens or antibodies, and other signal-generating reagents, such as enzyme substrates, cofactors and chromogens. Other components of a kit can easily be determined by one of skill in the art. Such components may include coating reagents, polyclonal or monoclonal capture antibodies specific for a peptide of the invention, or a cocktail of two or more of the antibodies, purified or semipurified extracts of these antigens as standards, monoclonal antibody detector antibodies, an anti-mouse, anti-dog, anti-chicken, or anti-human antibody with indicator molecule conjugated thereto, indicator charts for colorimetric comparisons, disposable gloves, decontamination instructions, applicator sticks or containers, a sample preparatory cup, etc. In one embodiment, a kit comprises buffers or other reagents appropriate for constituting a reaction medium allowing the formation of a peptide-antibody complex.

[0125] Such kits provide a convenient, efficient way for a clinical laboratory to diagnose infection by a pathogenic *Borrelia*, such as a *B. burgdorferi*. Thus, in certain embodiments, the kits further comprise an instruction. For example, in certain embodiments, the kits comprise an instruction indicating how to use a peptide of the invention to detect an antibody to a *Borrelia* antigen or to diagnose Lyme disease. In certain embodiments, the kits comprise an instruction indicating how to use a population of beads, a plate, or a device (e.g., comprising a mixture of different peptides of the invention) to detect an antibody to a *Borrelia* antigen or to diagnose Lyme disease.

[0126] The peptides, compositions and devices comprising the peptides, kits and methods described herein offer a number of advantages. For example, they allow for simple, inexpensive, rapid, sensitive and accurate detection of Lyme disease, and avoid serologic cross-reactivity with other conditions with "Lyme-like" symptoms, such as myalgias, arthralgias, malaise or fever, including conditions such as syphilis, chronic arthritis, and multiple sclerosis. This allows for an accurate diagnosis. Furthermore, a diagnostic test of the invention (e.g., an ELISA assay, lateral flow immunoassay, or agglutination assay) is useful in serum samples that contain anti-OspA antibodies or other antibodies produced in response to a vaccine based on the outer surface proteins of *Borrelia*. A VlsE IR6 peptide of the invention does not cross-react with such antibodies, thereby allowing the differentiation of vaccinated individuals from individuals who were naturally infected with *B. burgdorferi*.

SEQUENCE LISTING

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<110> Abaxis, Inc. Mehra, Rajesh K. Aron, Kenneth P. Bleile, Dennis M.

<120> PEPTIDES AND METHODS FOR THE DETECTION OF LYME DISEASE ANTIBODIES

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30 **Claims**

1. A mixture of isolated peptides comprising three or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO:1, M-K-X₃-X₄-D-X₆-I-A-A-X₁₀-X₁₁-V-L-X₁₄-G-M-A-K-X₁₉-G-X₂₁-F-A-X₂₄-X₂₅ (SEQ ID NO: 1)

35 wherein X₃ is an amino acid selected from the group consisting of R and Q, X₄ is an amino acid selected from the group consisting of N and D, X₆ is an amino acid selected from the group consisting of N and E, X₁₀ is an amino acid selected from the group consisting of V, F and L, X₁₁ is an amino acid selected from the group consisting of I and M, X₁₄ is an amino acid selected from the group consisting of K, Q and H, X₁₉ is an amino acid selected from the group consisting of N, Q and E, X₂₁ is an amino acid selected from the group consisting of E and Q, X₂₄ is an amino acid selected from the group consisting of I, V, Y and W, and X₂₅ is an amino acid selected from the group consisting of K and R.
2. The mixture of claim 1 comprising all possible peptides defined by SEQ ID NO: 1.
3. The mixture of claim 1, wherein the mixture comprises different isolated peptides having a sequence selected from SEQ ID NOs: 3-70.
4. The mixture of claim 1, wherein each isolated peptide comprises an additional N-terminal peptide sequence, wherein the additional N-terminal peptide sequence is a native VlsE sequence or non-VlsE *Borrelia* antigen.

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5. The mixture of claim 4, wherein the additional N-terminal peptide sequence is a sequence of SEQ ID NO: 73, n₁-n₂-S-P-n₅-n₆-P (SEQ ID NO: 73) or a fragment thereof, wherein n₁ is an amino acid selected from the group consisting of A and V, n₂ is an amino acid selected from the group consisting of E and D, n₅ is an amino acid selected from the group consisting of K and R, and n₆ is an amino acid selected from the group consisting of K and R.

