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- (54) **PEPTIDES, DEVICES, AND METHODS FOR THE DETECTION OF EHRLICHIA ANTIBODIES**
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- (52) **U.S. Cl.**
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- (58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The invention provides compositions (e.g., peptide compositions) useful for the detection of antibodies that bind to *Ehrlichia* antigens. The peptide compositions comprise polypeptide sequences based on an immunogenic fragment of the *Ehrlichia* Outer Membrane Protein 1 (OMP-1) protein. The invention also provides devices, methods, and kits comprising such peptide compositions and useful for the detection of antibodies that bind to *Ehrlichia* antigens and the diagnosis of monocytic ehrlichiosis.

22 Claims, 3 Drawing Sheets-

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Fig. 1

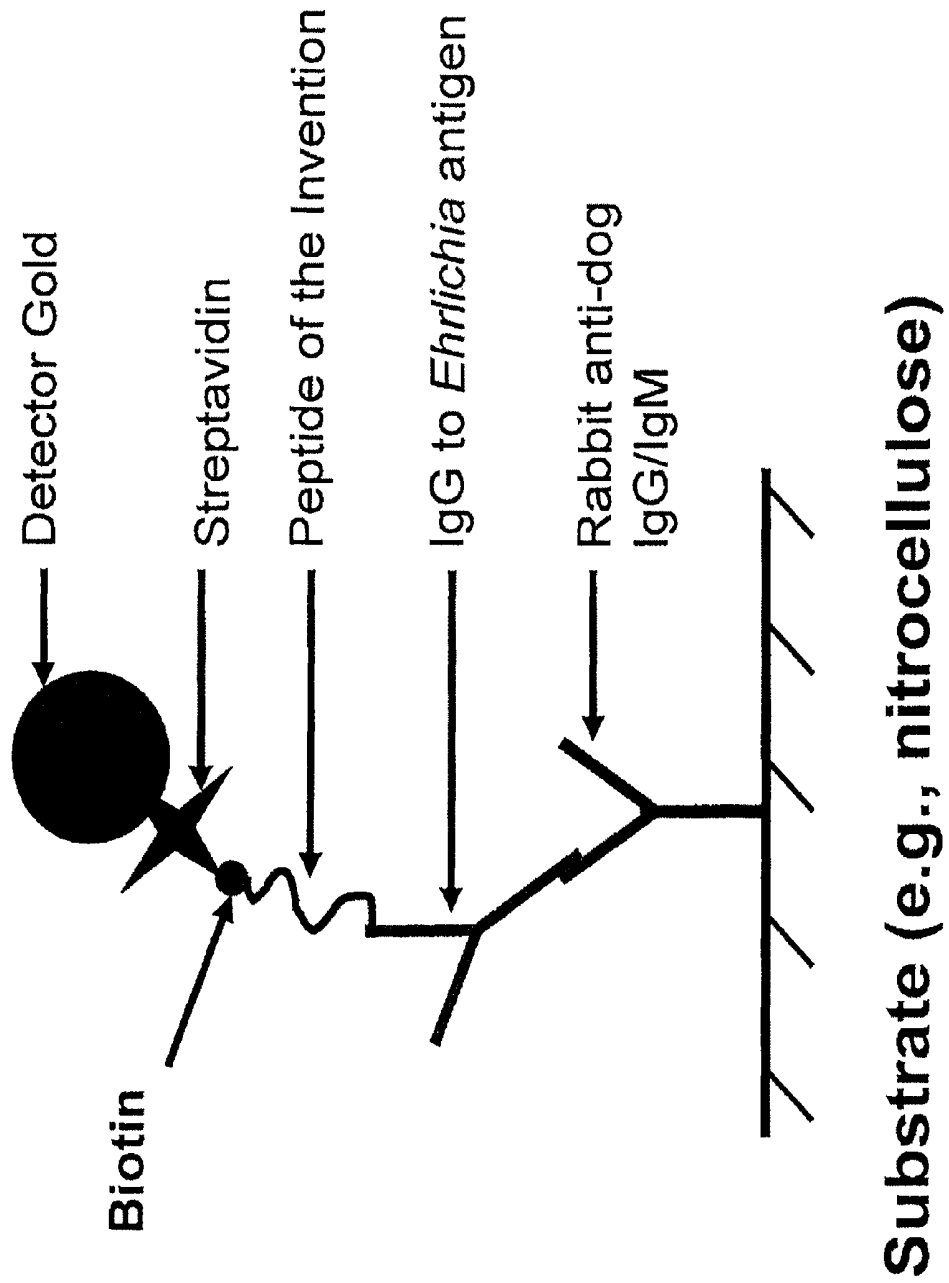


Fig. 2

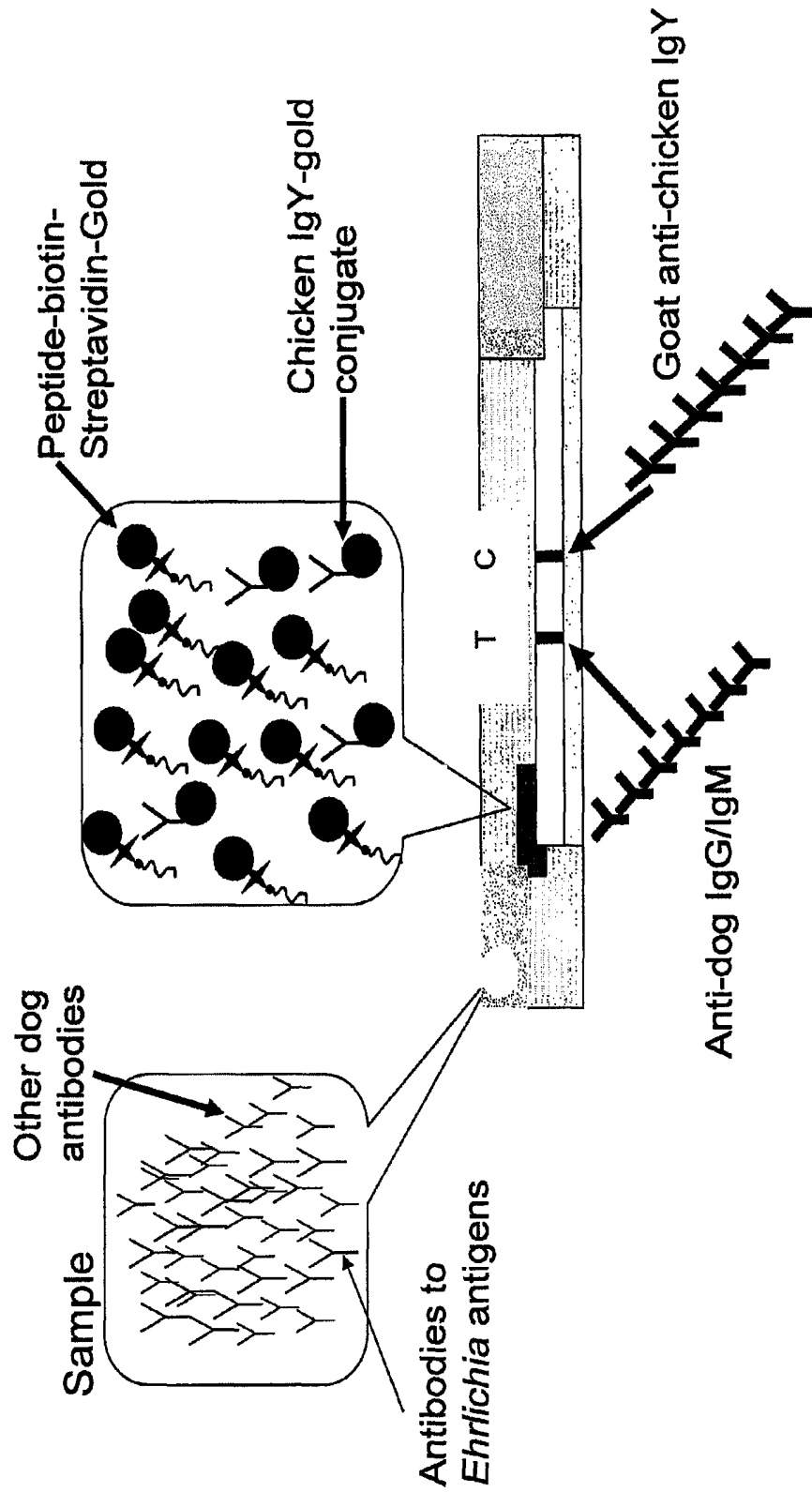
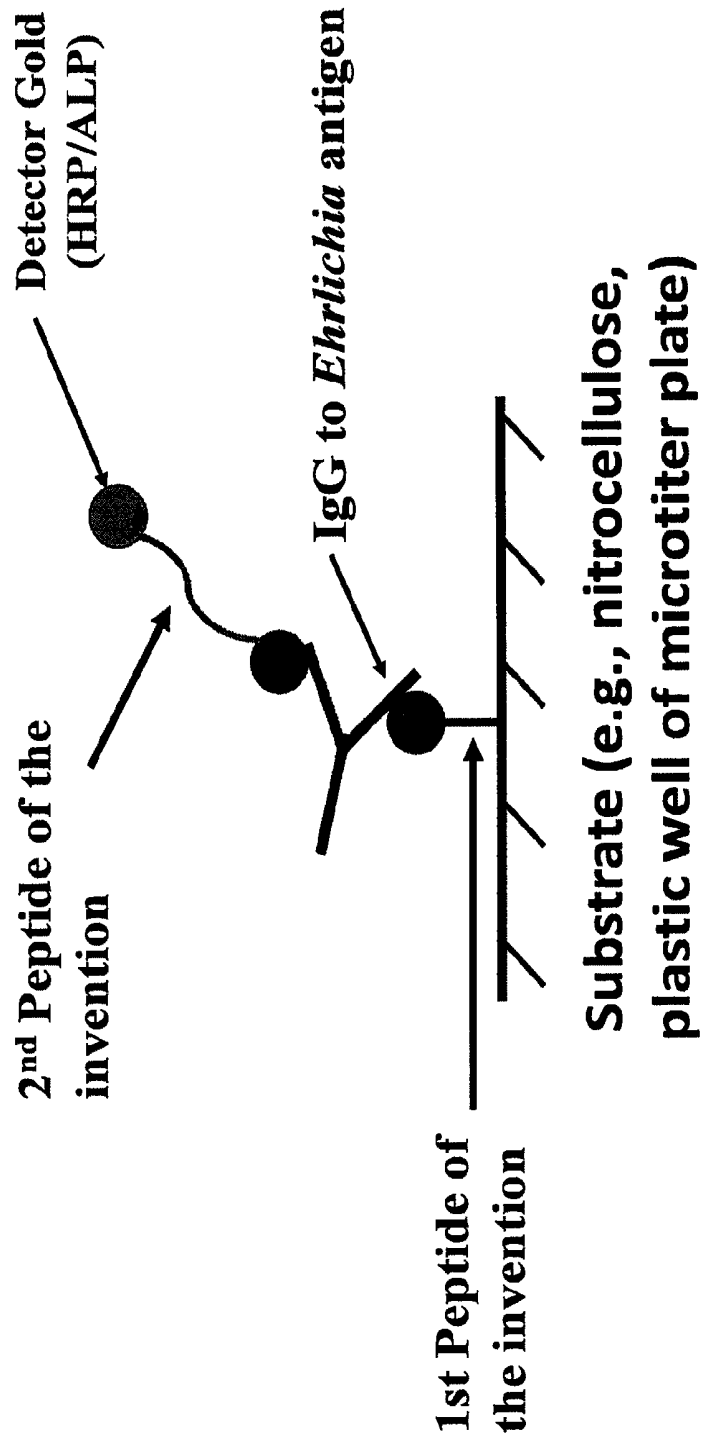


