



(43) International Publication Date  
17 April 2014 (17.04.2014)

(10) International Publication Number  
**WO 2014/059274 A1**

(51) International Patent Classification:

G01N 33/554 (2006.01) G01N 33/53 (2006.01)  
G01N 33/567 (2006.01)

(21) International Application Number:

PCT/US2013/064536

(22) International Filing Date:

11 October 2013 (11.10.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/712,578 11 October 2012 (11.10.2012) US

(71) Applicant: **ABAXIS, INC.** [US/US]; 3240 Whipple Road,  
Union City, California 94587 (US).

(72) Inventors: **MEHRA, Rajesh K.**; c/o 3240 Whipple Road,  
Union City, California 94587 (US). **ARON, Kenneth P.**;  
c/o 3240 Whipple Road, Union City, California 94587  
(US). **BLEILE, Dennis M.**; c/o 3240 Whipple Road, Uni-  
on City, California 94587 (US). **FORSYTH, Timothy P.**;  
c/o 3240 Whipple Road, Union City, California 94587  
(US). **WALKER, Jeremy D.**; c/o 3240 Whipple Road,  
Union City, California 94587 (US). **CUESICO, Cristina  
R.**; c/o 3240 Whipple Road, Union City, California 94587  
(US).

(74) Agents: **PURCELL, Angela L.** et al.; Cooley LLP, 1299  
Pennsylvania Avenue, NW, Suite 700, Washington, Dis-  
trict of Columbia 20004 (US).

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,  
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,  
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,  
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,  
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,  
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,  
ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,  
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,  
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,  
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,  
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,  
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,  
KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a  
patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the  
earlier application (Rule 4.17(iii))

**Published:**

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: PEPTIDES, DEVICES, AND METHODS FOR THE DETECTION OF EHRLICHIA ANTIBODIES

(57) Abstract: The invention provides peptide compositions and mixtures useful for the detection of antibodies that bind to Ehrlichia antigens. The peptide compositions and mixtures 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 mixtures useful for the detection of antibodies that bind to Ehrlichia antigens and the diagnosis of monocytic and/or granulocytic ehrlichiosis.



WO 2014/059274 A1

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

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/712,578, filed October 11, 2012, which is hereby incorporated by reference in its entirety.

### DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] 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\_040\_01WO\_SeqList\_ST25.txt, date recorded October 10, 2013, file size 62 kilobytes).

### BACKGROUND OF THE INVENTION

[0003] *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. Another species of *Ehrlichia* known as *Ehrlichia ewingii* has tropism for granulocytes and causes granulocytic ehrlichiosis. The canine disease is characterized by fever, epilepsy, incoordination, lethargy, bleeding episodes, 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.

[0004] 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.

[0005] Accordingly, there remains a need in the art for additional assays for detecting *Ehrlichia* antigens and serodiagnosis of monocytic ehrlichiosis and granulocytic ehrlichiosis.

## SUMMARY OF THE INVENTION

[0006] 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 and/or granulocytic ehrlichiosis.

[0007] 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 S-X<sub>2</sub>-K-E-D-K-Q-T-T-X<sub>10</sub>-X<sub>11</sub>-I-W-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-P-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-X<sub>32</sub>-X<sub>33</sub>-X<sub>34</sub>-X<sub>35</sub>-X<sub>36</sub>-X<sub>37</sub>-X<sub>38</sub>-X<sub>39</sub>-C (SEQ ID NO: 1), or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>18</sub> is an amino acid selected from the group consisting of E and Q, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>27</sub> is any amino acid except H, N, S, or A, X<sub>28</sub> is any amino acid except A, S, or P, X<sub>29</sub> is any amino acid except D, P, N, or S, X<sub>30</sub> is any amino acid except A, E, D, or S, X<sub>31</sub> is any amino acid except D, N, V, or H, X<sub>32</sub> is any amino acid except F or T, X<sub>33</sub> is any amino acid except N, F or I, X<sub>34</sub> is any amino acid except N, T or D, X<sub>35</sub> is any amino acid except K, V or P, X<sub>36</sub> is any amino acid except G, P, or S, X<sub>37</sub> is any amino acid except Y, N, or T, X<sub>38</sub> is any amino acid except S, Y, or I, and X<sub>39</sub> is any amino acid except F or S.

**[0008]** In some embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-D-K-Q-T-T-T-X<sub>11</sub>-I-W-G-L-K-Q-X<sub>18</sub>-W-D-G-X<sub>22</sub>-P-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-X<sub>32</sub>-X<sub>33</sub>-X<sub>34</sub>-X<sub>35</sub>-X<sub>36</sub>-X<sub>37</sub>-X<sub>38</sub>-X<sub>39</sub>-C (SEQ ID NO: 83), or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>18</sub> is an amino acid selected from the group consisting of E and Q, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S and N, X<sub>27</sub> is any amino acid except H, N, S, or A, X<sub>28</sub> is any amino acid except A, S, or P, X<sub>29</sub> is any amino acid except D, P, N, or S, X<sub>30</sub> is any amino acid except A, E, D, or S, X<sub>31</sub> is any amino acid except D, N, V, or R, X<sub>32</sub> is any amino acid except F or T, X<sub>33</sub> is any amino acid except N, F or I, X<sub>34</sub> is any amino acid except N, T or D, X<sub>35</sub> is any amino acid except K, V or P, X<sub>36</sub> is any amino acid except G, P, or S, X<sub>37</sub> is any amino acid except Y, N, or T, X<sub>38</sub> is any amino acid except S, Y, or I, and X<sub>39</sub> is any amino acid except F or S.

**[0009]** In other embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>39</sub>-A-X<sub>41</sub>-T-R-X<sub>44</sub>-T-F-G-X<sub>48</sub>-X<sub>49</sub>-K-Q-Y-D-G-A-X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 3) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>41</sub> is an amino acid selected from the group consisting of D and N, X<sub>44</sub> is any amino acid, X<sub>48</sub> is an amino acid selected from the group consisting of V and A, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid.

[0010] In related embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-N-F-S-A-K-E-E-K-X<sub>40</sub>-A-D-T-R-X<sub>45</sub>-T-F-G-L-X<sub>50</sub>-K-Q-T-D-G-A-X<sub>57</sub>-I-X<sub>59</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 85) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>40</sub> is any amino acid, X<sub>45</sub> is any amino acid, X<sub>50</sub> is any amino acid, X<sub>57</sub> is any amino acid, and X<sub>59</sub> is any amino acid.

[0011] In still other embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-N-F-S-A-K-E-E-X<sub>39</sub>-A-E-T-R-X<sub>44</sub>-T-F-G-L-X<sub>49</sub>-K-Q-Y-D-G-A-X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 72) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>44</sub> is any amino acid, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid.

[0012] In certain embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>39</sub> is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>44</sub> is an amino acid selected from the group consisting of K and R and X<sub>49</sub> is an amino acid selected from the group consisting of E and D. In still other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>56</sub> is an amino acid selected from the group consisting of K and Q and X<sub>58</sub> is an amino acid selected from the group consisting of E and T.

[0013] In some embodiments, peptides of the invention comprise a fragment of SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 72. Such fragments may comprise at least 10, 15, 20, 25, 30, or 35 contiguous amino acids from SEQ ID NO: 1 or at least 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from SEQ ID NO: 3 or SEQ ID NO: 72. In certain embodiments, a peptide of the invention may comprise or consist of a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 71, SEQ ID NO: 84, or SEQ ID NO: 86.

[0014] In certain embodiments, peptides of the invention described herein may 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 other embodiments, peptides of the invention described herein may 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 some 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).

[0015] 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,

keyhole limpet hemocyanin (KLH), 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).

[0016] In certain embodiments, peptides of the invention are attached to or immobilized on a solid support. In one embodiment, the peptides of the invention are attached to a solid support through a metallic nanolayer. In certain embodiments, the solid support is a bead (*e.g.*, a colloidal particle, metallic nanoparticle or nanoshell, latex bead, etc.), a flow path in a lateral flow immunoassay device (*e.g.*, a porous membrane), a blot (Western blot, a slot blot, or dot blot), a flow path in an analytical or centrifugal rotor, or a tube or well (*e.g.*, in a plate suitable for an ELISA assay).

[0017] 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 or population 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 or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 3. In other embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 72. In yet other embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 7. In still other embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 70. In some embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 83. In other embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 85. In certain embodiments, the composition comprises a mixture or population of two, three, four, or more different peptides of the invention, wherein each peptide comprises a sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86.

[0018] 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.

[0019] 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 or centrifugal 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.

[0020] 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: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, or SEQ ID NO: 86. In certain embodiments, the peptides are attached to or immobilized upon the device.

[0021] 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*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, or *Ehrlichia muris*. In certain embodiments, the methods comprise contacting the sample with a mixture or population of different peptides of the invention (*i.e.* a mixture of two, three, four, or more different peptides of the



invention). In some embodiments, the methods provide for detection of antibodies to antigens from multiple *Ehrlichia* species (e.g., *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and *Ehrlichia muris*) in a sample simultaneously.