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6. The mixture of claim 1, wherein each isolated peptide comprises an additional C-terminal peptide sequence, wherein the additional C-terminal peptide sequence is a native VlsE sequence or non-VlsE *Borrelia* antigen.

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7. The mixture of claim 6, wherein the additional C-terminal peptide sequence is a sequence of SEQ ID NO: 74, V-c₂-E-G-c₅-Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 74) or a fragment thereof, wherein c₂ is an amino acid selected from the group consisting of Q and R, and c₅ is an amino acid selected from the group consisting of V and A.
- 5 8. The mixture of claim 6, wherein the additional C-terminal peptide sequence is a sequence of SEQ ID NO: 76, A-V-c₃-E-G-c₆-Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 76) or a fragment thereof, wherein c₃ is an amino acid selected from the group consisting of Q and R, and c₆ is an amino acid selected from the group consisting of V and A.
9. The mixture of any one of claims 1 to 8, wherein each isolated peptide is conjugated to a ligand.
- 10 10. The mixture of any one of claims 1 to 8, wherein one or more of the isolated peptides is biotinylated.
11. The mixture of any one of claims 1 to 8, wherein one or more of the isolated peptides is conjugated to:
- 15 a) streptavidin or avidin,
b) biotin, for associating the peptide with streptavidin or avidin, or
c) serum albumin,
- optionally wherein the one or more isolated peptides is conjugated to the streptavidin, avidin, biotin or serum albumin
20 via a cysteine residue.
12. The mixture of any one of claims 1 to 8, wherein each isolated peptide is immobilized to a solid support.
13. The mixture of claim 12, wherein the solid support is a bead, a flow path in a lateral flow immunoassay device, a
25 well in a microtiter plate, or a flow path in a rotor.
14. A kit comprising the mixture of isolated peptides of any one of claims 1 to 13 and a labeling reagent capable of binding to an antibody that recognizes an epitope of said three or more isolated peptides.
- 30 15. The kit of claim 14, wherein the labeling reagent is an anti-human IgG antibody or an anti-canine IgG antibody conjugated to a detectable label.
16. The kit of claim 15, wherein the detectable label is colloidal gold particles.
- 35 17. A method for detecting in a sample an antibody to an epitope of a *Borrelia* antigen, the method comprising:
- contacting a sample with the mixture of isolated peptides of any one of claims 1 to 12; and
detecting formation of an antibody-peptide complex comprising one or more of said isolated peptides in the
mixture,
40 wherein formation of said complex is indicative of an antibody to an epitope of a *Borrelia* antigen being present in said sample.
18. The method of claim 17, wherein said *Borrelia* antigen is from a *Borrelia burgdorferi*, *Borrelia afzelli*, or *Borrelia garinii* species.
- 45 19. A method for diagnosing Lyme disease in a subject, the method comprising:
- contacting a sample from the subject with the mixture of isolated peptides of any one of claims 1 to 12; and
detecting formation of an antibody-peptide complex comprising one or more of said isolated peptides in the
50 mixture, wherein formation of the complex is indicative of the subject having Lyme disease.
20. The method of any one of claims 17 to 19, wherein said detecting step comprises (i) performing an ELISA assay, (ii) running a lateral flow assay, (iii) performing an agglutination assay, or (iv) running the sample through an analytical rotor.
- 55 21. The method of any one of claims 17 to 20, wherein said sample is from a human or canine subject or is selected from the group consisting of blood, serum, cerebral spinal fluid, urine, or saliva.