Fig. 3



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PEPTIDES, DEVICES, AND METHODS FOR THE DETECTION OF EHRLICHIA ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/263,329, filed Nov. 20, 2009, which is herein incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: ABAX_036_01US_SeqList_ST25.txt, date recorded: Nov. 19, 2010, file size 41 kilobytes).

BACKGROUND OF THE INVENTION

Ehrlichia bacteria are obligate intracellular pathogens that infect circulating lymphocytes in mammalian hosts. *Ehrlichia canis* and *Ehrlichia chaffeensis* are members of the same sub-genus group that infect canines and humans and cause canine monocytic ehrlichiosis (CME) and human monocytic ehrlichiosis (HME), respectively. The canine disease is characterized by fever, lymphadenopathy, weight loss, and pancytopenia. In humans the disease is characterized by fever, headache, myalgia, and leukopenia. Early detection and treatment are important for treating both canine and human ehrlichiosis.

Indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) have typically been used in the diagnosis of these diseases. These assays measure or otherwise detect the binding of anti-*Ehrlichia* antibodies from a subject's blood, plasma, or serum to infected cells, cell lysates, or partially purified whole *Ehrlichia* proteins. However, currently known assays for detecting anti-*Ehrlichia* antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the *Ehrlichia* antigen(s) used in these tests. That is, the currently known assays use mixtures of many whole *Ehrlichia* antigens or antigens that are not species specific.

Accordingly, there remains a need in the art for additional assays for detecting *Ehrlichia* antigens and serodiagnosis of monocytic ehrlichiosis.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that certain sequence variants in a fragment of the *Ehrlichia* Outer Membrane Protein 1 (OMP-1) proteins provide for robust detection of an antibody response against a range of *Ehrlichia* species. Accordingly, the invention provides compositions, devices, methods, and kits useful for the detection of antibodies that bind to *Ehrlichia* antigens and the diagnosis of monocytic ehrlichiosis.

In one aspect, the invention provides peptides capable of binding to antibodies that recognize *Ehrlichia* antigens. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-T-X₁₀-X₁₁-X₁₂-X₁₃-G-L-K-Q-X₁₈-W-X₂₀-G-X₂₂-X₂₃-X₂₄-X₂₅-X₂₆-X₂₇-X₂₈-X₂₉-X₃₀-X₃₁-X₃₂-X₃₃-X₃₄-X₃₅-X₃₆-X₃₇-X₃₈-X₃₉-X₄₀ (SEQ ID NO: 1) wherein each of X₁-X₆ and

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X₂₇-X₄₀ is any amino acid, X₇ is an amino acid selected from the group consisting of N and Q, X₈ is an amino acid selected from the group consisting of T and P, X₁₀ is an amino acid selected from the group consisting of T and V, X₁₁ is an amino acid selected from the group consisting of G and A, X₁₂ is an amino acid selected from the group consisting of L and V, X₁₃ is an amino acid selected from the group consisting of Y and F, X₁₈ is an amino acid selected from the group consisting of D and N, X₂₀ is an amino acid selected from the group consisting of D and N, X₂₂ is an amino acid selected from the group consisting of S and V, X₂₃ is an amino acid selected from the group consisting of A, S, and T, X₂₄ is an amino acid selected from the group consisting of A and I, X₂₅ is an amino acid selected from the group consisting of S, T, and P, and X₂₆ is an amino acid selected from the group consisting of S, N, and K.

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₇ is Q, and X₂₅ is an amino acid selected from the group consisting of T and P. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₇ is N, and X₂₅ is S. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is an amino acid selected from the group consisting of S and K, X₂ is an amino acid selected from the group consisting of A, V, and R, X₃ is an amino acid selected from the group consisting of K and D, X₄ is E, X₅ is an amino acid selected from the group consisting of E, D, and N, and X₆ is an amino acid selected from the group consisting of K and Q. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, and X₆ is K. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2). In certain embodiments, X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In other embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 59, F-S-A-K-X₅-X₆-X₇-A-E-T-X₁₁-X₁₂-T-F-G-X₁₆-X₁₇-X₁₈-X₁₉-X₂₀-D-G-A-X₂₄-X₂₅-X₂₆-X₂₇-N-X₂₉-V-X₃₁-N-X₃₃-F-T-I-S-N (SEQ ID NO: 59) wherein X₅ is an amino acid selected from the group consisting of E and Q, X₆ is an amino acid selected from the group consisting of E and Q, X₇ is any amino acid, X₁₁ is an amino acid selected from the group consisting of K and R, X₁₂ is any amino acid, X₁₆ is an amino acid selected from the group consisting of L and I, X₁₇ is any amino acid, X₁₈ is an amino acid selected from the group consisting of R and K, X₁₉ is an amino acid selected from the group consisting of Q and N, X₂₀ is an amino acid selected from the group consisting of Y and T, X₂₄ is any amino acid, X₂₅ is an amino acid selected from the group consisting of I and L, X₂₆ is any amino acid, X₂₇ is an amino acid selected from the group consisting of D and E, X₂₉ is an amino acid selected from the group consisting of E and Q, X₃₁ is an amino acid selected from the group consisting of E and Q, and X₃₃ is an amino acid selected from the group consisting of K and R.

In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 59, wherein X₅ is E, X₆ is E, X₁₆ is L, X₁₈ is K, X₂₀ is Y, X₂₅ is I, X₂₉ is Q, X₃₁ is Q, and X₃₃ is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 59, wherein X₇ is K, X₁₂ is an amino acid selected from the

group consisting of K and R, X₁₇ is an amino acid selected from the group consisting of E and D, X₂₄ is an amino acid selected from the group consisting of K and Q, and X₂₆ is an amino acid selected from the group consisting of E and T.

In other embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 92, G-X₂-F-S-A-K-X₇-X₈-K-X₁₀-A-D-T-R-X₁₅-T-F-G-L-X₂₀-K-Q-T-D-G-A-X₂₇-I-X₂₉-E-N-X₃₂-V-X₃₄-N-X₃₆-F-T-I-S-N (SEQ ID NO: 92) wherein X₂ is an amino acid selected from the group consisting of D and N, X₇ is an amino acid selected from the group consisting of E and Q, X₈ is an amino acid selected from the group consisting of E and Q, X₁₀ is any amino acid, X₁₅ is any amino acid, X₂₀ is any amino acid, X₂₇ is any amino acid, X₂₉ is any amino acid, X₃₂ is an amino acid selected from the group consisting of E and Q, X₃₄ is an amino acid selected from the group consisting of E and Q, and X₃₆ is an amino acid selected from the group consisting of K and R. In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 92, wherein X₂ is N, X₇ is E, X₈ is E, X₃₂ is Q, X₃₄ is Q, and X₃₆ is K.

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92 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, SEQ ID NO: 59, or SEQ ID NO: 92 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-OMP-1 *Ehrlichia* antigen (e.g., *Ehrlichia* p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3).

In certain embodiments, peptides of the invention comprise at least 25, 30, 35, 40, 45, 50, or more amino acids. In certain embodiments, 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 streptavidin, avidin, 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 are part of a multiple antigenic peptides system (MAPS).

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).

In another aspect, the invention provides compositions comprising two or more peptides of the invention. For example, in certain embodiments, the composition comprises a mixture of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 1. In some embodiments, the composition comprises a mixture of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 59. In other embodiments, the composition

comprises a mixture of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 92.

In certain embodiments, the peptides are conjugated to a ligand. For example, in certain embodiments, the peptides are biotinylated. In other embodiments, the peptides are conjugated to streptavidin, avidin, 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 are part of a multiple antigenic peptides system (MAPS).

In another aspect, the invention provides nucleic acids comprising a sequence encoding a peptide of the invention. In addition, the invention provides vectors comprising such nucleic acids, and host cells comprising such vectors. In certain embodiments, the vector is a shuttle vector. In other embodiments, the vector is an expression vector (e.g., a bacterial or eukaryotic expression vector). In certain embodiments, the host cell is a bacterial cell. In other embodiments, the host cell is a eukaryotic cell.

In another aspect, the invention provides devices. In certain embodiments, the devices are useful for performing an immunoassay. For example, in certain embodiments, the device is a lateral flow immunoassay device. In other embodiments, the device is an analytical rotor. In other embodiments, the device is a tube or a well, e.g., in a plate suitable for an ELISA assay. In still other embodiments, the device is an electrochemical, optical, or opto-electronic sensor.

In certain embodiments, the device comprises a peptide of the invention. In other embodiments, the device comprises a mixture of different peptides of the invention. For example, in certain embodiments, the device comprises two, three, four, or more different peptides of the invention. In certain embodiments, the peptide or each peptide in the mixture comprises a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. In certain embodiments, the peptides are attached to or immobilized upon the device.