[0022] 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 or population of peptides is attached to or immobilized upon a solid support. In one embodiment, the peptide or mixture or population of peptides is attached to the solid support through a metallic (e.g., gold) nanolayer. In certain embodiments, the solid support is a bead or plurality of beads (e.g., a colloidal particle, a metallic nanoparticle or nanoshell, a latex bead, etc.), a flow path in a lateral flow immunoassay device (e.g., a porous membrane), a flow path in an analytical or centrifugal rotor, a blot (Western blot, a slot blot, or dot blot), 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 or population of different peptides is attached to a dendrimer and/or incorporated into a MAPS system. In certain other embodiments, the peptide or mixture or population of different peptides is attached to BSA.

[0023] 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 or centrifugal rotor. In other embodiments, the detecting step comprises analyzing the sample using a Western blot, a slot blot, or a dot blot. 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 detecting step comprises performing a wavelength shift assay.

[0024] 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.

[0025] In another aspect, the invention provides methods of diagnosing monocytic and/or granulocytic 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 and/or granulocytic ehrlichiosis. In certain embodiments, the methods comprise contacting the sample with a mixture or population of different peptides of the invention (*i.e.* a mixture of two, three, four, or more different peptides of the invention).

[0026] 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: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, or SEQ ID NO: 86. In certain embodiments, the peptides are attached to or immobilized on a solid support optionally through a metallic nanolayer. In certain embodiments, the solid support is a bead (*e.g.*, a colloidal particle, a metallic nanoparticle or nanoshell, a latex bead, etc.), a flow path in a lateral flow immunoassay device, a flow path in an analytical or centrifugal 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 other embodiments, the peptide or mixture of different peptides is attached to BSA.

[0027] 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 or centrifugal rotor, a Western blot, a dot blot, a slot blot, 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.

[0028] 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 and/or granulocytic 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 one or more *Ehrlichia* antigens or to diagnose monocytic and/or granulocytic ehrlichiosis.

[0029] Additional aspects and embodiments of the invention will be apparent from the detailed description that follows.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **Figure 1** 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 to *Ehrlichia* antigens 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 detectable label (*e.g.*, metallic nanoparticle or nanoshell (*e.g.*, colloidal gold), horse radish peroxidase (HRP), alkaline phosphatase (ALP), fluorophore, colored latex particle), which detects the presence of the antibodies bound to the first set of peptides immobilized at the test site. In certain embodiments, to amplify the detection signal, protein A and/or protein G molecules conjugated to a detectable label (*e.g.*, metallic nanoparticle or nanoshell (*e.g.*, colloidal gold), HRP, ALP, fluorophore, colored latex particle) may be applied to the test site where they will bind to the Fc region of any antibodies to *Ehrlichia* antigens captured by the immobilized peptides of the invention.

[0031] **Figure 2** is a diagram of one type of indirect sandwich assay which can be used to detect antibodies to *Ehrlichia* antigens. In this embodiment, anti-human IgG/IgM, anti-dog IgG/IgM, or anti-cat IgG/IgM antibodies are immobilized to a suitable substrate (*e.g.*, nitrocellulose membrane, well of an ELISA plate) at a test site. Antibodies to *Ehrlichia* antigens 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 that are conjugated to a detectable label (*e.g.*, metallic nanoparticle or nanoshell (*e.g.*, colloidal gold), HRP, ALP, fluorophore, colored latex particle).

[0032] **Figure 3** is a diagram of another type of indirect sandwich assay which can be used to detect antibodies to *Ehrlichia* antigens. In this embodiment, peptides of the invention can be immobilized to a substrate (*e.g.*, nitrocellulose membrane, well of an ELISA plate) to capture anti-*Ehrlichia* antibodies in a test sample. Anti-human IgG/IgM, anti-dog IgG/IgM, or anti-cat IgG/IgM antibodies conjugated to a detectable label (*e.g.*, metallic nanoparticle or nanoshell (*e.g.*, colloidal gold), HRP, ALP, fluorophore, colored latex particle) can be used to detect the presence of the antibodies bound to the immobilized peptides at the test site.

[0033] **Figure 4** is a diagram of an immunoassay device which can be used to detect antibodies to *Ehrlichia* antigens. In this embodiment of an immunoassay device, peptides of the invention are immobilized to a suitable substrate (*e.g.*, nitrocellulose membrane, well of an ELISA plate) at a test site. Anti-*Ehrlichia* antibodies in a test sample are bound by the immobilized peptides of the invention. Protein A, Protein G, or a Protein A/G fusion protein conjugated to a detectable label (*e.g.*, metallic nanoparticle or nanoshell (*e.g.*, colloidal gold), HRP, ALP, fluorophore, colored latex particle) is added to the system and binds to the Fc portion of the captured anti-*Ehrlichia* antibody, thereby producing a positive signal. In this embodiment, the device can further comprise a control site at which binding partners that recognize the detectable label-conjugated protein A, detectable label-conjugated protein G, and/or detectable label-conjugated protein A/G fusion are immobilized. Such binding partners may include, but are not limited to, anti-protein A, anti-protein G, mouse IgG, and/or other similar IgG molecules.

[0034] **Figure 5** depicts one example of a lateral flow assay device that can be used to detect antibodies to *Ehrlichia* antigens. Peptides of the invention are linked to a carrier protein (*e.g.*

bovine serum albumin) and the resulting BSA-peptide conjugates are immobilized on a nitrocellulose (NC) membrane at a test site (T). The same BSA-peptide conjugates are conjugated to a detectable label (*e.g.*, colloidal gold) and deposited in a conjugate pad positioned upstream of the test site. Gold-conjugated protein A and gold-conjugated protein G (*i.e.* amplifier) is added to the conjugate pad to enhance the signal by binding to the Fc portion of the captured anti-*Ehrlichia* antibody. The device further comprises a control site (C) at which binding partners that recognize the gold-conjugated protein A and/or gold-conjugated protein G are immobilized.

### DETAILED DESCRIPTION

[0035] As used herein, the following terms shall have the following meanings:

[0036] 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.

[0037] 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. In certain embodiments, epitopes may comprise non-contiguous regions.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] Additional terms shall be defined, as required, in the detailed description that follows.

### **Compositions and Devices**

[0042] 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, including *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*. Accordingly, in one aspect, the invention provides peptides capable of binding to antibodies that recognize *Ehrlichia* antigens.

[0043] In certain embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-D-K-Q-T-T-X<sub>10</sub>-X<sub>11</sub>-I-W-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-P-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-X<sub>32</sub>-X<sub>33</sub>-X<sub>34</sub>-X<sub>35</sub>-X<sub>36</sub>-X<sub>37</sub>-X<sub>38</sub>-X<sub>39</sub>-C (SEQ ID NO: 1), or a fragment thereof, wherein SEQ ID NO: 1, as used throughout the specification unless further specified, has the following characteristics: X<sub>2</sub> is an amino acid selected from the group consisting of A and V; X<sub>10</sub> is an amino acid selected from the group consisting of T and V; X<sub>11</sub> is an amino acid selected from the group consisting of G and A; X<sub>18</sub> is an amino acid selected from the group consisting of E and Q; X<sub>20</sub> is an amino acid selected from the group consisting of D and N; X<sub>22</sub> is an amino acid selected from the group consisting of S and V; X<sub>24</sub> is an amino acid selected from the group consisting of A and I; X<sub>25</sub> is an amino acid selected from the group consisting of T and P; X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K; X<sub>27</sub> is any amino acid except H, N, S, or A; X<sub>28</sub> is any amino acid except A, S, or P; X<sub>29</sub> is any amino acid except D, P, N, or S; X<sub>30</sub> is any amino acid except A, E, D, or S; X<sub>31</sub> is any amino acid except D, N, V, or H; X<sub>32</sub> is any amino acid except F or T; X<sub>33</sub> is any amino acid except N, F or I; X<sub>34</sub> is any amino acid except N, T or D; X<sub>35</sub> is any amino acid except K, V or P; X<sub>36</sub> is any amino acid except G, P, or S; X<sub>37</sub> is any amino acid except Y, N, or T; X<sub>38</sub> is any amino acid except S, Y, or I; and X<sub>39</sub> is any amino acid except F or S.

[0044] In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein X<sub>2</sub> is V and X<sub>10</sub> is T. In some embodiments, peptides of the invention

comprise a sequence of SEQ ID NO: 1, wherein  $X_{10}$  is T and  $X_{26}$  is selected from the group consisting of S and N. In other embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein  $X_{24}$  is A,  $X_{25}$  is selected from the group consisting of T and P, and  $X_{26}$  is selected from the group consisting of S and N. In still other embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein  $X_{26}$  is selected from the group consisting of S and N and  $X_{31}$  is any amino acid except D, N, V, R, or H. In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, wherein  $X_{27}$ – $X_{39}$  has a sequence selected from the group consisting of Q-R-K-N-E-P-S-E-T-N-P-G-Q (SEQ ID NO: 74), M-V-E-F-E-E-L-Q-R-N-W-H-P (SEQ ID NO: 75), M-L-E-V-S-W-L-I-D-F-M-A-P (SEQ ID NO: 76), and Q-D-E-N-L-Y-S-S-I-F-F-V-P (SEQ ID NO: 77).