Patentansprüche

- 5 1. Gemisch aus isolierten Peptiden, umfassend drei oder mehr unterschiedliche isolierte Peptide, worin jedes isolierte Peptid eine Sequenz von Seq.-ID Nr. 1, M-K-X₃-X₄-D-X₆-I-A-A-X₁₀-X₁₁-V-L-X₁₄-G-M-A-K-X₁₉-G-X₂₁-F-A-X₂₄-X₂₅ (Seq.-ID Nr. 1), umfasst,
- 10 worin X₃ eine aus der aus R und Q bestehenden Gruppe ausgewählte Aminosäure, X₄ eine aus der aus N und D bestehenden Gruppe ausgewählte Aminosäure, X₆ eine aus der aus N und E bestehenden Gruppe ausgewählte Aminosäure, X₁₀ eine aus der aus V, F und L bestehenden Gruppe ausgewählte Aminosäure, X₁₁ eine aus der aus I und M bestehenden Gruppe ausgewählte Aminosäure, X₁₄ eine aus der aus K, Q und H bestehenden Gruppe ausgewählte Aminosäure, X₁₉ eine aus der aus N, Q und E bestehenden Gruppe ausgewählte Aminosäure, X₂₁ eine aus der aus E und Q bestehenden Gruppe ausgewählte Aminosäure, X₂₄ eine aus der aus I, V, Y und W bestehenden Gruppe ausgewählte Aminosäure und X₂₅ eine aus der aus K und R bestehenden Gruppe ausgewählte Aminosäure ist.
- 15 2. Gemisch nach Anspruch 1, umfassend alle möglichen durch Seq.-ID Nr. 1 definierten Peptide.
3. Gemisch nach Anspruch 1, worin das Gemisch unterschiedliche isolierte Peptide umfasst, die eine aus Seq.-ID Nr. 3-70 ausgewählte Sequenz aufweisen.
- 20 4. Gemisch nach Anspruch 1, worin jedes isolierte Peptid eine zusätzliche N-terminale Peptidsequenz umfasst, worin die zusätzliche N-terminale Peptidsequenz eine native V1sE-Sequenz oder ein Nicht-V1sE-*Borrelia*-Antigen ist.
- 25 5. Gemisch nach Anspruch 4, worin die zusätzliche N-terminale Peptidsequenz eine Sequenz von Seq.-ID Nr. 73, n₁-n₂-S-P-n₅-n₆-P (Seq.-ID Nr. 73), oder ein Fragment davon ist, worin n₁ eine aus der aus A und V bestehenden Gruppe ausgewählte Aminosäure, n₂ eine aus der aus E und D bestehenden Gruppe ausgewählte Aminosäure, n₅ eine aus der aus K und R bestehenden Gruppe ausgewählte Aminosäure und n₆ eine aus der aus K und R bestehenden Gruppe ausgewählte Aminosäure ist.
- 30 6. Gemisch nach Anspruch 1, worin jedes isolierte Peptid eine zusätzliche C-terminale Peptidsequenz umfasst, worin die zusätzliche C-terminale Peptidsequenz eine native V1sE-Sequenz oder ein Nicht-V1sE-*Borrelia*-Antigen ist.
- 35 7. Gemisch nach Anspruch 6, worin die zusätzliche C-terminale Peptidsequenz eine Sequenz von Seq.-ID Nr. 74, V-c₂-E-G-c₅-Q-Q-E-G-A-Q-Q-P-S (Seq.-ID Nr. 74), oder ein Fragment davon ist, worin c₂ eine aus der aus Q und R bestehenden Gruppe ausgewählte Aminosäure und c₅ eine aus der aus V und A bestehenden Gruppe ausgewählte Aminosäure ist.
- 40 8. Gemisch nach Anspruch 6, worin die zusätzliche C-terminale Peptidsequenz eine Sequenz von Seq.-ID Nr. 76, A-V-c₃-E-G-c₆-Q-Q-E-G-A-Q-Q-P-S (Seq.-ID Nr. 76), oder ein Fragment davon ist, worin c₃ eine aus der aus Q und R bestehenden Gruppe ausgewählte Aminosäure und c₆ eine aus der aus V und A bestehenden Gruppe ausgewählte Aminosäure ist.
- 45 9. Gemisch nach einem der Ansprüche 1 bis 8, worin jedes isolierte Peptid an einen Liganden konjugiert ist.
10. Gemisch nach einem der Ansprüche 1 bis 8, worin eines oder mehrere der isolierten Peptide biotinyliert ist.
- 50 11. Gemisch nach einem der Ansprüche 1 bis 8, worin eines oder mehrere der isolierten Peptide an:
- a. Streptavidin oder Avidin,
 - b. Biotin zum Assoziieren des Peptids mit Streptavidin oder Avidin oder
 - c. Serumalbumin
- konjugiert ist, gegebenenfalls worin das eine oder die mehreren isolierten Peptide über einen Cysteinrest an das Streptavidin, Avidin, Biotin oder Serumalbumin konjugiert sind.
- 55 12. Gemisch nach einem der Ansprüche 1 bis 8, worin jedes isolierte Peptid auf einem festen Träger immobilisiert ist.
13. Gemisch nach Anspruch 12, worin der feste Träger ein Kügelchen, ein Flussweg in einer Lateralflossimmuntestvorrichtung, ein Well in einer Mikrotiterplatte oder ein Flussweg in einem Rotor ist.