In another aspect, the invention provides methods of detecting in a sample an antibody to an epitope of an *Ehrlichia* antigen. In certain embodiments, the methods comprise contacting a sample with a peptide of the invention, and detecting formation of an antibody-peptide complex comprising said peptide, wherein formation of said complex is indicative of the presence of an antibody to an epitope of a *Ehrlichia* antigen in said sample. In certain embodiments, the *Ehrlichia* antigen is from an infectious *Ehrlichia* species, such as *Ehrlichia canis* or *Ehrlichia chaffeensis*. In certain embodiments, the methods comprise contacting the sample with a mixture of two, three, four, or more different peptides of the invention.

In certain embodiments, the peptide or each peptide in the mixture is an isolated (e.g., synthetic and/or purified) peptide. In certain embodiments, the peptide or 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, a nanoparticle, a 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). 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.

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.

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.

In another aspect, the invention provides methods of diagnosing monocytic ehrlichiosis in a subject. In certain embodiments, the methods comprise contacting a sample from the subject with a peptide of the invention, and detecting formation of an antibody-peptide complex comprising said peptide, wherein formation of said complex is indicative of the subject having monocytic ehrlichiosis. In certain embodiments, the methods comprise contacting the sample with a mixture of two, three, four, or more different peptides of the invention.

In certain embodiments, the peptide or each peptide in the mixture is an isolated (e.g., synthetic and/or purified) peptide. In certain embodiments, the peptide or mixture of different peptides is attached to or immobilized upon a solid support. In certain embodiments, the solid support is a bead (e.g., a colloidal particle, a nanoparticle, a 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.

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.

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.

In yet another aspect, the invention provides kits. In certain embodiments, the kits comprise a peptide of the invention. In certain embodiments, the kits comprise two, three, four, or more different peptides of the invention. The peptides can

comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. 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, a nanoparticle, a 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 peptides are attached to a dendrimer and/or incorporated into a MAPS system.

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 different peptides of the invention is attached to or immobilized on the beads, the plate, or the device.

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 an *Ehrlichia* antigen or to diagnose monocytic ehrlichiosis. 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 an *Ehrlichia* antigen or to diagnose monocytic ehrlichiosis.

Additional aspects and embodiments of the invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram of an indirect sandwich assay which can be used to detect antibodies to *Ehrlichia* 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 *Ehrlichia* 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-*Ehrlichia* 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.

FIG. 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-*Ehrlichia* 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 the 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-*Ehrlichia* antigen antibodies present in the test sample bind to the labeled antibodies and such antibody complexes are captured by the immobilized *Ehrlichia* 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.

FIG. 3 is a diagram of a double antigen sandwich assay which can be used to detect antibodies to *Ehrlichia* 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 *Ehrlichia* 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

As used herein, the following terms shall have the following meanings:

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.

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.

The term “OMP-1 protein” refers to any of the Outer Membrane Protein 1 paralogs of *Ehrlichia*, including, but not limited to, *E. canis* P-30, *E. canis* P30-1, *E. chaffeensis* P28, *E. chaffeensis* OMP-1C, *E. chaffeensis* OMP-1D, *E. chaffeensis* OMP-1E, and *E. chaffeensis* OMP-1F.

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.

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.

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

Compositions and Devices

The present invention is based, in part, on the discovery that certain sequence variants in a fragment of the *Ehrlichia* OMP-1 proteins provide for robust detection of an antibody response against a range of *Ehrlichia* species. Accordingly, in one aspect, the invention provides peptides capable of binding to antibodies that recognize *Ehrlichia* antigens.

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, X₁-X₂-X₃-X₄-X₅-X₆-X₇-

X₈-T-X₁₀-X₁₁-X₁₂-X₁₃-G-L-K-Q-X₁₈-W-X₂₀-G-X₂₂-X₂₃-X₂₄-X₂₅-X₂₆-X₂₇-X₂₈-X₂₉-X₃₀-X₃₁-X₃₂-X₃₃-X₃₄-X₃₅-X₃₆-X₃₇-X₃₈-X₃₉-X₄₀ (SEQ ID NO: 1) wherein SEQ ID NO: 1, as used throughout the specification unless further specified, has the following characteristics: each of X₁-X₆ and X₂₇-X₄₀ is any amino acid, X₇ is an amino acid selected from the group consisting of N and Q, X₈ is an amino acid selected from the group consisting of T and P, X₁₀ is an amino acid selected from the group consisting of T and V, X₁₁ is an amino acid selected from the group consisting of G and A, X₁₂ is an amino acid selected from the group consisting of L and V, X₁₃ is an amino acid selected from the group consisting of Y and F, X₁₈ is an amino acid selected from the group consisting of D and N, X₂₀ is an amino acid selected from the group consisting of D and N, X₂₂ is an amino acid selected from the group consisting of S and V, X₂₃ is an amino acid selected from the group consisting of A, S, and T, X₂₄ is an amino acid selected from the group consisting of A and I, X₂₅ is an amino acid selected from the group consisting of S, T, and P, and X₂₆ is an amino acid selected from the group consisting of S, N, and K.

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₇ is Q, and X₂₅ is an amino acid selected from the group consisting of T and P. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₇ is N, and X₂₅ is S. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is an amino acid selected from the group consisting of S and K, X₂ is an amino acid selected from the group consisting of A, V, and R, X₃ is an amino acid selected from the group consisting of K and D, X₄ is E, X₅ is an amino acid selected from the group consisting of E, D, and N, and X₆ is an amino acid selected from the group consisting of K and Q. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, and X₆ is K. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2). In certain embodiments, X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, X₆ is K, X₇ is Q, and X₂₅ is an amino acid selected from the group consisting of T and P. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, X₆ is K, X₇ is N, and X₂₅ is S. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, X₆ is K, X₇ is Q, X₂₅ is an amino acid selected from the group consisting of T and P, and X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

6). In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁ is S, X₂ is an amino acid selected from the group consisting of A and V, X₃ is K, X₄ is E, X₅ is an amino acid selected from the group consisting of E and D, X₆ is K, X₇ is N, X₂₅ is S, and X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2), X₇ is Q, and X₂₅ is an amino acid selected from the group consisting of T and P. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2), X₇ is N, and X₂₅ is S. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2), X₇ is Q, X₂₅ is an amino acid selected from the group consisting of T and P, and X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6). In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X₁-X₆ has the sequence K-R-D-E-N-Q (SEQ ID NO: 2), X₇ is N, X₂₅ is S, and X₂₇-X₄₀ has a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In certain embodiments, a peptide of the invention comprises or consists of the sequence

(SEQ ID NO: 7)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 8)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 9)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 10)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 11)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 12)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 13)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 14)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 15)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

-continued

(SEQ ID NO: 16)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 17)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 18)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 19)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 20)
S-A-K-E-E-K-Q-P-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 21)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
or

(SEQ ID NO: 22)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-P-S-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E.

25 In any of the embodiments of this paragraph, the first six amino acid residues of the peptide can be replaced by a sequence selected from the group consisting of S-V-K-E-E-K (SEQ ID NO: 23), S-A-K-E-D-K (SEQ ID NO: 24), S-A-K-E-E-K (SEQ ID NO: 25), and K-R-D-E-N-Q (SEQ ID NO: 26). In any of the embodiments of this paragraph, the last fourteen amino acid residues of the peptide can be replaced by a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In certain embodiments, a peptide of the invention comprises or consists of the sequence

(SEQ ID NO: 27)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 28)
S-A-K-E-E-K-Q-P-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 29)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 30)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 31)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 32)
S-A-K-E-E-K-Q-P-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 33)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

(SEQ ID NO: 34)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-A-T-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

-continued

- (SEQ ID NO: 35)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 36) 5
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 37)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 38) 10
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 39) 15
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 40)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 41) 20
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
or
- (SEQ ID NO: 42) 25
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-N-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E.

In any of the embodiments of this paragraph, the first six amino acid residues of the peptide can be replaced by a sequence selected from the group consisting of S-V-K-E-E-K (SEQ ID NO: 23), S-A-K-E-D-K (SEQ ID NO: 24), S-A-K-E-E-K (SEQ ID NO: 25), and K-R-D-E-N-Q (SEQ ID NO: 26).

In certain embodiments, a peptide of the invention comprises or consists of the sequence

- (SEQ ID NO: 43)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 44)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 45) 45
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 46)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 47)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 48) 55
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 49)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 50)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-T-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 51) 65
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;

-continued

- (SEQ ID NO: 52)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 53)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 54)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-S-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 55)
S-A-K-E-E-K-Q-T-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 56)
S-A-K-E-E-K-Q-P-T-T-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
- (SEQ ID NO: 57)
S-A-K-E-E-K-Q-T-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E;
or
- (SEQ ID NO: 58)
S-A-K-E-E-K-Q-P-T-V-G-L-Y-G-L-K-Q-D-W-D-G-V-A-
A-P-K-Q-R-K-N-D-P-S-E-T-S-P-G-Q-E.

In any of the embodiments of this paragraph, the first six amino acid residues of the peptide can be replaced by a sequence selected from the list consisting of S-V-K-E-E-K (SEQ ID NO: 23), S-A-K-E-D-K (SEQ ID NO: 24), S-A-K-E-E-K (SEQ ID NO: 25), and K-R-D-E-N-Q (SEQ ID NO: 26). In any of the embodiments of this paragraph, the last fourteen amino acid residues of the peptide can be replaced by a sequence selected from the group consisting of Q-R-K-N-D-P-S-E-T-S-P-G-Q-E (SEQ ID NO: 3), M-A-P-F-H-E-L-D-V-N-N-H-P-N (SEQ ID NO: 4), S-L-N-V-S-F-L-I-D-P-M-A-P-F (SEQ ID NO: 5), and Q-D-S-N-L-Y-S-I-F-F-V-P-Q (SEQ ID NO: 6).