**[0045]** In related embodiments, peptides of the invention comprise a sequence of S- $X_2$ -K-E-D-K-Q-T-T-T- $X_{11}$ -I-W-G-L-K-Q- $X_{18}$ -W-D-G- $X_{22}$ -P- $X_{24}$ - $X_{25}$ - $X_{26}$ - $X_{27}$ - $X_{28}$ - $X_{29}$ - $X_{30}$ - $X_{31}$ - $X_{32}$ - $X_{33}$ - $X_{34}$ - $X_{35}$ - $X_{36}$ - $X_{37}$ - $X_{38}$ - $X_{39}$ -C (SEQ ID NO: 83), or a fragment thereof, wherein  $X_2$  is an amino acid selected from the group consisting of A and V,  $X_{11}$  is an amino acid selected from the group consisting of G and A,  $X_{18}$  is an amino acid selected from the group consisting of E and Q,  $X_{22}$  is an amino acid selected from the group consisting of S and V,  $X_{24}$  is an amino acid selected from the group consisting of A and I,  $X_{25}$  is an amino acid selected from the group consisting of T and P,  $X_{26}$  is an amino acid selected from the group consisting of S and N,  $X_{27}$  is any amino acid except H, N, S, or A,  $X_{28}$  is any amino acid except A, S, or P,  $X_{29}$  is any amino acid except D, P, N, or S,  $X_{30}$  is any amino acid except A, E, D, or S,  $X_{31}$  is any amino acid except D, N, V, or R,  $X_{32}$  is any amino acid except F or T,  $X_{33}$  is any amino acid except N, F or I,  $X_{34}$  is any amino acid except N, T or D,  $X_{35}$  is any amino acid except K, V or P,  $X_{36}$  is any amino acid except G, P, or S,  $X_{37}$  is any amino acid except Y, N, or T,  $X_{38}$  is any amino acid except S, Y, or I, and  $X_{39}$  is any amino acid except F or S. In some embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 83, wherein  $X_{27}$ – $X_{39}$  has a sequence selected from the group consisting of Q-R-K-N-E-P-S-E-T-N-P-G-Q (SEQ ID NO: 74), M-V-E-F-E-E-L-Q-R-N-W-H-P (SEQ ID NO: 75), M-L-E-V-S-W-L-I-D-F-M-A-P (SEQ ID NO: 76), and Q-D-E-N-L-Y-S-S-I-F-F-V-P (SEQ ID NO: 77).

**[0046]** In certain other embodiments of the invention, peptides of the invention comprise a sequence of S- $X_2$ -K-E- $X_5$ -K-Q- $X_8$ -T- $X_{10}$ - $X_{11}$ - $X_{12}$ - $X_{13}$ -G-L-K-Q- $X_{18}$ -W- $X_{20}$ -G- $X_{22}$ - $X_{23}$ - $X_{24}$ - $X_{25}$ - $X_{26}$ -G-G-G-G-G-N-F-S-A-K-E-E- $X_{39}$ -A- $X_{41}$ -T-R- $X_{44}$ -T-F-G- $X_{48}$ - $X_{49}$ -K-Q-Y-D-G-A-

X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 3) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>41</sub> is an amino acid selected from the group consisting of D and N, X<sub>44</sub> is any amino acid, X<sub>48</sub> is an amino acid selected from the group consisting of V and A, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid.

[0047] In still other embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-G-N-F-S-A-K-E-E-K-X<sub>40</sub>-A-D-T-R-X<sub>45</sub>-T-F-G-L-X<sub>50</sub>-K-Q-T-D-G-A-X<sub>57</sub>-I-X<sub>59</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 85) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>40</sub> is any amino acid, X<sub>45</sub> is any amino acid, X<sub>50</sub> is any amino acid, X<sub>57</sub> is any amino acid, and X<sub>59</sub> is any amino acid.



[0048] In particular embodiments, peptides of the invention comprise a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>39</sub>-A-E-T-R-X<sub>44</sub>-T-F-G-L-X<sub>49</sub>-K-Q-Y-D-G-A-X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 72) or a fragment thereof, wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>44</sub> is any amino acid, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid. In one particular embodiment, peptides comprising a sequence of SEQ ID NO: 72 enable the detection of antibodies to *Ehrlichia* antigens from multiple species (e.g., *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*) simultaneously.

[0049] In some embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>39</sub> is K. In other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>44</sub> is an amino acid selected from the group consisting of K and R and X<sub>49</sub> is an amino acid selected from the group consisting of E and D. In still other embodiments, peptides of the invention comprise or consist of a sequence of SEQ ID NO: 3 or SEQ ID NO: 72, wherein X<sub>56</sub> is an amino acid selected from the group consisting of K and Q and X<sub>58</sub> is an amino acid selected from the group consisting of E and T.

[0050] In another aspect of the invention, peptides of the invention comprise or consist of a sequence of S-V-K-X<sub>4</sub>-D-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-V-L-W-G-I-R-Q-N-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-A-X<sub>25</sub>-X<sub>26</sub>-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 7), wherein X<sub>4</sub> is an amino acid selected from the group consisting of E and N, X<sub>8</sub> is an amino acid selected from the group consisting of P and S, X<sub>10</sub> is an amino acid selected from the group consisting of A and S, X<sub>20</sub> is an

amino acid selected from the group consisting of E and Q, X<sub>22</sub> is an amino acid selected from the group consisting of P and T, X<sub>23</sub> is an amino acid selected from the group consisting of S and V, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, and X<sub>26</sub> is an amino acid selected from the group consisting of S and N.

[0051] In certain embodiments, a peptide of the invention comprises or consists of the sequence S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 8); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 9); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 10); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 11); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 12); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 13); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 14); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 15); S-V-K-E-D-K-Q-S-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 16); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 17); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 18); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 19); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 20); S-V-K-E-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 21); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 22); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 23); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 24); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 25); S-V-K-E-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 26); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 27); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-V-A-T-S-Q-V-

E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 28); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 29); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 30); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 31); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 32); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 33); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-T-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 34); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 35); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 36); S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 37); or S-V-K-E-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-V-A-P-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 38).

[0052] In other embodiments, a peptide of the invention comprises or consists of the sequence S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 39); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 40); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 41); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 42); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 43); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 44); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 45); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 46); S-V-K-N-D-K-Q-S-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 47); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 48); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 49); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 50); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ

ID NO: 51); S-V-K-N-D-K-Q-S-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 52); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 53); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 54); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 55); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 56); S-V-K-N-D-K-Q-P-T-S-V-L-W-G-I-R-Q-N-W-Q-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 57); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-T-S-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 58); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 59); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 60); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 61); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 62); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 63); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-T-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 64); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-T-S-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 65); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 66); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-P-S-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 67); S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-Q-G-P-V-A-T-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 68); or S-V-K-N-D-K-Q-P-T-A-V-L-W-G-I-R-Q-N-W-E-G-P-V-A-P-N-Q-V-E-V-E-W-Q-Q-R-G-W-G-G-C (SEQ ID NO: 69).

**[0053]** In one particular embodiment of the invention, peptides of the invention comprise or consist of a sequence of S-X<sub>2</sub>-K-D-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-X<sub>16</sub>-Q-X<sub>18</sub>-X<sub>19</sub>-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-X<sub>32</sub>-X<sub>33</sub>-X<sub>34</sub>-X<sub>35</sub>-X<sub>36</sub>-X<sub>37</sub>-X<sub>38</sub>-X<sub>39</sub>-C (SEQ ID NO: 70), wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of S, V, and A, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from

the group consisting of Y, F, and W, X<sub>16</sub> is an amino acid selected from the group consisting of K and R, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>19</sub> is an amino acid selected from the group consisting of W and F, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of T and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, and each of X<sub>27</sub>–X<sub>39</sub> is any amino acid. In certain embodiments, X<sub>27</sub>–X<sub>39</sub> has a sequence selected from the group consisting of Q-R-K-N-E-P-S-E-T-N-P-G-Q (SEQ ID NO: 74), M-V-E-F-E-E-L-Q-R-N-W-H-P (SEQ ID NO: 75), M-L-E-V-S-W-L-I-D-F-M-A-P (SEQ ID NO: 76), Q-D-E-N-L-Y-S-S-I-F-F-V-P (SEQ ID NO: 77), Q-R-K-N-D-P-S-E-T-S-P-G-Q (SEQ ID NO: 78), M-A-P-F-H-E-L-D-V-N-N-H-P (SEQ ID NO: 79), S-L-N-V-S-F-L-I-D-P-M-A-P (SEQ ID NO: 80), and Q-D-S-N-L-Y-S-S-I-F-F-V-P (SEQ ID NO: 81).