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14. Set, umfassend das Gemisch an isolierten Peptiden nach einem der Ansprüche 1 bis 13 und ein Markierungsreagens, das in der Lage ist, an einen Antikörper zu binden, der ein Epitop der drei oder mehr isolierten Peptide erkennt.

5 15. Set nach Anspruch 14, worin das Markierungsreagens eine Anti-Human-IgG-Antikörper oder ein Anti-Hunde-IgG-Antikörper ist, der an eine detektierbare Markierung konjugiert ist.

16. Set nach Anspruch 15, worin die detektierbare Markierung kolloidale Goldpartikel sind.

10 17. Verfahren zum Detektieren eines Antikörpers für ein Epitop eines *Borrelia*-Antigens in einer Probe, wobei das Verfahren Folgendes umfasst:

Kontaktieren einer Probe mit dem Gemisch an isolierten Peptiden nach einem der Ansprüche 1 bis 12; und Detektieren der Bildung eines Antikörper-Peptid-Komplexes, der eines oder mehrere der isolierten Peptide in dem Gemisch umfasst,

15 worin die Bildung des Komplexes indikativ für einen Antikörper für ein Epitop eines *Borrelia*-Antigens ist, das in der Probe vorliegt.

18. Verfahren nach Anspruch 17, worin das *Borrelia*-Antigen von einer *Borrelia burgdorferi*-, *Borrelia afzelli*- oder *Borrelia garinii*-Spezies ist.

20 19. Verfahren zum Diagnostizieren von Lyme-Krankheit in einem Individuum, wobei das Verfahren Folgendes umfasst:

Kontaktieren einer Probe von dem Individuum mit dem Gemisch an isolierten Peptiden nach einem der Ansprüche 1 bis 12; und

25 Detektieren der Bildung eines Antikörper-Peptid-Komplexes, der eines oder mehrere der isolierten Peptide in dem Gemisch umfasst, worin die Bildung des Komplexes indikativ dafür ist, dass das Individuum die Lyme-Krankheit hat.

30 20. Verfahren nach einem der Ansprüche 17 bis 19, worin der Detektierungsschritt (i) das Durchführen eines ELISA-Tests, (ii) das Durchführen eines Lateralflusstests, (iii) das Durchführen eines Agglutinationstests oder (iv) das Durchlaufenlassen der Probe durch einen Analyserotor umfasst.

35 21. Verfahren nach einem der Ansprüche 17 bis 20, worin die Probe von einem menschlichen oder einem hundeartigen Individuum stammt oder aus der aus Blut, Serum, zerebrospinaler Flüssigkeit, Urin oder Speichel bestehenden Gruppe ausgewählt ist.

Revendications

40 1. Mélange de peptides isolés comprenant trois peptides isolés différents ou plus, dans lequel chaque peptide isolé comprend une séquence de SEQ ID NO: 1, M-K-X₃-X₄-D-X₆-I-A-A-X₁₀-X₁₁-V-L-X₁₄-G-M-A-K-X₁₉-G-X₂₁-F-A-X₂₄-X₂₅ (SEQ ID NO: 1)

45 dans laquelle X₃ est un acide aminé sélectionné dans le groupe consistant en R et Q, X₄ est un acide aminé sélectionné dans le groupe consistant en N et D, X₆ est un acide aminé sélectionné dans le groupe consistant en N et E, X₁₀ est un acide aminé sélectionné dans le groupe consistant en V, F et L, X₁₁ est un acide aminé sélectionné dans le groupe consistant en I et M, X₁₄ est un acide aminé sélectionné dans le groupe consistant en K, Q et H, X₁₉ est un acide aminé sélectionné dans le groupe consistant en N, Q et E, X₂₁ est un acide aminé sélectionné dans le groupe consistant en E et Q, X₂₄ est un acide aminé sélectionné dans le groupe consistant en I, V, Y et W, et X₂₅ est un acide aminé sélectionné dans le groupe consistant en K et R.