In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 59, F-S-A-K-X₅-X₆-X₇-A-E-T-X₁₁-X₁₂-T-F-G-X₁₆-X₁₇-X₁₈-X₁₉-X₂₀-D-G-A-X₂₄-X₂₅-X₂₆-X₂₇-N-X₂₉-V-X₃₁-N-X₃₃-F-T-I-S-N (SEQ ID NO: 59) wherein X₅ is an amino acid selected from the group consisting of E and Q, X₆ is an amino acid selected from the group consisting of E and Q, X₇ is any amino acid, X₁₁ is an amino acid selected from the group consisting of K and R, X₁₂ is any amino acid, X₁₆ is an amino acid selected from the group consisting of L and I, X₁₇ is any amino acid, X₁₈ is an amino acid selected from the group consisting of R and K, X₁₉ is an amino acid selected from the group consisting of Q and N, X₂₀ is an amino acid selected from the group consisting of Y and T, X₂₄ is any amino acid, X₂₅ is an amino acid selected from the group consisting of I and L, X₂₆ is any amino acid, X₂₇ is an amino acid selected from the group consisting of D and E, X₂₉ is an amino acid selected from the group consisting of E and Q, X₃₁ is an amino acid selected from the group consisting of E and Q, and X₃₃ is an amino acid selected from the group consisting of K and R.

In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 59, wherein X₅ is E, X₆ is E, X₁₆ is L, X₁₈ is K, X₂₀ is Y, X₂₅ is I, X₂₉ is Q, X₃₁ is Q, and X₃₃ is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 59, wherein X₇ is K, X₁₂ is an amino acid selected from the group consisting of K and R, X₁₇ is an amino acid selected from the group consisting of E and D, X₂₄ is an amino acid selected from the group consisting of K and Q, and X₂₆ is an amino acid selected from the group consisting of E and T.

In certain embodiments, a peptide of the invention comprises or consists of the sequence

(SEQ ID NO: 60) 5
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-E-K-N-Y-D-G-A-K-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 61) 10
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-E-K-N-Y-D-G-A-K-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 62) 15
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-E-K-N-Y-D-G-A-Q-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 63) 20
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-E-K-N-Y-D-G-A-Q-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 64) 25
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-D-K-N-Y-D-G-A-K-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 65) 30
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-D-K-N-Y-D-G-A-K-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 66) 35
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-D-K-N-Y-D-G-A-Q-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 67) 40
 F-S-A-K-E-E-K-A-E-T-K-K-T-F-G-L-D-K-N-Y-D-G-A-Q-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 68) 45
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-E-K-N-Y-D-G-A-K-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 69) 50
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-E-K-N-Y-D-G-A-K-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 70) 55
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-E-K-N-Y-D-G-A-Q-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 71) 60
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-E-K-N-Y-D-G-A-Q-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 72) 65
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-D-K-N-Y-D-G-A-K-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 73) 70
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-D-K-N-Y-D-G-A-K-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 74) 75
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-D-K-N-Y-D-G-A-Q-I-E-D-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 75) 80
 F-S-A-K-E-E-K-A-E-T-K-R-T-F-G-L-D-K-N-Y-D-G-A-Q-I-T-D-N-Q-V-Q-N-K-F-T-I-S-N.

In other embodiments, a peptide of the invention comprises or consists of the sequence

(SEQ ID NO: 76) 85
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-E-K-Q-Y-D-G-A-K-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 77) 90
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-E-K-Q-Y-D-G-A-K-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

-continued

(SEQ ID NO: 78)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-E-K-Q-Y-D-G-A-Q-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 79)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-E-K-Q-Y-D-G-A-Q-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 80)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-E-K-Q-Y-D-G-A-K-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 81)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-D-K-Q-Y-D-G-A-K-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 82)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-D-K-Q-Y-D-G-A-Q-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 83)
 F-S-A-K-E-E-K-A-E-T-R-K-T-F-G-L-D-K-Q-Y-D-G-A-Q-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 84)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-E-K-Q-Y-D-G-A-K-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 85)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-E-K-Q-Y-D-G-A-K-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 86)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-E-K-Q-Y-D-G-A-Q-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 87)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-E-K-Q-Y-D-G-A-Q-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 88)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-D-K-Q-Y-D-G-A-K-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 89)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-D-K-Q-Y-D-G-A-K-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 90)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-D-K-Q-Y-D-G-A-Q-I-E-E-N-Q-V-Q-N-K-F-T-I-S-N;

(SEQ ID NO: 91)
 F-S-A-K-E-E-K-A-E-T-R-R-T-F-G-L-D-K-Q-Y-D-G-A-Q-I-T-E-N-Q-V-Q-N-K-F-T-I-S-N.

In some embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 92, G-X₂-F-S-A-K-X₇-X₈-K-X₁₀-A-D-T-R-X₁₅-T-F-G-L-X₂₀-K-Q-T-D-G-A-X₂₇-I-X₂₉-E-N-X₃₂-V-X₃₄-N-X₃₆-F-T-I-S-N (SEQ ID NO: 92) wherein X₂ is an amino acid selected from the group consisting of D and N, X₇ is an amino acid selected from the group consisting of E and Q, X₈ is an amino acid selected from the group consisting of E and Q, X₁₀ is any amino acid, X₁₅ is any amino acid, X₂₀ is any amino acid, X₂₇ is any amino acid, X₂₉ is any amino acid, X₃₂ is an amino acid selected from the group consisting of E and Q, X₃₄ is an amino acid selected from the group consisting of E and Q, and X₃₆ is an amino acid selected from the group consisting of K and R. In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 92, wherein X₂ is N, X₇ is E, X₈ is E, X₃₂ is Q, X₃₄ is Q, and X₃₆ is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 93, DNQVQNKF^TISNYSFKYEDNP (SEQ ID NO: 93).

In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92 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. In one embodiment, the N-terminal peptide sequence can be G-N or G-D. For instance, in certain embodiments, the peptides of the invention comprise a sequence of SEQ ID NO: 59 and an additional N-terminal peptide sequence of G-N or G-D. 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 *Ehrlichia* OMP-1 sequence, or a variant thereof. In certain embodiments, the peptide sequence is a fragment of a naturally-occurring *Ehrlichia* OMP-1 sequence. The peptide sequence can be, e.g., from a conserved or non-conserved region of OMP-1. 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. OMP-1 proteins and peptides thereof have been described, e.g., in U.S. Pat. Nos. 6,544,517, 6,893,640, 6,923,963, 7,063,846, and 7,407,770, U.S. Patent Applications 2004/0265333 and 2009/0075368, and European Patent No. 1026949, the contents of which are incorporated herein by reference.

Variant polypeptides are at least about 80, 85, 90, 95, 98, or 99% identical to a peptide shown in SEQ ID NO: 1, SEQ ID NO: 7-22, and SEQ ID NO: 27-94 and are also polypeptides of the invention. 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.

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.

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 sub-

stitutions) 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.

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.

In certain embodiments, the additional N-terminal peptide sequence can comprise or consist of another peptide having a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. Thus, in some embodiments, a peptide of the invention can be a multimer of sequences having a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. In other embodiments, the N-terminal peptide sequence is a native OMP-1 peptide sequence that is naturally adjacent to the N-terminal end of a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. For instance, in one embodiment, the peptide can comprise a multimer of SEQ ID NO: 94, (KEEKAETRKTFGLEKQYDGAKEENQVQNKGGGGG)_N, wherein N=1-10. In other embodiments, the peptide can comprise a fusion of sequences of SEQ ID NO: 1, SEQ ID NO: 59, SEQ ID NO: 92, or SEQ ID NO: 94 optionally through one or more linking amino acids. For example, in one embodiment, the peptide can comprise a sequence of SEQ ID NO: 1 linked to SEQ ID NO: 94 optionally through one or more linking amino acids (e.g. glycine residues). In another embodiment, the peptide can comprise a sequence of SEQ ID NO: 1 linked to SEQ ID NO: 92 optionally through one or more linking amino acids (e.g. glycine residues).

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 an *Ehrlichia* protein or otherwise, other than a native OMP-1 peptide sequence. In certain embodiments, the additional N-terminal peptide sequence comprises an epitope of an *Ehrlichia* surface antigen. In certain embodiments, the additional N-terminal peptide sequence comprises an epitope of an *Ehr-*

lichia antigen, such as p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3. Protein and peptide sequences corresponding to *Ehrlichia* antigens have been described. See, e.g., U.S. Pat. Nos. 6,306,402, 6,355,777, 7,204,992, and 7,407,770, and WO2006/138509, the contents of which are incorporated herein by reference. Polypeptides or peptides derived from other microorganisms can also be used.

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 sequence, a non-native sequence, or any combination of such sequences (e.g., two or more native sequences, two or more non-native sequence, or one or more native sequences in combination with one or more non-native sequences).

In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92 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. In certain embodiments, the additional C-terminal 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 C-terminal peptide sequence can be a native OMP-1 sequence. In certain embodiments, the C-terminal peptide sequence is a fragment of a naturally-occurring *Ehrlichia* OMP-1 sequence. The peptide sequence can be, e.g., from a conserved or non-conserved region of OMP-1. 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. In certain embodiments, the additional C-terminal peptide sequence can comprise or consist of another peptide having a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. For example, in certain embodiments, a peptide of the invention can be a multimer of sequences each having a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. In other embodiments, the native sequence is a OMP-1 sequence that is naturally adjacent to the C-terminal end of a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92.

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 an *Ehrlichia* surface antigen other than OMP-1. In certain embodiments, the additional C-terminal peptide sequence comprises an epitope of an *Ehrlichia* antigen, such as p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3. Polypeptides or peptides derived from other microorganisms can also be used.

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, or one or more native sequences in combination with one or more non-native sequences).

In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92 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 of the invention do not consist of a full-length OMP-1 protein. However, in certain embodiments, peptides of the invention can com-

prise a full-length OMP-1 protein. In other embodiments, peptides of the invention do not comprise a full-length OMP-1 protein.

A peptide of the invention comprising an additional N-terminal and/or C-terminal peptide sequence can be designed for diagnosing *Ehrlichia* infections early after infection (e.g., within one to two weeks after the onset of infection). For example, in certain embodiments, the additional N-terminal and/or C-terminal peptide sequence comprises an antigen or epitope associated with early stages of *Ehrlichia* infection.

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.

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.

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, SEQ ID NO: 7 through SEQ ID NO: 22, SEQ ID NO: 27 through SEQ ID NO: 94.