**[0054]** In certain embodiments, peptides of the invention comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85 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 one or more linking residues (*e.g.* one or more glycine, cysteine, or serine residues). For instance, in certain embodiments, the carboxyl-terminal cysteine residue in any of the sequences described herein can be located at the amino terminus instead. Accordingly, in some embodiments, peptides of the invention comprise or consist of a sequence of C-S-X<sub>3</sub>-K-E-D-K-Q-T-T-X<sub>11</sub>-X<sub>12</sub>-I-W-G-L-K-Q-X<sub>19</sub>-W-X<sub>21</sub>-G-X<sub>23</sub>-P-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-X<sub>32</sub>-X<sub>33</sub>-X<sub>34</sub>-X<sub>35</sub>-X<sub>36</sub>-X<sub>37</sub>-X<sub>38</sub>-X<sub>39</sub>-X<sub>40</sub> (SEQ ID NO: 2), wherein X<sub>3</sub> is an amino acid selected from the group consisting of A and V, X<sub>11</sub> is an amino acid selected from the group consisting of T and V, X<sub>12</sub> is an amino acid selected from the group consisting of G and A, X<sub>19</sub> is an amino acid selected from the group consisting of E and Q, X<sub>21</sub> is an amino acid selected from the group consisting of D and N, X<sub>23</sub> is an amino acid selected from the group consisting of S and V, X<sub>25</sub> is an amino acid selected from the group consisting of A and I, X<sub>26</sub> is an amino

acid selected from the group consisting of T and P, X<sub>27</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>28</sub> is any amino acid except H, N, S, or A, X<sub>29</sub> is any amino acid except A, S, or P, X<sub>30</sub> is any amino acid except D, P, N, or S, X<sub>31</sub> is any amino acid except A, E, D, or S, X<sub>32</sub> is any amino acid except D, N, V, or H, X<sub>33</sub> is any amino acid except F or T, X<sub>34</sub> is any amino acid except N, F or I, X<sub>35</sub> is any amino acid except N, T or D, X<sub>36</sub> is any amino acid except K, V or P, X<sub>37</sub> is any amino acid except G, P, or S, X<sub>38</sub> is any amino acid except Y, N, or T, X<sub>39</sub> is any amino acid except S, Y, or I, and X<sub>40</sub> is any amino acid except F or S. In still other embodiments, peptides of the invention comprise or consist of a sequence of C-S-X<sub>3</sub>-K-E-X<sub>6</sub>-K-Q-X<sub>9</sub>-T-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-G-L-K-Q-X<sub>19</sub>-W-X<sub>21</sub>-G-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>40</sub>-A-X<sub>42</sub>-T-R-X<sub>45</sub>-T-F-G-X<sub>49</sub>-X<sub>50</sub>-K-Q-Y-D-G-A-X<sub>57</sub>-I-X<sub>59</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N (SEQ ID NO: 4), wherein X<sub>3</sub> is an amino acid selected from the group consisting of A and V, X<sub>6</sub> is an amino acid selected from the group consisting of E and D, X<sub>9</sub> is an amino acid selected from the group consisting of T and P, X<sub>11</sub> is an amino acid selected from the group consisting of T and V, X<sub>12</sub> is an amino acid selected from the group consisting of G and A, X<sub>13</sub> is an amino acid selected from the group consisting of L and V, X<sub>14</sub> is an amino acid selected from the group consisting of Y and F, X<sub>19</sub> is an amino acid selected from the group consisting of D and N, X<sub>21</sub> is an amino acid selected from the group consisting of D and N, X<sub>23</sub> is an amino acid selected from the group consisting of S and V, X<sub>24</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>25</sub> is an amino acid selected from the group consisting of A and I, X<sub>26</sub> is an amino acid selected from the group consisting of T and P, X<sub>27</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>40</sub> is any amino acid, X<sub>42</sub> is an amino acid selected from the group consisting of D and N, X<sub>45</sub> is any amino acid, X<sub>49</sub> is an amino acid selected from the group consisting of V and A, X<sub>50</sub> is any amino acid, X<sub>57</sub> is any amino acid, and X<sub>59</sub> is any amino acid. In some embodiments, peptides of the invention comprise or consist of a sequence of C-S-X<sub>3</sub>-K-E-X<sub>6</sub>-K-Q-X<sub>9</sub>-T-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-G-L-K-Q-X<sub>19</sub>-W-X<sub>21</sub>-G-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>40</sub>-A-E-T-R-X<sub>45</sub>-T-F-G-L-X<sub>50</sub>-K-Q-Y-D-G-A-X<sub>57</sub>-I-X<sub>59</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 73), wherein X<sub>3</sub> is an amino acid selected from the group consisting of A and V, X<sub>6</sub> is an amino acid selected from the group consisting of E and D, X<sub>9</sub> is an amino acid selected from the group consisting of T and P, X<sub>11</sub> is an amino acid selected from the group consisting of T and V, X<sub>12</sub> is an amino acid selected from the group consisting of G and A, X<sub>13</sub> is an amino acid selected from the group consisting of L and V, X<sub>14</sub> is an amino acid selected from the group consisting of Y and F,

X<sub>19</sub> is an amino acid selected from the group consisting of D and N, X<sub>21</sub> is an amino acid selected from the group consisting of D and N, X<sub>23</sub> is an amino acid selected from the group consisting of S and V, X<sub>24</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>25</sub> is an amino acid selected from the group consisting of A and I, X<sub>26</sub> is an amino acid selected from the group consisting of T and P, X<sub>27</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>40</sub> is any amino acid, X<sub>45</sub> is any amino acid, X<sub>50</sub> is any amino acid, X<sub>57</sub> is any amino acid, and X<sub>59</sub> is any amino acid.

[0055] 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. Patent 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 in their entirety.

[0056] Variant polypeptides are at least about 80, 85, 90, 95, 98, or 99% identical to a peptide shown in SEQ ID NOs: 1-73 and 83-86 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.

[0057] 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.

[0058] Variants of the peptide sequences can be readily selected by one of skill in the art, based in part on known properties of the sequence. For example, a variant peptide can include amino acid substitutions (*e.g.*, conservative amino acid substitutions) and/or deletions (*e.g.*, small, single amino acid deletions, or deletions encompassing 2, 3, 4, 5, 10, 15, 20, or more contiguous amino acids). Thus, in certain embodiments, a variant of a native peptide sequence is one that differs from a naturally-occurring sequence by (i) one or more (*e.g.*, 2, 3, 4, 5, 6, or more) conservative amino acid substitutions, (ii) deletion of 1 or more (*e.g.*, 2, 3, 4, 5, 6, or more) amino acids, or (iii) a combination thereof. Deleted amino acids can be contiguous or non-contiguous. Conservative amino acid substitutions are those that take place within a family of amino acids that are related in their side chains and chemical properties. These include, *e.g.*, (1) acidic amino acids: aspartate, glutamate; (2) basic amino acids: lysine, arginine, histidine; (3) nonpolar amino acids: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; (4) uncharged polar amino acids: glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine; (5) aliphatic amino acids: glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally grouped separately as aliphatic-hydroxyl; (6) aromatic amino acids: phenylalanine, tyrosine, tryptophan; (7) amide amino acids: asparagine, glutamine; and (9) sulfur-containing amino acids: cysteine and methionine. *See, e.g.*, Biochemistry, 2nd ed., Ed. by L. Stryer, W H Freeman and Co.: 1981. Methods for confirming that variant peptides are suitable are conventional and routine.



[0059] 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.

[0060] 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85. 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85. 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85. In other embodiments, the peptide can comprise a fusion of sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85 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: 3 or SEQ ID NO: 85 optionally through one or more linking amino acids (*e.g.* glycine, serine, or cysteine residues). In another embodiment, the peptide can comprise a sequence of SEQ ID NO: 7 linked to SEQ ID NO: 3 or SEQ ID NO: 85 optionally through one or more linking amino acids (*e.g.* glycine, serine, or cysteine residues). In another embodiment, the peptide can comprise a sequence of SEQ ID NO: 83 linked to SEQ ID NO: 3 or SEQ ID NO: 85 optionally through one or more linking amino acids (*e.g.* glycine, serine, or cysteine residues). In still another embodiment, the peptide can comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 7, or SEQ ID NO: 83 linked to SEQ ID NO: 72 optionally through one or more linking amino acids (*e.g.* glycine, serine, or cysteine residues). In yet another embodiment, the peptide can comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 7, or SEQ ID NO: 83 linked to SEQ ID NO: 71 optionally through one or more linking amino acids (*e.g.* glycine, serine, or cysteine residues).

[0061] 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 *Ehrlichia* 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. Patent 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 in their entirety. Polypeptides or peptides derived from other microorganisms can also be used.

[0062] 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).

[0063] In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ

ID NO: 85. 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85. 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85.

[0064] 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.

[0065] 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).

[0066] In certain embodiments, peptides of the invention comprise a sequence defined by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85 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 comprise a full-length OMP-1 protein. In other embodiments, peptides of the invention do not comprise a full-length OMP-1 protein.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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 NOs: 1-73 and 83-86.