50 2. Mélange selon la revendication 1, comprenant tous les peptides possibles définis par SEQ ID NO: 1.

55 3. Mélange selon la revendication 1, où le mélange comprend différents peptides isolés possédant une séquence sélectionnée parmi SEQ ID NO: 3-70.

4. Mélange selon la revendication 1, dans lequel chaque peptide isolé comprend une séquence peptidique N-terminale supplémentaire, où la séquence peptidique N-terminale supplémentaire est une séquence VlsE native ou un antigène de *Borrelia* non-VlsE.

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5. Mélange selon la revendication 4, dans lequel la séquence peptidique N-terminale supplémentaire est une séquence de SEQ ID NO: 73, n_1 - n_2 -S-P- n_5 - n_6 -P (SEQ ID NO: 73) ou un fragment de celle-ci, dans laquelle n_1 est un acide aminé sélectionné dans le groupe consistant en A et V, n_2 est un acide aminé sélectionné dans le groupe consistant en E et D, n_5 est un acide aminé sélectionné dans le groupe consistant en K et R, et n_6 est un acide aminé sélectionné dans le groupe consistant en K et R.
6. Mélange selon la revendication 1, dans lequel chaque peptide isolé comprend une séquence peptidique C-terminale supplémentaire, où la séquence peptidique C-terminale supplémentaire est une séquence VIsE native ou un antigène de *Borrelia* non-VIsE.
7. Mélange selon la revendication 6, dans lequel la séquence peptidique C-terminale supplémentaire est une séquence de SEQ ID NO: 74, V- c_2 -E-G- c_5 -Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 74) ou un fragment de celle-ci, dans laquelle c_2 est un acide aminé sélectionné dans le groupe consistant en Q et R, et c_5 est un acide aminé sélectionné dans le groupe consistant en V et A.
8. Mélange selon la revendication 6, dans lequel la séquence peptidique C-terminale supplémentaire est une séquence de SEQ ID NO: 76, A-V- c_3 -E-G- c_6 -Q-Q-E-G-A-Q-Q-P-S (SEQ ID NO: 76) ou un fragment de celle-ci, dans laquelle c_3 est un acide aminé sélectionné dans le groupe consistant en Q et R, et c_6 est un acide aminé sélectionné dans le groupe consistant en V et A.
9. Mélange selon l'une quelconque des revendications 1 à 8, dans lequel chaque peptide isolé est conjugué à un ligand.
10. Mélange selon l'une quelconque des revendications 1 à 8, dans lequel un ou plusieurs des peptides isolés est biotinylé.
11. Mélange selon l'une quelconque des revendications 1 à 8, dans lequel un ou plusieurs des peptides isolés est conjugué à :
- a) la streptavidine ou l'avidine,
 - b) la biotine, pour associer le peptide avec la streptavidine ou l'avidine, ou
 - c) la sérumalbumine,
- facultativement dans lequel le un ou plusieurs peptides isolés est conjugué à la streptavidine, l'avidine, la biotine ou la sérumalbumine via un résidu cystéine.
12. Mélange selon l'une quelconque des revendications 1 à 8, dans lequel chaque peptide isolé est immobilisé sur un support solide.
13. Mélange selon la revendication 12, où le support solide est une bille, un trajet d'écoulement dans un dispositif de test immunologique à flux latéral, un puits dans une plaque de microtitration, ou un trajet d'écoulement dans un rotor.
14. Kit comprenant le mélange de peptides isolés selon l'une quelconque des revendications 1 à 13 et un réactif de marquage capable de se lier à un anticorps qui reconnaît un épitope desdits trois peptides isolés ou plus.
15. Kit selon la revendication 14, dans lequel le réactif de marquage est un anticorps anti-IgG humaine ou un anticorps anti-IgG canine conjugué à un marqueur détectable.
16. Kit selon la revendication 15, dans lequel le marqueur détectable est des particules d'or colloïdal.
17. Procédé pour détecter, dans un échantillon, un anticorps dirigé contre un épitope d'un antigène de *Borrelia*, le procédé comprenant :
- la mise en contact d'un échantillon avec le mélange de peptides isolés selon l'une quelconque des revendications 1 à 12 ; et
 - la détection de la formation d'un complexe anticorps-peptide comprenant un ou plusieurs desdits peptides isolés dans le mélange,
- où la formation dudit complexe indique qu'un anticorps dirigé contre un épitope d'un antigène de *Borrelia* est présent dans ledit échantillon.