In certain embodiments, peptides of the invention comprise a fragment of a peptide sequence described herein. For example, in certain embodiments, peptides of the invention comprise a fragment of a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 7 through SEQ ID NO: 22, and SEQ ID NO: 27 through SEQ ID NO: 94. 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). In certain embodiments, the fragment comprises a sequence set forth in U.S. Pat. No. 6,306,402, 6,355,777, 7,204,992, or 7,407,770, or in WO2006/138509. In certain embodiments, the fragment does not consist of a sequence set forth in one or more of U.S. Pat. Nos. 6,306,402, 6,355,777, 7,204,992, and 7,407,770, and WO2006/138509. Peptides of the invention 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.

Peptides of the invention comprising an additional N-terminal or C-terminal peptide sequence can further comprise a linker connecting the peptide (e.g., a peptide of SEQ ID NO: 1, SEQ ID NO: 59, SEQ ID NO: 92, or a fragment thereof) 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.

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). In certain embodiments, 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. In certain embodiments, an isolated peptide of the invention is 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.

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-*Ehrlichia* antibodies (e.g., antibodies to OMP-1 proteins and, optionally, other *Ehrlichia* 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.

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.

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, both of which are herein incorporated by reference), or neutravidin. Avidin, streptavidin, polymeric streptavidin, or neutravidin, in turn, can be linked to a signaling moiety (e.g., an enzyme, such as horse radish peroxidase (HRP) or alkaline phosphatase, or other moiety that can be visualized, such as colloidal gold or a fluorescent moiety) 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.

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), horse radish peroxidase (HRP), 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.

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 cross-links, 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.

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.

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.

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.

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.

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, N. Mex.). 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.

Accordingly, in another aspect, the invention provides devices. In certain embodiments, the devices are useful for performing an immunoassay. For example, in certain embodiments, the device is a lateral flow immunoassay device. In other embodiments, the device is an analytical rotor. In other embodiments, the device is a dot blot. In other embodiments, the device is a tube or a well, e.g., in a plate suitable for an ELISA assay. In still other embodiments, the device is an electrochemical sensor, an optical sensor, or an opto-electronic sensor.

In certain embodiments, the device comprises a peptide of the invention. In other embodiments, the device comprises a mixture of different peptides of the invention. For example, in certain embodiments, the device comprises two, three, four, or more different peptides of the invention. In certain embodiments, the peptide or each peptide in the mixture comprises a

sequence of SEQ ID NO: 1, SEQ ID NO: 59 or SEQ ID NO: 92. In other embodiments, the peptide or each peptide in the mixture comprises a sequence of SEQ ID NO: 1. In certain embodiments, the peptides are attached to or immobilized upon the device.

In another aspect, the invention provides compositions comprising one or more peptides of the invention. For example, in certain embodiments, the invention provides a composition comprising a peptide comprising a sequence of SEQ ID NO: 1, or mixtures thereof. In certain embodiments, the composition comprises a mixture of 2, 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). In certain embodiments, the peptides comprise an N-terminal and/or C-terminal addition, and/or are modified (e.g., by association with one or more further moieties), as described herein. In certain embodiments, the peptides comprise the same N-terminal and/or C-terminal additions. In other embodiments, the peptides comprise different N-terminal and/or C-terminal additions. In still other embodiments, the invention provides a composition comprising a peptide comprising a sequence of SEQ ID NO: 59 or SEQ ID NO: 92, or mixtures thereof. In certain embodiments, the composition comprises a mixture of 2, 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: 59 or SEQ ID NO: 92).

In certain embodiments, the compositions comprise one or more peptides of the invention and one or more additional peptides, such as an *Ehrlichia* peptide or antigen, a peptide or antigen from one or more infectious *Ehrlichia* species, or a peptide or antigen from one or more causative agents of monocytic ehrlichiosis. The *Ehrlichia* peptide or antigen can be any *Ehrlichia* surface peptide or antigen, or any peptide or antigen described herein (e.g., any peptide or antigen of an OMP-1, p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3 protein, or any fragment or epitope thereof). The combination may comprise a cocktail (a simple mixture) of individual peptides or polypeptides, 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). 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 an *Ehrlichia* peptide or antigen, a peptide or antigen from an infectious *Ehrlichia* species, or a peptide or antigen from a causative agent of monocytic ehrlichiosis.

In another aspect, the invention provides nucleic acids comprising a sequence encoding a peptide of the invention. Nucleic acids of the invention 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 of the invention encode the peptides described herein. In certain embodiments, the nucleic acids encode a peptide having the sequence of SEQ ID NO: 1, SEQ ID NOS: 7-22, or SEQ ID NOS: 27-94, or combinations thereof. Nucleic acids of the invention 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.

Nucleic acids of the invention 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 of the invention can also comprise fragments that encode immunogenic peptides. Nucleic acids of the invention can encode full-length polypeptides, peptide fragments, and variant or fusion peptides.

Nucleic acids of the invention 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.

Nucleic acids of the invention 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.

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 of the invention 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.

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 compositions or meth-

ods of the invention, 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.

Accordingly, the invention also provides vectors comprising nucleic acids of the invention, and host cells comprising such vectors. In certain embodiments, the vector is a shuttle vector. In other embodiments, the vector is an expression vector (e.g., a bacterial or eukaryotic expression vector). In certain embodiments, the host cell is a bacterial cell. In other embodiments, the host cell is a eukaryotic cell.

Suitable host cells or cell lines for the recombinant nucleic acids or vectors of the invention 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 of the invention can be expressed in yeast, insect, mammalian, or other cell types, using conventional procedures.

The present invention also provides 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 of the invention 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

In another aspect, the invention provides methods of detecting in a sample an antibody to an epitope of an *Ehrlichia* antigen. In certain embodiments, the methods comprise contacting a sample with a peptide of the invention, and detecting formation of an antibody-peptide complex comprising said peptide, wherein formation of said complex is indicative of the presence of an antibody to an epitope of an *Ehrlichia* antigen in said sample. In certain embodiments, the *Ehrlichia* antigen is from an infectious *Ehrlichia* species. In certain embodiments, the *Ehrlichia* antigen is from a pathogenic *Ehrlichia* species, such as *Ehrlichia chaffeensis* or *Ehrlichia canis*. Other species of *Ehrlichia* which have been implicated in monocytic ehrlichiosis 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 *Ehrlichia*,” as used herein, refers to any such *Ehrlichia* species that causes monocytic ehrlichiosis.

In certain embodiments, the methods comprise contacting the sample with a mixture of two, 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., an *Ehrlichia* peptide, or antigenic fragment or epitope thereof, such as an *Ehrlichia* surface antigen, or an OMP-1, p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3 protein).

In certain embodiments, the peptide or each peptide in the mixture is an isolated (e.g., synthetic and/or purified) peptide. In certain embodiments, the peptide or 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, a nanoparticle, a 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).

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 (e.g., a hemagglutination or particle/bead 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.

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.

In one embodiment, the methods involve detecting the presence of naturally occurring antibodies against an *Ehrlichia* antigen (e.g., the antigen of a pathogenic *Ehrlichia*, such as *E. chaffeensis* or *E. canis*) 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.

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 peptide 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 *Ehrlichia* species. A peptide, including a modified form thereof, which “binds specifically”

to (e.g., “is specific for” or binds “preferentially” to) an antibody against an *Ehrlichia* 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 monocytic ehrlichiosis.

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 *Ehrlichia* is present in a sample, irrespective of whether or not it is detected.

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).

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. 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 whole blood, plasma, or serum 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.

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.

In certain embodiments of the invention, the assay comprises: immobilizing the antibody(s) in the sample; adding a peptide of the invention; and detecting the degree of antibody bound to the peptide, e.g., by the peptide being labeled or by adding a labeled substance, such as a labeled binding partner (e.g., streptavidin-HRP or streptavidin-colloidal gold complex) or a labeled antibody which specifically recognizes the peptide. See, e.g., FIG. 1. In other embodiments, the assay comprises: immobilizing a peptide of the invention; adding the sample containing antibodies; and detecting the amount of antibody bound to the peptide, e.g., by adding another peptide 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., FIG. 3. In still other embodiments, the assay comprises: reacting the peptide 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-HRP or streptavidin-colloidal gold complex) or a labeled antibody which specifically recognizes the peptide.

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: 95) 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 particles 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.

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.

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.

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.

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 labels include, but are not limited to, enzymes (e.g., HRP, beta-galactosidase, etc.), fluorescent labels, radioactive labels, and metal-conjugated labels (e.g., colloidal gold-conjugated labels). Suitable detection methods include, e.g., detection of an agent which is tagged, directly or indirectly, with a colorimetric assay (e.g., for detection of HRP or beta-galactosidase activity), visual inspection using light microscopy, immunofluorescence microscopy, including confocal microscopy, or by flow cytometry (FACS), autoradiography (e.g., for detection of a radioactively labeled agent), 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.

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.

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.

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, G, or L proteins, 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. As a variation on this assay format, the sample can be applied to the sample application pad in a manner that allows antibodies in the sample to travel to and bind peptides on the diagnostic strip, and a second "developer" solution can be added to the sample application pad, wherein the developer solution contains labeled reporter (or, e.g., solubilizes labeled reporter present in the sample application pad or a conjugate pad). The developer solution then carries, by capillary action, the labeled reporter to the diagnostic strip, where the labeled reporter can bind any sample antibodies bound to the peptides located at the diagnostic strip.

An alternative format for the lateral flow immunoassay comprises the peptides or compositions of the invention 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 FIG. 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 FIG. 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. This format can also be modified to have a "developer" solution. For example, the developer solution could contain (or solubilize) the labeled ligand receptor.

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 contain-

ing specific anti-*Ehrlichia* (e.g., anti-*E. chaffeensis* or anti-*E. canis*) 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 ehrlichiosis 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.

In one embodiment of an ELISA, a peptide of the invention is 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.

An alternative format for the ELISA assay features the peptide(s) of the invention being attached (e.g., fused) to an appropriate enzyme, such as HRP. Steps for carrying out such an ELISA include: coating the wells of a plate with anti-dog or anti-human IgG/IgM; incubating samples suspected of containing antibodies to the peptide of the invention with the immobilized anti-species IgG/IgM; removing unreacted sample and washing the wells with a suitable wash buffer; applying enzyme-coupled (e.g., HRP-coupled) peptide of the invention and allowing it to react with any captured anti-*Ehrlichia* antibodies; and visualizing the enzyme-coupled peptide by applying an appropriate enzyme substrate (e.g., TMB).