[0071] 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 NOs: 1-73 and 83-86. 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). For

instance, in one embodiment, peptides of the invention comprise a fragment of SEQ ID NO: 1. Such fragments may comprise at least 10, 15, 20, 25, 30, or 35 contiguous amino acids from SEQ ID NO: 1. In some embodiments, the fragments comprise amino acids 1 to 26 of SEQ ID NO: 1. Thus, in one embodiment, a peptide of the invention comprises or consists of a sequence of S-X<sub>2</sub>-K-E-D-K-Q-T-T-X<sub>10</sub>-X<sub>11</sub>-I-W-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-P-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub> (SEQ ID NO: 84), wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>18</sub> is an amino acid selected from the group consisting of E and Q, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, and X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K.

**[0072]** In one particular embodiment, peptides of the invention comprise a fragment of SEQ ID NO: 3 or SEQ ID NO: 72. Such fragments may comprise at least 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids from SEQ ID NO: 3 or SEQ ID NO: 72. For instance, in certain embodiments, such fragments may comprise amino acids 1 to 26 of SEQ ID NO: 3 or SEQ ID NO: 72. Thus, in one embodiment, a peptide of the invention comprises or consists of a sequence of S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub> (SEQ ID NO: 86), wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, and X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K. In other embodiments, the fragments may comprise

amino acids 33 to 71 of SEQ ID NO: 3 or SEQ ID NO: 72. Thus, in one embodiment, a peptide of the invention comprises or consists of a sequence of F-S-A-K-E-E-X<sub>7</sub>-A-X<sub>9</sub>-T-R-X<sub>12</sub>-T-F-G-X<sub>16</sub>-X<sub>17</sub>-K-Q-Y-D-G-A-X<sub>24</sub>-I-X<sub>26</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 5), wherein X<sub>7</sub> is any amino acid, X<sub>9</sub> is an amino acid selected from the group consisting of D and N, X<sub>12</sub> is any amino acid, X<sub>16</sub> is an amino acid selected from the group consisting of V and A, X<sub>17</sub> is any amino acid, X<sub>24</sub> is any amino acid, and X<sub>26</sub> is any amino acid. In another embodiment, a peptide of the invention comprises a sequence of C-F-S-A-K-E-E-X<sub>8</sub>-A-X<sub>10</sub>-T-R-X<sub>13</sub>-T-F-G-X<sub>17</sub>-X<sub>18</sub>-K-Q-Y-D-G-A-X<sub>25</sub>-I-X<sub>27</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N (SEQ ID NO: 6), wherein X<sub>8</sub> is any amino acid, X<sub>10</sub> is an amino acid selected from the group consisting of D and N, X<sub>13</sub> is any amino acid, X<sub>17</sub> is an amino acid selected from the group consisting of V and A, X<sub>18</sub> is any amino acid, X<sub>25</sub> is any amino acid, and X<sub>27</sub> is any amino acid. In still another embodiment, a peptide of the invention comprises a sequence of F-S-A-K-E-E-X<sub>7</sub>-A-E-T-R-X<sub>12</sub>-T-F-G-L-X<sub>17</sub>-K-Q-Y-D-G-A-X<sub>24</sub>-I-X<sub>26</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 71), wherein X<sub>7</sub> is any amino acid, X<sub>12</sub> is any amino acid, X<sub>17</sub> is any amino acid, X<sub>24</sub> is any amino acid, and X<sub>26</sub> is any amino acid.

**[0073]** In certain embodiments, the fragment comprises a sequence set forth in U.S. Patent 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. Patent 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.

**[0074]** 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 85, 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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, keyhole limpet hemocyanin (KLH) 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 (ALP), or other moiety that can be visualized, such as a metallic nanoparticle or nanoshell (*e.g.*, 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.

[0079] 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, KLH), enzymes (*e.g.*, horse radish peroxidase (HRP), beta-galactosidase, glutathione-S-transferase, alkaline phosphatase), 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 certain embodiments, a mixture of peptides of the invention can be linked by a dendrimer, *e.g.*, as in a MAPS structure.

[0080] 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.

[0081] 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.

[0082] 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. In some embodiments, the peptide can be associated with a fusion partner, *e.g.*, bovine serum albumin (BSA), which facilitates the attachment of the peptide to a substrate. In other embodiments, the peptides of the invention are attached to or immobilized on a substrate via a metallic nanolayer. In one embodiment, the metallic nanolayer is comprised of cadmium, zinc, mercury, or a noble metal, such as gold, silver, copper, and platinum. The peptide or mixture of peptides can be attached to or immobilized on the substrate either prior to or after the addition of a sample containing antibody during an immunoassay.

**[0083]** In certain embodiments, the substrate is a bead, such as a colloidal particle (*e.g.*, a colloidal nanoparticle made from gold, silver, platinum, copper, cadmium, 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, cadmium, metal composites, or other soft metals, or metallic nanoshells (*e.g.*, gold hollow spheres, gold-coated silica nanoshells, and silica-coated gold shells).

**[0084]** 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.

**[0085]** In certain embodiments, the substrate is a flow path in an analytical or centrifugal rotor. In other embodiments, the substrate is a tube or a well, such as a well in a plate (*e.g.*, a microtiter plate) suitable for use in an ELISA assay. Such substrates can comprise glass,

cellulose-based materials, thermoplastic polymers, such as polyethylene, polypropylene, or polyester, sintered structures composed of particulate materials (*e.g.*, glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone, or the like. A substrate can be sintered, fine particles of polyethylene, commonly known as porous polyethylene, for example, 0.2-15 micron porous polyethylene from Chromex Corporation (Albuquerque, NM). All of these substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

[0086] 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 some embodiments, the device is a slide comprised of a plurality of beads to which a peptide or population of peptides is attached. In other embodiments, the device is an analytical or centrifugal rotor. In other embodiments, the device is a dot blot, slot blot, or Western 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.

[0087] In certain embodiments, the device comprises a peptide or population of peptides 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: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 85 or a fragment thereof. In other embodiments, the peptide or each peptide in the mixture comprises a sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 72, or a fragment thereof. In certain embodiments, the mixture or population of peptides are attached to or immobilized upon the device optionally through a metallic nanolayer. The devices may be used to detect the presence of antibodies to *Ehrlichia* antigens from multiple species (*e.g.*, *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*) in a sample simultaneously. In one embodiment, the device comprises

a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 72. In related embodiments, the population of isolated peptides further comprises peptides comprising a sequence of SEQ ID NO: 3 and/or SEQ ID NO: 71. In another embodiment, the device comprises a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 3. In another embodiment, the device comprises a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 1. In still another embodiment, the device comprises a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 71. In other embodiments, the device comprises a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 83, or SEQ ID NO: 85.

**[0088]** 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). Thus, the present invention provides a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 1. In certain embodiments, the peptides in the population or mixture 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.

**[0089]** In still other embodiments, the invention provides a composition comprising a peptide comprising a sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 85, 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: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85). Thus, the invention provides a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 3. In another embodiment, the invention provides a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 72. In other embodiments, the invention provides a population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 83, or SEQ ID NO: 85. The peptides in the population or mixture may comprise an N-terminal and/or C-terminal addition, and/or be modified (*e.g.*, by association with one or more further moieties), as described herein.

[0090] In certain embodiments, the compositions comprise one or more peptides (or one or more populations of 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 and/or granulocytic 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) optionally through a linking residue (*e.g.* lysine or cysteine residue). For instance, in certain embodiments, a composition comprises one or more peptides of the invention (*e.g.*, a peptide having a sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85) and one or more antigenic *Ehrlichia* peptides having a sequence of F-S-A-K-E-E-X<sub>7</sub>-A-E-T-R-X<sub>12</sub>-T-F-G-L-X<sub>17</sub>-K-Q-Y-D-G-A-X<sub>24</sub>-I-X<sub>26</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 71) or a fragment thereof, wherein X<sub>7</sub> is any amino acid, X<sub>12</sub> is any amino acid, X<sub>17</sub> is any amino acid, X<sub>24</sub> is any amino acid, and X<sub>26</sub> is any amino acid. In some embodiments, X<sub>7</sub> is K. In other embodiments, X<sub>12</sub> is an amino acid selected from the group

consisting of K and R, and X<sub>17</sub> is an amino acid selected from the group consisting of E and D. In still other embodiments, X<sub>24</sub> is an amino acid selected from the group consisting of K and Q, and X<sub>26</sub> is an amino acid selected from the group consisting of E and T.