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18. Procédé selon la revendication 17, dans lequel ledit antigène de *Borrelia* provient d'une espèce *Borrelia burgdorferi*, *Borrelia afzelii* ou *Borrelia garinii*.

19. Procédé pour diagnostiquer la maladie de Lyme chez un sujet, le procédé comprenant :

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la mise en contact d'un échantillon du sujet avec le mélange de peptides isolés selon l'une quelconque des revendications 1 à 12 ; et
la détection de la formation d'un complexe anticorps-peptide comprenant un ou plusieurs desdits peptides isolés dans le mélange, où la formation du complexe indique que le sujet est atteint de la maladie de Lyme.

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20. Procédé selon l'une quelconque des revendications 17 à 19, dans lequel ladite étape de détection comprend (i) la réalisation d'un test ELISA, (ii) la réalisation d'un test à flux latéral, (iii) la réalisation d'un test d'agglutination, ou (iv) le traitement de l'échantillon dans un rotor analytique.

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21. Procédé selon l'une quelconque des revendications 17 à 20, dans lequel ledit échantillon provient d'un sujet humain ou canin ou est sélectionné dans le groupe consistant en du sang, du sérum, du liquide céphalo-rachidien, de l'urine, ou de la salive.

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Fig. 1

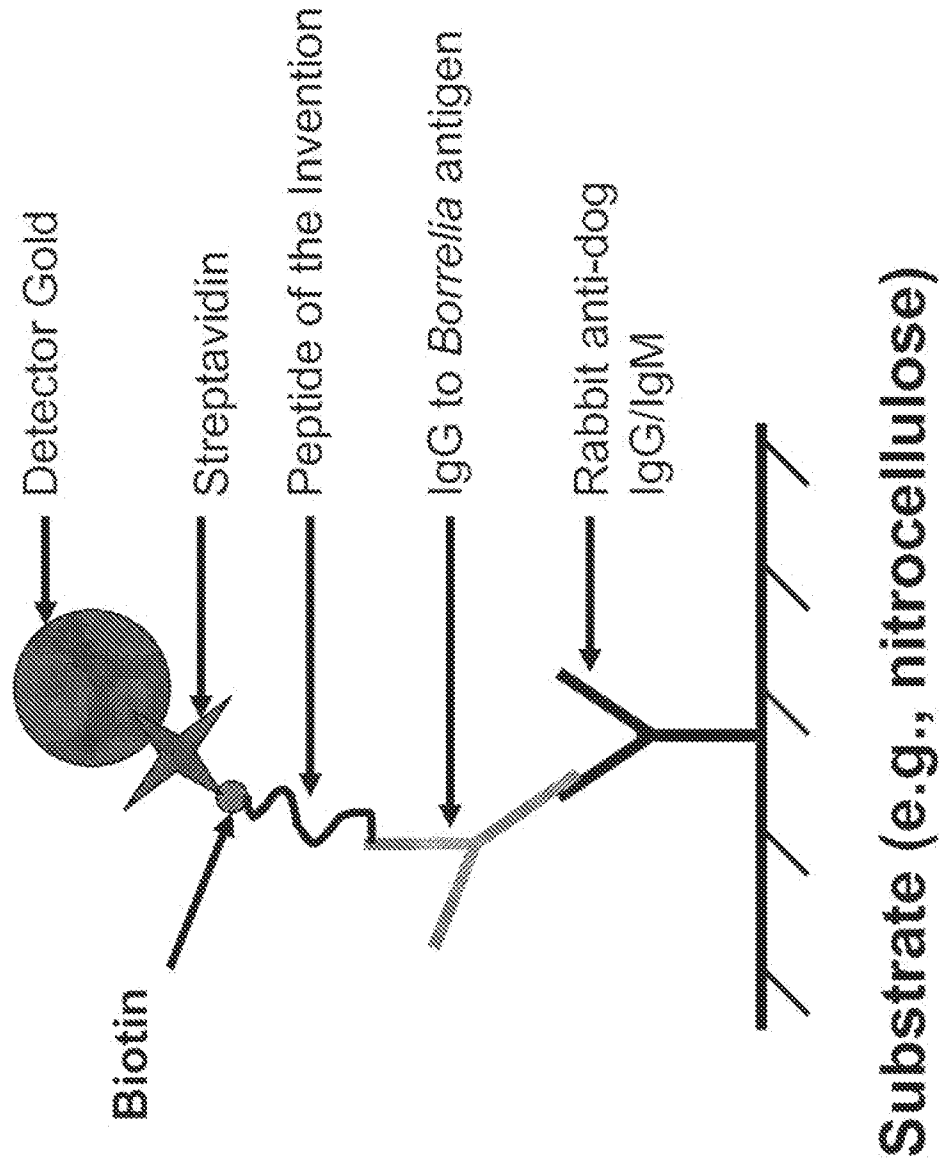


Fig. 2

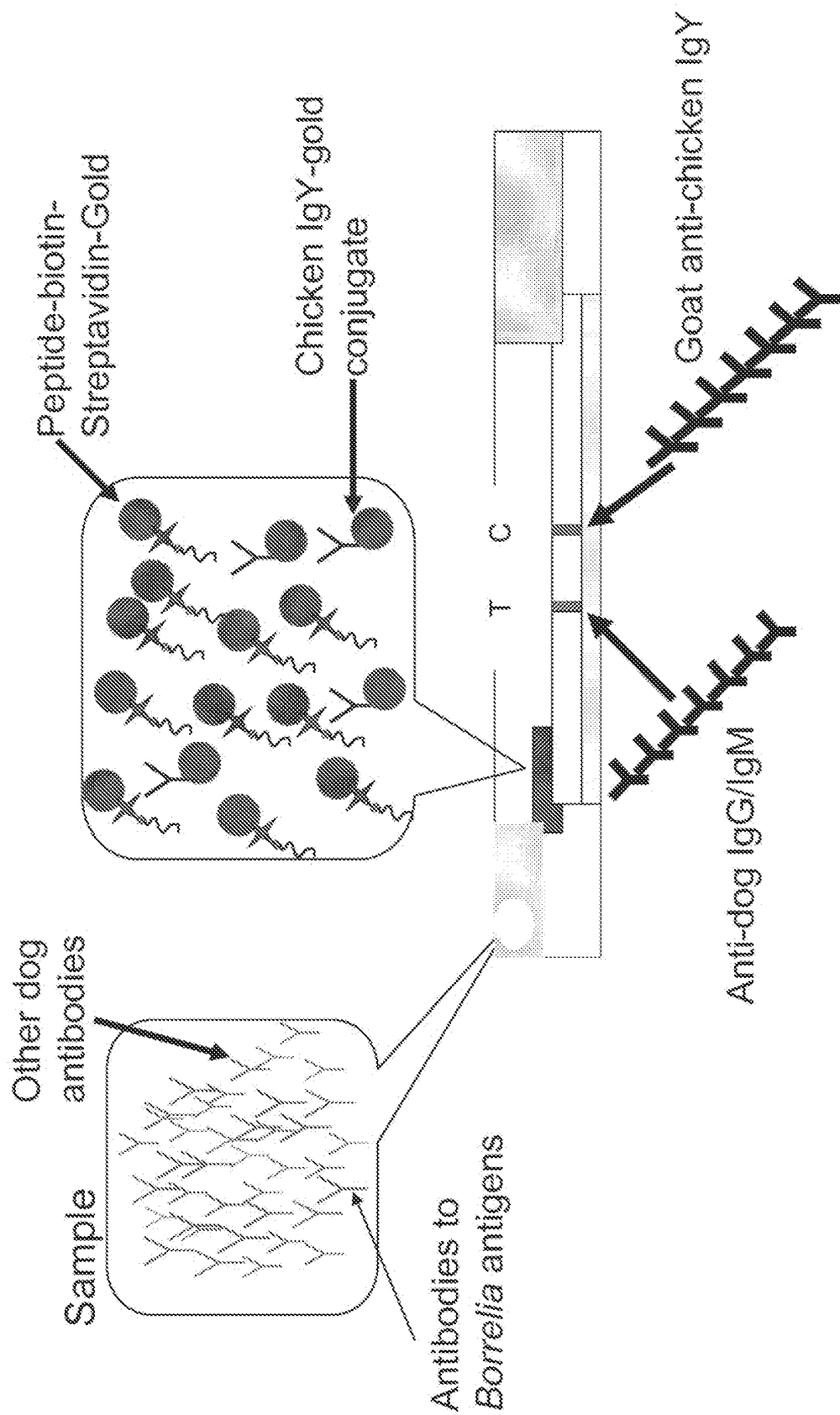
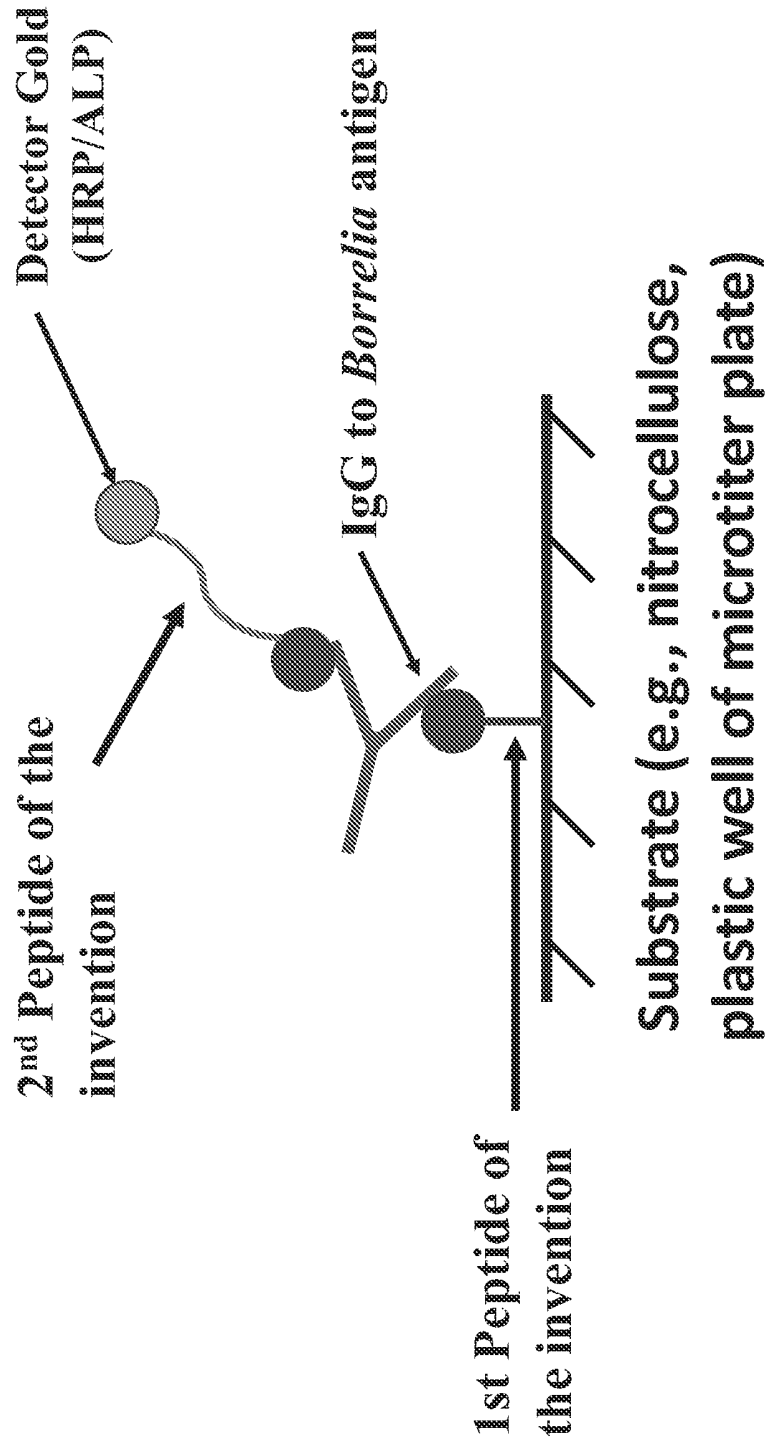


Fig. 3



REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于检测莱姆病抗体的肽和方法		
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摘要(译)

本发明提供了用于检测与疏螺旋体抗原结合的抗体的组合物(例如肽组合物)。肽组合物包含多肽序列,其包含疏螺旋体VisE蛋白的IR6结构域中的变体。本发明还提供了包含此类肽组合物的装置,方法和试剂盒,其可用于检测与疏螺旋体抗原结合的抗体和莱姆病的诊断。