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 or compositions 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 compositions 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.

In still other embodiment, peptide or compositions 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 immuno-enzymatic 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.

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 *Ehrlichia* antibodies and infection by pathogenic *Ehrlichia* (e.g., *E. chaffeensis* or *E. canis*) 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.

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.

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

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.

When a method of the invention is carried out in vivo, any of a variety of conventional assays can be used. 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 *Ehrlichia* capable of causing monocytic ehrlichiosis, 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.

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

In certain embodiments, the methods comprise contacting a sample from the subject with a peptide of the invention, and detecting formation of an antibody-peptide complex comprising said peptide, wherein formation of said complex is indicative of the subject having ehrlichiosis disease. In certain embodiments, the methods comprise contacting the sample with a mixture of two, 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 or the invention and one or more other peptides (e.g., an *Ehrlichia* peptide, or antigenic fragment or epitope thereof, such as from an *Ehrlichia* surface protein or an *Ehrlichia* OMP-1, p38, p43, p120, p140, p153, p156, p200, gp19, gp36, gp47, gp200, or HGE-3 protein).

In certain embodiments, the peptide or each peptide in the mixture is an isolated (e.g., synthetic and/or purified) peptide. In certain embodiments, the peptide or 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).

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.

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

In yet another aspect, the invention provides kits. In certain embodiments, the kits comprise a peptide of the invention. In certain embodiments, the kits comprise two, three, four, or more different peptides of the invention. The peptides can comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 59, or SEQ ID NO: 92. 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).

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 peptide, a mixture of different peptides of the invention, or a peptide composition of the invention is attached to or immobilized on the beads, the plate, or the device.

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 deter-

mined 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 semi-purified 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.

Such kits provide a convenient, efficient way for a clinical laboratory to diagnose infection by a pathogenic *Ehrlichia*, such as *E. chaffeensis* or *E. canis*. 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 an *Ehrlichia* antigen or to diagnose monocytic ehrlichiosis. 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 an *Ehrlichia* antigen or to diagnose monocytic ehrlichiosis.

The peptides, compositions and devices comprising the peptides, kits and methods of the invention offer a number of advantages. For example, they allow for simple, inexpensive, rapid, sensitive and accurate detection of monocytic ehrlichiosis, and avoid serologic cross-reactivity with other conditions with similar symptoms. 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-OMP-1 antibodies or other antibodies produced in response to a vaccine based on the outer surface proteins of *Ehrlichia*.

The following examples illustrates various aspects of the invention. The examples should, of course, be understood to be merely illustrative of only certain embodiments of the invention and not to constitute limitations upon the scope of the invention.

EXAMPLES

Example 1

Dipstick tests were performed using serum samples known to be positive or negative for *Ehrlichia*. The dipsticks consisted of 2 mm wide HF180 nitrocellulose, with a 17 mm CF6 upper wick and a capture line containing AIRM-2 peptides attached to the nitrocellulose membrane. The AIRM-2 peptides were a mixture of biotinylated peptides each having a sequence of SEQ ID NO: 1, and were attached to the membrane via streptavidin. The lower halves of the dipsticks were blocked with a solution of PBS, 1% BSA, 1% TRITON X-100 detergent, pH 7.4.

Initially, the amount of AIRM-2 peptides attached to a dipstick was varied between 0.1 mg/ml, 0.5 mg/ml, and 1.0 mg/ml, and the ratio of peptide:streptavidin was further varied between 1:1, 1:4, and 1:8 for each of the peptide concentrations.

The test consisted of: placing each dipstick into a well containing 40 µl of TBS and allowing the well to empty (wash step); placing 1 µl spot of sample onto the blocked, lower half of each dipstick; placing each dipstick into a well containing 40 µl of conjugate and allowing the well to empty; and reading any signal present at the capture line of each dipstick. The conjugated consisted of gold-conjugated rabbit anti-dog IgG, at OD1 or OD2.

The results for the *Ehrlichia* positive sample (R09266-007 (BH19)) are shown in Tables 1 and 2.

TABLE 1

OD1 Conj. + 0.1 mg/ml AIRM-2		OD1 Conj. + 0.5 mg/ml AIRM-2		OD1 Conj. + 1.0 mg/ml AIRM-2	
SA ratio	(Op1)	SA ratio	(Op1)	SA ratio	(Op1)
1:1	8	1:1	9	1:1	11
1:4	9	1:4	11	1:4	11
1:8	9	1:8	11	1:8	11

TABLE 2

OD2 Conj. + 0.1 mg/ml AIRM-2		OD2 Conj. + 0.5 mg/ml AIRM-2		OD2 Conj. + 1.0 mg/ml AIRM-2	
SA ratio	(Op1)	SA ratio	(Op1)	SA ratio	(Op1)
1:1	10	1:1	8	1:1	11
1:4	4	1:4	11	1:4	11
1:8	11	1:8	11	1:8	5

The corresponding results for the *Ehrlichia* negative sample (R09266-008) were all 0. Although all of the results with *Ehrlichia* positive sample shown in Tables 1 and 2 produced a positive signal at the capture line, the 0.5 mg/ml AIRM-2 peptides with a 1:4 or 1:8 ratio of peptide:streptavidin appeared to provide the optimal capture conditions for further testing.

Example 2

Performing dipstick tests according to the method of Example 1, with 0.5 mg/ml AIRM-2 peptides, a peptide: streptavidin ratio of 1:4, and OD1 conjugate, a panel of *Ehrlichia* positive and negative serum samples were tested. The results are shown in Table 3.

TABLE 3

Sample	Dipstick 1 (Op1)	Dipstick 2 (Op1)	Average	St Dev
BH1	10	10	10	0
BH2	10	10	10	0
BH5	10	10	10	0
BH9	10	10	10	0
BH15	10	10	10	0
BH16	10	10	10	0
BH17	10	10	10	0
BH18	10	10	10	0
BH19	10	10	10	0
Neg: R09266-008	0	0	0	0
Neg: <i>Borrelia</i> sample 2	0	0	0	0
Neg: <i>Borrelia</i> sample 5	0	0	0	0
Neg: <i>Borrelia</i> sample 8	0	0	0	0
Neg: <i>Borrelia</i> sample 9	0	0	0	0
Neg: <i>Borrelia</i> sample 10	0	0	0	0
Conjugate alone	0	0	0	0

The results of Table 3 show that, under the conditions of the tests, all *Ehrlichia* positive samples were detected with signals of rann 10, while no false positive signals were observed with the *Ehrlichia* negative samples.

Example 3

Performing dipstick tests according to the method of Example 1, serial dilutions of the *Ehrlichia* positive BH19

serum sample with *Ehrlichia* negative R09266-008 serum sample were analyzed. The results are shown in Table 4.

TABLE 4

Dilution Factor	Dipstick 1 (Op1)	Dipstick 2 (Op1)	Dipstick 3 (Op1)	Average	St Dev
0	10	10	10	10	0
2	10	10	10	10	0
10	10	9	10	9.7	0.6
50	9	9	9	9	0
100	8	8	8	8	0
150	6	6	6	6	0
200	6	6	6	6	0
300	5	5	5	5	0
500	4	4	4	4	0
1000	3	3	3	3	0
2000	3	3	3	3	0

As shown in Table 4, increased dilution of the *Ehrlichia* positive serum with the *Ehrlichia* negative serum resulted in decreased signal intensities being observed. This indicates that *Ehrlichia* specific signals are being detected as a result of the antibody present in the positive sample, and not due to matrix interference or other non-specific binding.

Example 4

Two different mixtures of biotinylated peptides each having a sequence of SEQ ID NO: 96 or SEQ ID NO: 97 were synthesized using standard synthesis procedures:

SEQ ID NO: 96:

F-S-A-K-E-E-X₇-A-E-T-K-X₁₂-T-F-G-L-X₁₇-K-N-Y-D-G-A-X₂₄-I-X₂₆-D-N-Q-V-Q-N-K-F-T-I-S-N

SEQ ID NO: 97:

F-S-A-K-E-E-X₇-A-E-T-R-X₁₂-T-F-G-L-X₁₇-K-Q-Y-D-G-A-X₂₄-I-X₂₆-E-N-Q-V-Q-N-K-F-T-I-S-N,
wherein X₇, X₁₂, X₁₇, X₂₄, and X₂₆ is any amino acid.

Each mixture was an equimolar mixture of all the natural L-amino acids at the "X" positions. These two peptide mix-

tures were individually dissolved in distilled water at 1 mg/ml. Heating to 40° C. facilitated dissolution. Commercially purchased streptavidin was dissolved in water at room temperature. Streptavidin was mixed with individual peptides to produce a final concentration of 5 µg/ml of streptavidin and 2.5 µg/ml of peptides with SEQ ID NO: 96 or SEQ ID NO: 97 using Tris buffer.

The streptavidin-peptide complexes thus formed were used to coat wells of ELISA plates. The unbound mixture was poured off and the plates were blocked to prevent undesirable non-specific binding. Dog serum samples positive to *Ehrlichia* species, as determined by indirect immunofluorescence assays, were then allowed to react with the peptides comprising sequences of SEQ ID NO: 96 or SEQ ID NO: 97. After one hour incubation, unbound materials were removed and the plates washed. The binding of dog IgG to the wells was realized by reacting the bound IgG with (i) the goat anti-dog HRP conjugate and (ii) visualizing the bound HRP with a commercial TMB substrate.

Two of the dog serum samples were found to react with both mixtures of peptides comprising sequences of SEQ ID NO: 96 or SEQ ID NO: 97 but not with peptides designed specifically to identify *E. chaffeensis*. Similarly, 13 dog serum samples reacted with the peptides designed to detect *E. chaffeensis* but not with peptides comprising SEQ ID NO: 96 or SEQ ID NO: 97. Thus, a combination of peptides comprising SEQ ID NO: 96, peptides comprising SEQ ID NO: 97 and peptides that detect *E. chaffeensis* is capable of detecting all animals harboring antibodies to the *Ehrlichial* species *canis*, *chaffeensis* and *ewingii*.