[0091] In certain embodiments, the mixture or population of peptides comprises one or more peptides having a sequence of SEQ ID NO: 3 and one or more peptides having a sequence of SEQ ID NO: 71. In still other embodiments, the mixture or population of peptides comprises one or more peptides having a sequence of SEQ ID NO: 1 and one or more peptides having a sequence of SEQ ID NO: 71. In one embodiment, the mixture or population of peptides comprises one or more peptides having a sequence of SEQ ID NO: 3 and one or more peptides having a sequence of SEQ ID NO: 72. In another embodiment, the mixture or population of peptides comprises one or more peptides having a sequence of SEQ ID NO: 72 and one or more peptides having a sequence of SEQ ID NO: 71. In one particular embodiment, the mixture or population of peptides comprises one or more peptides having a sequence of SEQ ID NO: 3, one or more peptides having a sequence of SEQ ID NO: 72, and one or more peptides having a sequence of SEQ ID NO: 71. Such mixtures enable the detection of antibodies to *Ehrlichia* antigens from multiple species (e.g., *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*) in a sample simultaneously.

[0092] 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 and/or granulocytic ehrlichiosis.

[0093] 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 NOs: 1-73 and 83-86, 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.

[0094] 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 with which it is naturally associated. 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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 methods 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.



[0099] 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.

[00100] 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. Cell-free *in vitro* synthesis and/or enzyme-mediated synthetic machineries may also be used.

[00101] 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)), amino-terminal amino acid analysis, and quantitative amino acid analysis.

## **Methods**

[00102] 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 some 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*, *E. ewingii*, *E. muris*, or *Ehrlichia canis*. Other species of *Ehrlichia* which have been implicated in monocytic and/or granulocytic 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 and/or granulocytic ehrlichiosis. In particular embodiments, the methods provide detection of antibodies to *Ehrlichia* antigens from multiple species (e.g., *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*) in a sample simultaneously.

[00103] In certain embodiments, the methods comprise contacting the sample with a mixture or population 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. For instance, in one particular embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 1. In another particular embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 3. In still another embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 72. In some embodiments, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 72. In other embodiments, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated

peptide comprises a sequence of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 83, or SEQ ID NO: 85.

**[00104]** 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). For instance, in some embodiments, the methods comprise contacting the sample with a mixture of one or more peptides of the invention (*e.g.*, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85) and one or more *Ehrlichia* antigenic peptides having a sequence of SEQ ID NO: 71. In one particular embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 3 and one or more peptides having a sequence of SEQ ID NO: 71. In another embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72 and one or more peptides having a sequence of SEQ ID NO: 71. In another embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72 and one or more peptides having a sequence of SEQ ID NO: 3. In one embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72, one or more peptides having a sequence of SEQ ID NO: 3, and one or more peptides having a sequence of SEQ ID NO: 71. Mixtures of peptides of the invention allow, in some embodiments, for the detection of antibodies to antigens from multiple *Ehrlichia* species (*e.g.*, *E. canis*, *E. muris*, *E. chaffeensis*, and *E. ewingii*) in a sample simultaneously.

**[00105]** In certain embodiments, the peptide or each peptide in the mixture or population is an isolated (*e.g.*, synthetic and/or purified) peptide. In certain embodiments, the peptide or mixture of peptides (*i.e.* population of peptides) is attached to or immobilized upon a solid support. In certain embodiments, the solid support is a bead (*e.g.*, a metallic nanoparticle or nanoshell, 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 or centrifugal rotor, a blot (Western blot, dot blot, or slot blot), 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 some

embodiments, the peptide or mixture of peptides is attached to or immobilized upon a solid support through a metallic nanolayer that, in some embodiments, may be comprised of cadmium, zinc, mercury, or a noble metal (*e.g.*, gold, silver, copper, and platinum).

[00106] In certain embodiments, the detecting step comprises performing an ELISA or immunofluorescence 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 or centrifugal rotor. In some embodiments, the detecting step comprises performing a wavelength shift assay. Such wavelength shift assays may entail measuring or determining a change in the surface plasmon resonance or localized surface plasmon resonance wavelength resulting from binding of antibodies to peptides attached to metallic nanolayers or metallic nanoparticle/nanoshells. In still other embodiments, the detecting step comprises analyzing the sample with an electrochemical, optical, or opto-electronic sensor.

[00107] 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 an immunofluorescence assay, performing a lateral flow immunoassay, performing an agglutination assay, performing a wavelength shift assay, performing a Western blot, slot blot, or dot blot, analyzing the sample in an analytical or centrifugal rotor, or analyzing the sample with an electrochemical, optical, or opto-electronic sensor. These different assays are described herein and/or are well-known to those skilled in the art.

[00108] In one embodiment, the methods involve detecting the presence of naturally occurring antibodies against one or more *Ehrlichia* antigens (*e.g.*, the antigen of a pathogenic *Ehrlichia*, such as *E. chaffeensis*, *E. muris*, *E. ewingii*, 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.

[00109] 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 and/or granulocytic ehrlichiosis.

[00110] 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.

[00111] 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).

[00112] 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. The sample may also be a tissue extract or a cell lysate.

[00113] 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.

[00114] 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.*, Figure 2. 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.*, metallic nanoparticle or metallic nanoshell, 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, anti-cat IgG antibodies, anti-cat IgM antibodies, protein A, protein G, protein A/G fusion proteins, protein L, or combinations thereof, etc.). *See, e.g.*, Figures 1, 3, and 4. 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 a first binding partner which specifically recognizes the

sample antibodies (*e.g.*, anti-human IgG antibodies, anti-human IgM antibodies, anti-dog IgG antibodies, anti-dog IgM antibodies, anti-cat IgG antibodies, anti-cat IgM antibodies, protein A, protein G, protein A/G fusion proteins, protein L, etc.), and further adding a second binding partner (*e.g.*, protein A, protein G, protein A/G fusion proteins, protein L, etc.), wherein the second binding partner is labeled and recognizes said first binding partner. 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.

**[00115]** 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-His (SEQ ID NO: 82) and the carrier comprises a Nitrilotriacetic Acid (NTA) derivative charged with  $\text{Ni}^{++}$  or  $\text{Co}^{++}$  ions. In certain embodiments, the moiety is a fusion partner, *e.g.*, BSA. In exemplary embodiments, peptides of the invention may be conjugated to BSA via N-terminal and/or C-terminal residues of the peptides. In one embodiment, one, two, three, four, five, 10, 15, 20, 25, 30 or more peptides of the invention may be substituted into, *e.g.*, conjugated with BSA. As would be understood by one skilled in the art, substitution levels may impact the sensitivity of the assay. Lower concentrations of highly substituted BSA are needed to achieve sensitivity offered by high concentrations of BSA-peptide containing fewer molecules of peptide. In certain other embodiments, the fusion partner may be MAPS. In certain exemplary embodiments, MAPS may consist of 4, 8, or more asymmetric branches.

[00116] Suitable carriers, supports, and surfaces include, but are not limited to, metallic nanolayers, beads (*e.g.*, magnetic beads, colloidal particles or metallic nanoparticles or nanoshells, such as colloidal gold, or particles or nanoparticles comprising silica, latex, polystyrene, polycarbonate, or PDVF), latex of co-polymers 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.

[00117] 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.

[00118] 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.

[00119] 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.

**[00120]** 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, alkaline phosphatase, etc.), fluorescent labels, radioactive labels, colored latex particles, and metal-conjugated labels (*e.g.*, metallic nanolayers, metallic nanoparticle- or metallic nanoshell-conjugated labels). Suitable metallic nanoparticle or metallic nanoshell labels include, but are not limited to, gold particles, silver particles, copper particles, platinum particles, cadmium particles, composite particles, gold hollow spheres, gold-coated silica nanoshells, and silica-coated gold shells. Metallic nanolayers suitable for detectable layers include nanolayers comprised of cadmium, zinc, mercury, and noble metals, such as gold, silver, copper, and platinum.

**[00121]** 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 or combinations thereof) which binds to the first antibody. This secondary antibody or other binding agent can be labeled, *e.g.*, with a radioactive, enzymatic, fluorescent, luminescent, metallic nanoparticle or metallic nanoshell (*e.g.* colloidal gold), 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 or other signaling moiety. 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.

[00122] 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.

[00123] In some 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.

[00124] 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.

[00125] 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 A/G fusion proteins, 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. Another embodiment of this type of lateral flow device in which the peptides of the invention are used both as the immobilized capture agent at a test site and as a soluble labeled complex to react with antibodies in a sample is shown in Figure 1. In such embodiments, to amplify the detection signal, protein A, protein G, and/or protein A/G fusion proteins conjugated to a detectable label (*e.g.*, metallic nanoparticle or nanoshell, HRP, ALP, fluorophore, colored latex particle) may be applied to the test site where they will bind to the Fc region of any antibodies to *Ehrlichia* antigens captured by the immobilized peptides of the invention. 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.

[00126] 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 mixtures or populations of peptides of the invention are adsorbed to the surface of a microtiter well directly or through a capture matrix (*e.g.*, an antibody). Residual, non-specific protein-binding sites on the surface are then blocked with an appropriate agent, such as bovine serum albumin (BSA), heat-inactivated normal goat serum (NGS), or BLOTTO (a buffered solution of nonfat dry milk which also contains a preservative, salts, and an antifoaming agent). The well is then incubated with a biological sample suspected of containing specific anti-*Ehrlichia* (*e.g.*, anti-*E. chaffeensis*, anti-*E. muris*, anti-*E. ewingii*, 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 ( $\alpha$ 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 (ALP), 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.

[00127] 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.

[00128] In another embodiment of an ELISA, a peptide or a mixture of peptides of the invention is immobilized on a surface, such as a ninety-six-well ELISA plate or equivalent solid phase via a fusion partner, *e.g.*, BSA or MAPS. A sample is then added and the assay proceeds as above.

[00129] 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, anti-cat, 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).

[00130] In another embodiment, the methods comprise an agglutination assay. For example, in certain embodiments, metallic nanoparticles or metallic nanoshells (*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 metallic nanoparticles or metallic nanoshells (*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, anti-cat 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.

[00131] In still other embodiments, 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 immunoenzymatic methods or by visualization using metallic nanoparticles or nanoshells coupled to secondary antibodies or other antibody binding agents, such as protein A, protein G, protein A/G fusion proteins, protein L, or combinations thereof.

[00132] 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*, *E. muris*, *E. ewingii*, 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.

[00133] 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.

[00134] 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*.

[00135] 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- $\gamma$ . These methods are well-known in the art.

[00136] 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 and/or granulocytic 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.

[00137] In another aspect, the invention provides methods of diagnosing monocytic and/or granulocytic ehrlichiosis in a subject. The subject can be a subject suspected of having antibody against a causative agent of monocytic and/or granulocytic ehrlichiosis. The diagnostic method is useful for diagnosing subjects exhibiting the clinical symptoms of monocytic and/or granulocytic ehrlichiosis. Clinical symptoms of human monocytic/granulocytic ehrlichiosis include, but are not limited to, fever, headache, malaise, myalgia, rash, thrombocytopenia, leukopenia, and elevated serum transaminase levels. Clinical symptoms of ehrlichiosis in animals (*e.g.* canines) include, but are not limited to, fever, petechiae, bleeding disorders, vasculitis, lymphadenopathy, discharge from the nose and eyes, edema of the legs and scrotum, weight loss, pale gums due to anemia, bleeding due to thrombocytopenia, vasculitis, lymphadenopathy, dyspnea, coughing, polyuria, polydipsia, and lameness.

[00138] 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 or population 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. For instance, in one particular embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 1. In another particular embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 3. In still another embodiment, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 72. In some embodiments, the methods comprise contacting the sample with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 72. In other embodiments, the methods comprise contacting the sample

with a mixture or population of two or more different isolated peptides, wherein each isolated peptide comprises a sequence of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 83, or SEQ ID NO: 85.

**[00139]** 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. For instance, in some embodiments, the methods comprise contacting the sample with a mixture of one or more peptides of the invention (*e.g.*, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, or SEQ ID NO: 85) and one or more *Ehrlichia* antigenic peptides having a sequence of SEQ ID NO: 71. In one particular embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 3 and one or more peptides having a sequence of SEQ ID NO: 71. In another embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72 and one or more peptides having a sequence of SEQ ID NO: 71. In another embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72 and one or more peptides having a sequence of SEQ ID NO: 3. In one embodiment, the methods comprise contacting the sample with a mixture of one or more peptides having a sequence of SEQ ID NO: 72, one or more peptides having a sequence of SEQ ID NO: 3, and one or more peptides having a sequence of SEQ ID NO: 71.

**[00140]** In certain embodiments, the peptide or each peptide in the mixture or population is an isolated (*e.g.*, synthetic and/or purified) peptide. In certain embodiments, the peptide or mixture of different peptides (*i.e.* population of 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 metallic nanoparticle or nanoshell), a flow path in a lateral flow immunoassay device (*e.g.*, a porous membrane), a flow path in an analytical or centrifugal rotor, a blot (*e.g.*, a Western blot, dot blot, or slot blot), 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 some embodiments, the peptide or



mixture of peptides is attached to or immobilized upon a solid support through a metallic nanolayer that, in some embodiments, may be comprised of cadmium, zinc, mercury, or a noble metal (*e.g.*, gold, silver, copper, and platinum).

[00141] 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, performing a wavelength shift assay, analyzing the sample using a Western blot, a slot blot, or a dot blot, analyzing the sample in an analytical or centrifugal 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.

[00142] 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**

[00143] 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 or a mixture or population of the peptides of the invention. The peptides can comprise a sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 85, or fragments thereof. In certain embodiments, the peptides are attached to or immobilized on a solid support. In some embodiments, the peptides are attached to or immobilized on a solid support through a metallic nanolayer (*e.g.*, cadmium, zinc, mercury, gold, silver, copper, or platinum nanolayer). In certain embodiments, the solid support is a bead (*e.g.*, a colloidal particle or a metallic nanoparticle or nanoshell), a flow path in a lateral flow immunoassay device, a flow

path in an analytical or centrifugal rotor, a tube or a well (*e.g.*, in a plate), or a sensor (*e.g.*, an electrochemical, optical, or opto-electronic sensor).

[00144] 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 or centrifugal rotor, a Western blot, a dot blot, a slot blot, 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 (*i.e.* population of peptides) of the invention, or a peptide composition of the invention is attached to or immobilized on the beads, the plate, or the device.

[00145] In addition, the kits can include various diluents and buffers, labeled conjugates or other agents for the detection of specifically bound antigens or antibodies (*e.g.* labeling reagents), and other signal-generating reagents, such as enzyme substrates, cofactors and chromogens. In some embodiments, the kit comprises an anti-human, anti-canine, or anti-feline IgG/IgM antibody conjugated to a detectable label (*e.g.*, a metallic nanoparticle, metallic nanoshell, metallic nanolayer, fluorophore, colored latex particle, or enzyme) as a labeling reagent. In other embodiments, the kit comprises protein A, protein G, protein A/G fusion proteins, protein L, or combinations thereof conjugated to a detectable label (*e.g.*, a metallic nanoparticle, metallic nanoshell, metallic nanolayer, fluorophore, colored latex particle, or enzyme) as a labeling reagent. An exemplary protein A/G fusion protein combines four Fc-binding domains from protein A with two from protein G. *See, e.g.*, Sikkema, J.W.D., *Amer. Biotech. Lab*, 7:42, 1989 and Eliasson *et al.*, *J. Biol. Chem.* 263, 4323-4327, 1988, both which are hereby incorporated by reference in their entireties. In still other embodiments, the labeling reagents of the kit are a second population of peptides of the invention conjugated to a detectable label (*e.g.*, a metallic nanoparticle, metallic nanoshell, metallic nanolayer, fluorophore, colored latex particle, or enzyme). The second population of peptides can be the same as or different than the first population of peptides, which may optionally be attached to or immobilized upon a solid support.

[00146] Other components of a kit can easily be determined by one of skill in the art. Such components may include coating reagents, polyclonal or monoclonal capture antibodies specific for a peptide of the invention, or a cocktail of two or more of the antibodies, purified or semi-purified extracts of these antigens as standards, monoclonal antibody detector antibodies, an anti-mouse, anti-dog, anti-cat, anti-chicken, or anti-human antibody conjugated to a detectable label, 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.

[00147] Such kits provide a convenient, efficient way for a clinical laboratory to diagnose infection by a pathogenic *Ehrlichia*, such as *E. chaffeensis*, *E. muris*, *E. ewingii*, 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 or population of peptides of the invention to detect an antibody to one or more *Ehrlichia* antigens or to diagnose monocytic and/or granulocytic 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 one or more *Ehrlichia* antigens or to diagnose monocytic and/or granulocytic ehrlichiosis.

[00148] 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 and/or granulocytic 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*.

[00149] The following examples illustrate 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-ELISA Assay

[00150] Three different populations of peptides were synthesized using standard synthesis procedures. Each peptide in the first population of peptides (ECHEW1) contained a sequence of SEQ ID NO: 72. The first population of peptides specifically binds to antibodies elicited by multiple *Ehrlichia* spp. (e.g., *canis*, *chaffeensis*, and *ewingii*). Each peptide in the second population of peptides (EE12EW1) contained a sequence of SEQ ID NO: 3. The second population of peptides specifically binds to antibodies elicited primarily by *E. canis* and *E. chaffeensis* with some cross-reactivity to *E. ewingii*. Each peptide in the third population of peptides (EE13) contained a sequence of SEQ ID NO: 71. The third population of peptides specifically binds to antibodies elicited primarily by *E. ewingii* with some cross-reactivity to *E. canis* and *E. chaffeensis*.

[00151] Each peptide in the three populations was linked separately to the carrier protein bovine serum albumin (BSA) using thio-ether chemistry. The resulting BSA-peptide conjugates were used as capture entities in 96-well ELISA plates to create three separate ELISA assays (one population of peptides per plate). The plates were then blocked to prevent undesirable non-specific binding.