To the extent that any definitions in documents incorporated by reference are inconsistent with the definitions provided herein, the definitions provided herein are controlling. Although the invention has been described with reference to the presently preferred embodiments and the foregoing non-limiting examples, it should be understood that various changes and modifications, as would be obvious to one skilled in the art, can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 97

<210> SEQ ID NO 1
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<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.
<220> FEATURE:
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<222> LOCATION: (1)..(6)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa may be Asn or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa may be Thr or Pro
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa may be Thr or Val
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa may be Gly or Ala
<220> FEATURE:
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<221> NAME/KEY: misc_feature
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<223> OTHER INFORMATION: Xaa may be Leu or Val
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa may be Tyr or Phe
<220> FEATURE:
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<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa may be Asp or Asn
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<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa may be Asp or Asn
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<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa may be Ser or Val
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa may be Ala, Ser, or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa may be Ala or Ile
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa may be Ser, Thr, or Pro
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: Xaa may be Ser, Asn, or Lys
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(40)
<223> OTHER INFORMATION: Xaa may be any amino acid

<400> SEQUENCE: 1

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Thr Xaa Xaa Xaa Xaa Gly Leu Lys
1          5          10          15

Gln Xaa Trp Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          20          25          30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
          35          40

<210> SEQ ID NO 2
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 2

Lys Arg Asp Glu Asn Gln
1          5

<210> SEQ ID NO 3
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 3

Gln Arg Lys Asn Asp Pro Ser Glu Thr Ser Pro Gly Gln Glu
1          5          10

<210> SEQ ID NO 4
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 4

Met Ala Pro Phe His Glu Leu Asp Val Asn Asn His Pro Asn
 1 5 10

<210> SEQ ID NO 5

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 5

Ser Leu Asn Val Ser Phe Leu Ile Asp Pro Met Ala Pro Phe
 1 5 10

<210> SEQ ID NO 6

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 6

Gln Asp Ser Asn Leu Tyr Ser Ser Ile Phe Phe Val Pro Gln
 1 5 10

<210> SEQ ID NO 7

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 7

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 8

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 8

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 9

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 9

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

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<210> SEQ ID NO 10
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 10

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 11
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 11

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 12
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 12

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 13
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 13

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 14
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 14

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Ser Gln Arg Lys Asn Asp Pro

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20 25 30
 Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 15
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 15

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 16
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 16

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 17
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 17

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 18
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 18

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 19
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 19

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 20

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 20

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 21

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 21

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 22

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 22

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Ser Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 23

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 23

Ser Val Lys Glu Glu Lys
 1 5

<210> SEQ ID NO 24

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 24

Ser Ala Lys Glu Asp Lys
 1 5

<210> SEQ ID NO 25

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 25

Ser Ala Lys Glu Glu Lys
 1 5

<210> SEQ ID NO 26

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 26

Lys Arg Asp Glu Asn Gln
 1 5

<210> SEQ ID NO 27

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 27

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 28

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 28

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 29

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 29

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

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<210> SEQ ID NO 30

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 30

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
35 40

<210> SEQ ID NO 31

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 31

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
35 40

<210> SEQ ID NO 32

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 32

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
35 40

<210> SEQ ID NO 33

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 33

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro
20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
35 40

<210> SEQ ID NO 34

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 34

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Asn Gln Arg Lys Asn Asp Pro

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20 25 30
 Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 35
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 35

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 36
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 36

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 37
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 37

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 38
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 38

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 39
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 39

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 40

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 40

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 41

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 41

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 42

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 42

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Asn Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 43

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 43

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Thr Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

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Gln Asp Trp Asp Gly Val Ala Ala Thr Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 49
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 49

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 50
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 50

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Thr Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 51
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 51

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 52
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 52

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 53
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 53

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 54

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 54

Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Ser Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 55

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 55

Ser Ala Lys Glu Glu Lys Gln Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 56

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 56

Ser Ala Lys Glu Glu Lys Gln Pro Thr Thr Thr Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 57

<211> LENGTH: 40

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 57

Ser Ala Lys Glu Glu Lys Gln Thr Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

Gln Asp Trp Asp Gly Val Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

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<210> SEQ ID NO 58
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

 <400> SEQUENCE: 58

 Ser Ala Lys Glu Glu Lys Gln Pro Thr Val Gly Leu Tyr Gly Leu Lys
 1 5 10 15

 Gln Asp Trp Asp Gly Val Ala Ala Pro Lys Gln Arg Lys Asn Asp Pro
 20 25 30

 Ser Glu Thr Ser Pro Gly Gln Glu
 35 40

<210> SEQ ID NO 59
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (5)..(5)
 <223> OTHER INFORMATION: Xaa may be Glu or Gln
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (6)..(6)
 <223> OTHER INFORMATION: Xaa may be Glu or Gln
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(7)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (11)..(11)
 <223> OTHER INFORMATION: Xaa may be Lys or Arg
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (12)..(12)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (16)..(16)
 <223> OTHER INFORMATION: Xaa may be Leu or Ile
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (17)..(17)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (18)..(18)
 <223> OTHER INFORMATION: Xaa may be Arg or Lys
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (19)..(19)
 <223> OTHER INFORMATION: Xaa may be Gln or Asn
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (20)..(20)
 <223> OTHER INFORMATION: Xaa may be Tyr or Thr
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (24)..(24)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (25)..(25)
 <223> OTHER INFORMATION: Xaa may be Ile or Leu
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (26)..(26)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (27)..(27)
 <223> OTHER INFORMATION: Xaa may be Asp or Glu

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33)..(33)
<223> OTHER INFORMATION: Xaa may be Lys or Arg

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<400> SEQUENCE: 59

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Phe Ser Ala Lys Xaa Xaa Xaa Ala Glu Thr Xaa Xaa Thr Phe Gly Xaa
1          5          10          15
Xaa Xaa Xaa Xaa Asp Gly Ala Xaa Xaa Xaa Xaa Asn Xaa Val Xaa Asn
          20          25          30
Xaa Phe Thr Ile Ser Asn
          35

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<210> SEQ ID NO 60
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 60

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Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
1          5          10          15
Glu Lys Asn Tyr Asp Gly Ala Lys Ile Glu Asp Asn Gln Val Gln Asn
          20          25          30
Lys Phe Thr Ile Ser Asn
          35

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<210> SEQ ID NO 61
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 61

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Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
1          5          10          15
Glu Lys Asn Tyr Asp Gly Ala Lys Ile Thr Asp Asn Gln Val Gln Asn
          20          25          30
Lys Phe Thr Ile Ser Asn
          35

```

```

<210> SEQ ID NO 62
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

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<400> SEQUENCE: 62

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Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
1          5          10          15
Glu Lys Asn Tyr Asp Gly Ala Gln Ile Glu Asp Asn Gln Val Gln Asn
          20          25          30
Lys Phe Thr Ile Ser Asn
          35

```

```

<210> SEQ ID NO 63
<211> LENGTH: 38
<212> TYPE: PRT

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-continued

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 63

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
 1 5 10 15
 Glu Lys Asn Tyr Asp Gly Ala Gln Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 64

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 64

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Asn Tyr Asp Gly Ala Lys Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 65

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 65

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Asn Tyr Asp Gly Ala Lys Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 66

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 66

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Asn Tyr Asp Gly Ala Gln Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 67

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 67

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Lys Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Asn Tyr Asp Gly Ala Gln Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn

-continued

35

<210> SEQ ID NO 68
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 68

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Glu Lys Asn Tyr Asp Gly Ala Lys Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 69
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 69

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Glu Lys Asn Tyr Asp Gly Ala Lys Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 70
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 70

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Glu Lys Asn Tyr Asp Gly Ala Gln Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 71
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 71

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Glu Lys Asn Tyr Asp Gly Ala Gln Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 72
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 72

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu

-continued

1 5 10 15
 Asp Lys Asn Tyr Asp Gly Ala Lys Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 73
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 73

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Asp Lys Asn Tyr Asp Gly Ala Lys Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 74
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 74

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Asp Lys Asn Tyr Asp Gly Ala Gln Ile Glu Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 75
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 75

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Lys Arg Thr Phe Gly Leu
 1 5 10 15

Asp Lys Asn Tyr Asp Gly Ala Gln Ile Thr Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 76
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 76

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Lys Ile Glu Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 77
 <211> LENGTH: 38

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<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 77

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Lys Ile Thr Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 78

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 78

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Gln Ile Thr Glu Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 79

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 79

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Gln Ile Thr Glu Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 80

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 80

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Asp Lys Gln Tyr Asp Gly Ala Lys Ile Thr Glu Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 81

<211> LENGTH: 38

<212> TYPE: PRT

<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 81

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
 1 5 10 15

Asp Lys Gln Tyr Asp Gly Ala Lys Ile Thr Glu Glu Asn Gln Val Gln Asn
 20 25 30

-continued

Lys Phe Thr Ile Ser Asn
35

<210> SEQ ID NO 82
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 82

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
1 5 10 15

Asp Lys Gln Tyr Asp Gly Ala Gln Ile Glu Glu Asn Gln Val Gln Asn
20 25 30

Lys Phe Thr Ile Ser Asn
35

<210> SEQ ID NO 83
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 83

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu
1 5 10 15

Asp Lys Gln Tyr Asp Gly Ala Gln Ile Thr Glu Asn Gln Val Gln Asn
20 25 30

Lys Phe Thr Ile Ser Asn
35

<210> SEQ ID NO 84
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 84

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Lys Ile Glu Glu Asn Gln Val Gln Asn
20 25 30

Lys Phe Thr Ile Ser Asn
35

<210> SEQ ID NO 85
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 85

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
1 5 10 15

Glu Lys Gln Tyr Asp Gly Ala Lys Ile Thr Glu Asn Gln Val Gln Asn
20 25 30

Lys Phe Thr Ile Ser Asn
35

<210> SEQ ID NO 86
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 86

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Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
 1 5 10 15
 Glu Lys Gln Tyr Asp Gly Ala Gln Ile Glu Glu Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 87
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 87

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
 1 5 10 15
 Glu Lys Gln Tyr Asp Gly Ala Gln Ile Thr Glu Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 88
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 88

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Gln Tyr Asp Gly Ala Lys Ile Thr Glu Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 89
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 89

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Gln Tyr Asp Gly Ala Lys Ile Glu Glu Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 90
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 90

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
 1 5 10 15
 Asp Lys Gln Tyr Asp Gly Ala Gln Ile Glu Glu Asn Gln Val Gln Asn
 20 25 30
 Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 91

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<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.

<400> SEQUENCE: 91

Phe Ser Ala Lys Glu Glu Lys Ala Glu Thr Arg Arg Thr Phe Gly Leu
1          5          10          15

Asp Lys Gln Tyr Asp Gly Ala Gln Ile Thr Glu Asn Gln Val Gln Asn
          20          25          30

Lys Phe Thr Ile Ser Asn
          35

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<210> SEQ ID NO 92
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia sp.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa may be Asp or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(27)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Xaa may be any amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34)..(34)
<223> OTHER INFORMATION: Xaa may be Glu or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(36)
<223> OTHER INFORMATION: Xaa may be Lys or Arg

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<400> SEQUENCE: 92

Gly Xaa Phe Ser Ala Lys Xaa Xaa Lys Xaa Ala Asp Thr Arg Xaa Thr
1          5          10          15

Phe Gly Leu Xaa Lys Gln Thr Asp Gly Ala Xaa Ile Xaa Glu Asn Xaa
          20          25          30

Val Xaa Asn Xaa Phe Thr Ile Ser Asn
          35          40

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<210> SEQ ID NO 93
<211> LENGTH: 21

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<212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.
 <400> SEQUENCE: 93
 Asp Asn Gln Val Gln Asn Lys Phe Thr Ile Ser Asn Tyr Ser Phe Lys
 1 5 10 15
 Tyr Glu Asp Asn Pro
 20

<210> SEQ ID NO 94
 <211> LENGTH: 35
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(35)
 <223> OTHER INFORMATION: May be repeated 1-10 times
 <400> SEQUENCE: 94

Lys Glu Glu Lys Ala Glu Thr Arg Lys Thr Phe Gly Leu Glu Lys Gln
 1 5 10 15
 Tyr Asp Gly Ala Lys Ile Glu Glu Asn Gln Val Gln Asn Lys Gly Gly
 20 25 30
 Gly Gly Gly
 35

<210> SEQ ID NO 95
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: His tag sequence
 <400> SEQUENCE: 95

His His His His His His
 1 5

<210> SEQ ID NO 96
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(7)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (12)..(12)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (17)..(17)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (24)..(24)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (26)..(26)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(38)
 <223> OTHER INFORMATION: May be biotinylated

<400> SEQUENCE: 96
 Phe Ser Ala Lys Glu Glu Xaa Ala Glu Thr Lys Xaa Thr Phe Gly Leu
 1 5 10 15

-continued

Xaa Lys Asn Tyr Asp Gly Ala Xaa Ile Xaa Asp Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

<210> SEQ ID NO 97
 <211> LENGTH: 38
 <212> TYPE: PRT
 <213> ORGANISM: Ehrlichia sp.
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(7)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (12)..(12)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (17)..(17)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (24)..(24)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (26)..(26)
 <223> OTHER INFORMATION: Xaa may be any amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(38)
 <223> OTHER INFORMATION: May be biotinylated

<400> SEQUENCE: 97

Phe Ser Ala Lys Glu Glu Xaa Ala Glu Thr Arg Xaa Thr Phe Gly Leu
 1 5 10 15

Xaa Lys Gln Tyr Asp Gly Ala Xaa Ile Xaa Glu Asn Gln Val Gln Asn
 20 25 30

Lys Phe Thr Ile Ser Asn
 35

What is claimed:

1. A mixture of isolated peptides, wherein each isolated peptide comprises the sequence of SEQ ID NO: 59,

(SEQ ID NO: 59)
 F-S-A-K-Xs-X6-X7-A-E-T-XII-X12-T-F-G-X16-X17-
 X18-X19-X20-D-G-A-X24-X25-X26-X27-N-X29-V-X31-
 N-X33-F-T-I-S-N

wherein X5 is an amino acid selected from the group consisting of E and Q, X6 is an amino acid selected from the group consisting of E and Q, X7 is any amino acid, X11 is an amino acid selected from the group consisting of K and R, X12 is any amino acid, X16 is an amino acid selected from the group consisting of L and I, X17 is any amino acid, X18 is an amino acid selected from the group consisting of R and K, X19 is an amino acid selected from the group consisting of Q and N, X20 is an amino acid selected from the group consisting of Y and T, X24 is any amino acid, X25 is an amino acid selected from the group consisting of I and L, X26 is any amino acid, X27 is an amino acid selected from the group consisting of D and E, X29 is an amino acid selected from the group consisting of E and Q, X31 is an amino acid selected from the group

consisting of E and Q, and X33 is an amino acid selected from the group consisting of K and R; and wherein any additional peptide sequence N-terminal or C-terminal to the sequence of SEQ ID NO: 59 is a non-native sequence, wherein each isolated peptide is conjugated to a ligand or wherein each isolated peptide is immobilized to a solid support.

2. A mixture of isolated peptides comprising three or more different isolated peptides, wherein each isolated peptide comprises the sequence of SEQ ID NO: 59,

(SEQ ID NO: 59)
 F-S-A-K-Xs-X6-X7-A-E-T-X11-X12-T-F-G-X16-X17-
 X18-X19-X20-D-G-A-X24-X25-Xa6-X27-N-X29-V-X31-
 N-X33-F-T-I-S-N

wherein X5 is an amino acid selected from the group consisting of E and Q, X6 is an amino acid selected from the group consisting of E and Q, X7 is any amino acid, X11 is an amino acid selected from the group consisting of K and R, X12 is any amino acid, Xa6 is an amino acid selected from the group consisting of L and I, X-7 is any amino acid, X18 is an amino acid selected from the group consisting of R and K, X19 is an amino acid selected from the group consisting of Q and N,

X20 is an amino acid selected from the group consisting of Y and T, X24 is any amino acid, X25 is an amino acid selected from the group consisting of I and L, X26 is any amino acid, X27 is an amino acid selected from the group consisting of D and E, X29 is an amino acid selected from the group consisting of E and Q, X31 is an amino acid selected from the group consisting of E and Q, and X33 is an amino acid selected from the group consisting of K and R; and wherein any additional peptide sequence N-terminal or C-terminal to the sequence of SEQ ID NO: 59 is a non-native sequence, wherein each isolated peptide is conjugated to a ligand or wherein each isolated peptide is immobilized to a solid support.

3. The mixture of claim 1 or 2, wherein one or more of the isolated peptides is biotinylated.

4. The mixture of claim 1 or 2, wherein one or more of the isolated peptides is conjugated to avidin, streptavidin, neutravidin, or serum albumin.

5. A method for detecting in a sample an antibody to an epitope of an *Ehrlichia* antigen, the method comprising:

contacting a sample with the mixture of isolated peptides of claim 1; and

detecting formation of an antibody-peptide complex comprising one or more of said isolated peptides in the mixture,

wherein formation of said complex is indicative of an antibody to an epitope of an *Ehrlichia* antigen being present in said sample.

6. The method of claim 5, wherein said *Ehrlichia* antigen is from an *Ehrlichia chaffeensis* or *Ehrlichia canis* species.

7. The method of claim 5, wherein said isolated peptide is immobilized to a solid support.

8. The method of claim 7, wherein said solid support is a bead, a flow path in a lateral flow assay device, a well in a microtiter plate, or a flow path in a rotor.

9. The method of claim 5, 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.

10. The method of claim 5, wherein said sample is from a human or canine subject.

11. The method of claim 5, wherein said sample is a blood, serum, cerebral spinal fluid, urine, or saliva sample.

12. A kit comprising the mixture of isolated peptides of claim 1 and a labeling reagent capable of binding to an antibody that recognizes an epitope of one or more isolated peptides in the mixture.

13. The kit of claim 12, wherein each isolated peptide is attached to a solid support.

14. The kit of claim 12, wherein each isolated peptide is attached to a bead, a tube or a well, a lateral flow assay device, or an analytical rotor.

15. The kit of claim 12, wherein the labeling reagent is an anti-human or anti-canine IgG antibody conjugated to a detectable label.

16. The kit of claim 15, wherein the detectable label is colloidal gold particles.

17. The mixture of claim 1 or 2, wherein the mixture comprises all possible peptides defined by SEQ ID NO: 59.

18. The mixture of claim 1 or 2, wherein X₅ is E, X₆ is E, X₁₆ is L, X₁₈ is K, X₂₀ is Y, X₂₅ is I, X₂₉ is Q, X₃₁ is Q, and X₃₃ is K.

19. The mixture of claim 1 or 2, wherein X₇ is K, X₁₂ is an amino acid selected from the group consisting of K and R, X₁₇ is an amino acid selected from the group consisting of E and D, X₂₄ is an amino acid selected from the group consisting of K and Q, and X₂₆ is an amino acid selected from the group consisting of E and T.

20. The mixture of claim 1 or 2, wherein X₁₁ is R, X₁₉ is Q, and/or X₂₇ is E.

21. The mixture of claim 1 or 2, wherein the mixture comprises different isolated peptides having a sequence selected from SEQ ID NOs: 60-91.

22. The mixture of claim 4, wherein one or more of the isolated peptides is conjugated to avidin, streptavidin, neutravidin, or serum albumin via a cysteine residue.

* * * * *

专利名称(译)	用于检测埃立克体抗体的肽，装置和方法		
公开(公告)号	US8828675	公开(公告)日	2014-09-09
申请号	US12/950707	申请日	2010-11-19
[标]申请(专利权)人(译)	艾巴希斯公司		
申请(专利权)人(译)	ABAXIS INC.		
当前申请(专利权)人(译)	ABAXIS INC.		
[标]发明人	MEHRA RAJESH K ARON KENNETH P BLEILE DENNIS M		
发明人	MEHRA, RAJESH K. ARON, KENNETH P. BLEILE, DENNIS M.		
IPC分类号	G01N33/53 G01N33/569		
CPC分类号	G01N2333/29 G01N33/54366 G01N33/54306 C07K14/195 G01N33/54353 G01N33/56911		
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外部链接	Espacenet USPTO		

摘要(译)

本发明提供了用于检测与埃立克体抗原结合的抗体的组合物(例如,肽组合物)。肽组合物包含基于埃立克体外膜蛋白1(OMP-1)蛋白的免疫原性片段的多肽序列。本发明还提供了包含此类肽组合物的装置,方法和试剂盒,其可用于检测与埃立克体抗原结合的抗体和单核细胞埃立克体病的诊断。