[00152] Dog plasma samples positive to *Ehrlichia* species, as determined by indirect immunofluorescence assays (IFA), IDEXX SNAP 4DX Plus<sup>TM</sup>, and/or SNAP 3Dx<sup>TM</sup>, were incubated with the immobilized capture peptides in each of the three ELISA plates. After one hour incubation, the unreacted materials were removed by washing the micro wells. The specifically captured dog IgG or IgM were detected by reaction with HRP-labeled Protein A. HRP was assayed using a commercial TMB substrate. The optical density of each well was read at 650 nm with a plate reader.

[00153] A total of 156 samples were evaluated, of which 152 tested positive in the ELISA plates with four samples testing negative. Thus, the percent sensitivity of the test was 97.4%. A summary of the results separated by infective *Ehrlichia* species is shown in Table 1 below.

These experimental results show that populations of peptides defined by SEQ ID NO: 72, SEQ ID NO: 3, or SEQ ID NO: 71 have a high degree of sensitivity in detecting the presence of antibodies to antigens from various *Ehrlichia* spp.

**Table 1-ELISA Results of Known Ehrlichia-Positive Samples**

	Positive by ELISA	Negative by ELISA	Total
No. of <i>E. canis</i> -positive samples <sup>1</sup>	44	1	45
No. of <i>E. chaffeensis</i> -positive samples <sup>2</sup>	38	2	40
No. of <i>E. ewingii</i> -positive samples <sup>3</sup>	46	1	47
No. of positive samples; indeterminate species <sup>4</sup>	24	0	24
Total Samples Tested	156		
Total No. of Positive Samples Correctly Identified (detected positive by ELISA)	152		
Total No. of Positive Samples Incorrectly Identified (detected negative by ELISA)	4		
% Sensitivity	97.4		

<sup>1</sup> These samples tested positive in the ELISA assays with ECHEW1 (SEQ ID NO:72) and EE12EW1 (SEQ ID NO: 3) populations of peptides and had a higher titer for *E. canis* by IFA.

<sup>2</sup> These samples tested positive in the ELISA assays with ECHEW1 (SEQ ID NO:72) and EE12EW1 (SEQ ID NO: 3) populations of peptides and had a higher titer for *E. chaffeensis* by IFA.

<sup>3</sup> These samples tested positive in the ELISA assays with ECHEW1 (SEQ ID NO:72) and EE13 (SEQ ID NO: 71) populations of peptides.

<sup>4</sup> The species of *Ehrlichia* in these samples could not be determined conclusively by IFA or the SNAP assays, but the samples tested positive in the ELISA assay with ECHEW1 (SEQ ID NO:72).

#### Example 2-Lateral Flow Assay

**[00154]** A lateral flow immunoassay in a double antigen sandwich format was constructed to detect the presence of antibodies specific for *Ehrlichia* antigens from multiple species. A population of peptides defined by SEQ ID NO: 72 was linked to BSA and the resulting complexes were used both as test conjugate (peptides labeled with gold nanoparticles) and as capture (immobilized at the test line of the device). The signal produced at the test line was enhanced by Protein A and Protein G-gold conjugates added to the labeled peptide conjugate. The device is depicted in Figure 5.

[00155] To conduct the assay, one drop of anti-coagulated whole blood, serum, or plasma is applied to the sample port of the device. The blood separation pad filters blood cells from whole blood. Plasma (or serum) mobilizes and binds specifically to the test conjugate present on the conjugate pad and any formed antibody-peptide complexes migrate to the nitrocellulose membrane containing the test and the control regions. The application of a chase buffer after sample application moves the free and the bound test conjugates through the nitrocellulose membrane towards the upper absorbent pad. The labeled peptide-antibody complexes move to the test line where immobilized peptides capture labeled peptide-antibody complexes via the second binding sites on the antibodies. Protein A-gold and Protein G-gold conjugates in the conjugate mixture bind to captured antibodies amplifying the detection signal. The appearance of one red line at the test site and a second red line at the control site indicates the presence of antibodies to *Ehrlichia* spp. (e.g., *canis*, *chaffeensis*, or *ewingii*) in the sample. The appearance of a red line at only the control site indicates the absence of antibodies to all of the *Ehrlichia* spp. in the sample. The test is considered invalid if (i) a signal at the test line appears but no signal at the control line is present or (ii) no signal is observed at either the control or test lines.

[00156] The same 156 known *Ehrlichia*-positive dog plasma samples evaluated by ELISA assay in Example 1 were tested in the lateral flow device. In addition, 120 dog samples (100 plasma and 20 whole blood samples) that were determined to be negative by indirect immunofluorescence assays or IDEXX SNAP 4DX Plus<sup>TM</sup>, were also evaluated. The results are summarized in Table 2 below. The lateral flow assay had a sensitivity of 97.4% with a 95% confidence interval of 93.6 – 99.3%. The specificity of the assay was 98.3% with a 95% confidence interval of 94.1 – 99.8%. This example demonstrates that a population of peptides defined by SEQ ID NO: 72 can effectively detect antibodies against *Ehrlichia* antigens when employed in a lateral assay format.

**Table 2-Lateral Flow Assay Results of Known Ehrlichia-Positive and Negative Samples**

	Negative by Lateral Flow	Positive by Lateral Flow
No. of known negative samples	118	2
No. of known positive samples	4	152

Example 3- Indirect Fluorescent Antibody Assay

[00157] An indirect fluorescent antibody test is constructed using latex beads coated with one or more peptides of the invention. In certain embodiments, the peptides defined by SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 3, or SEQ ID NO: 5 are used. The peptides of the invention are coated onto maleimide-derivatized latex beads using thio-ether chemistry. Alternatively, the peptides of the invention may be conjugated to BSA via thio-ether or similar chemistries and are passively absorbed on to latex beads. A population of such beads is then immobilized on a glass slide using known techniques.

[00158] To conduct the assay, one drop of serum or plasma (diluted appropriately with a suitable buffer) from dogs suspected of having anti-*Ehrlichia* antibodies, is applied to the glass slide coated with latex beads. Following a suitable incubation time, the unreacted materials are washed away and a drop of fluorescently labeled anti-dog IgG (or IgM) is applied and the slides are incubated for an additional time period. The final preparation is viewed under a fluorescent microscope to determine fluorescently tagged latex beads. The classification of the test serum/plasma as positive or negative is based on comparison with appropriate controls. An enzyme label may be used in place of the fluorescent label in which case the visualization step employs an enzyme substrate. For example, anti-dog IgG/IgM labeled with alkaline phosphatase can be visualized by exposing the slide to a BCIP-nitro BT substrate. Labeled Protein A, Protein G, or Protein A/G fusion can be used in place of labeled anti-dog IgG and anti-dog IgM to detect antibodies bound to the peptide-coated beads.

[00159] 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, 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.

[00160] The disclosures, including the claims, figures and/or drawings, of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entireties.

**What is claimed:**

1. A population of isolated peptides comprising three or more different peptides, wherein each peptide in the population comprises a sequence of:

(i) S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>39</sub>-A-E-T-R-X<sub>44</sub>-T-F-G-L-X<sub>49</sub>-K-Q-Y-D-G-A-X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 72) or fragment thereof,

wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>44</sub> is any amino acid, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid, or

(ii) S-X<sub>2</sub>-K-E-X<sub>5</sub>-K-Q-X<sub>8</sub>-T-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-G-L-K-Q-X<sub>18</sub>-W-X<sub>20</sub>-G-X<sub>22</sub>-X<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-G-G-G-G-G-N-F-S-A-K-E-E-X<sub>39</sub>-A-X<sub>41</sub>-T-R-X<sub>44</sub>-T-F-G-X<sub>48</sub>-X<sub>49</sub>-K-Q-Y-D-G-A-X<sub>56</sub>-I-X<sub>58</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 3) or a fragment thereof,

wherein X<sub>2</sub> is an amino acid selected from the group consisting of A and V, X<sub>5</sub> is an amino acid selected from the group consisting of E and D, X<sub>8</sub> is an amino acid selected from the group consisting of T and P, X<sub>10</sub> is an amino acid selected from the group consisting of T and V, X<sub>11</sub> is an amino acid selected from the group consisting of G and A, X<sub>12</sub> is an amino acid selected from the group consisting of L and V, X<sub>13</sub> is an amino acid selected from the group consisting of Y and F, X<sub>18</sub> is an amino acid selected from the group consisting of D and N, X<sub>20</sub> is an amino acid selected from the group consisting of D and N, X<sub>22</sub> is an amino acid selected from the group consisting of S and V, X<sub>23</sub> is an amino acid selected from the group consisting of A, S, and T, X<sub>24</sub> is an amino acid selected from the group consisting of A and I, X<sub>25</sub> is an amino acid selected from the group consisting of T and P, X<sub>26</sub> is an amino



acid selected from the group consisting of S, N, and K, X<sub>39</sub> is any amino acid, X<sub>41</sub> is an amino acid selected from the group consisting of D and N, X<sub>44</sub> is any amino acid, X<sub>48</sub> is an amino acid selected from the group consisting of V and A, X<sub>49</sub> is any amino acid, X<sub>56</sub> is any amino acid, and X<sub>58</sub> is any amino acid.

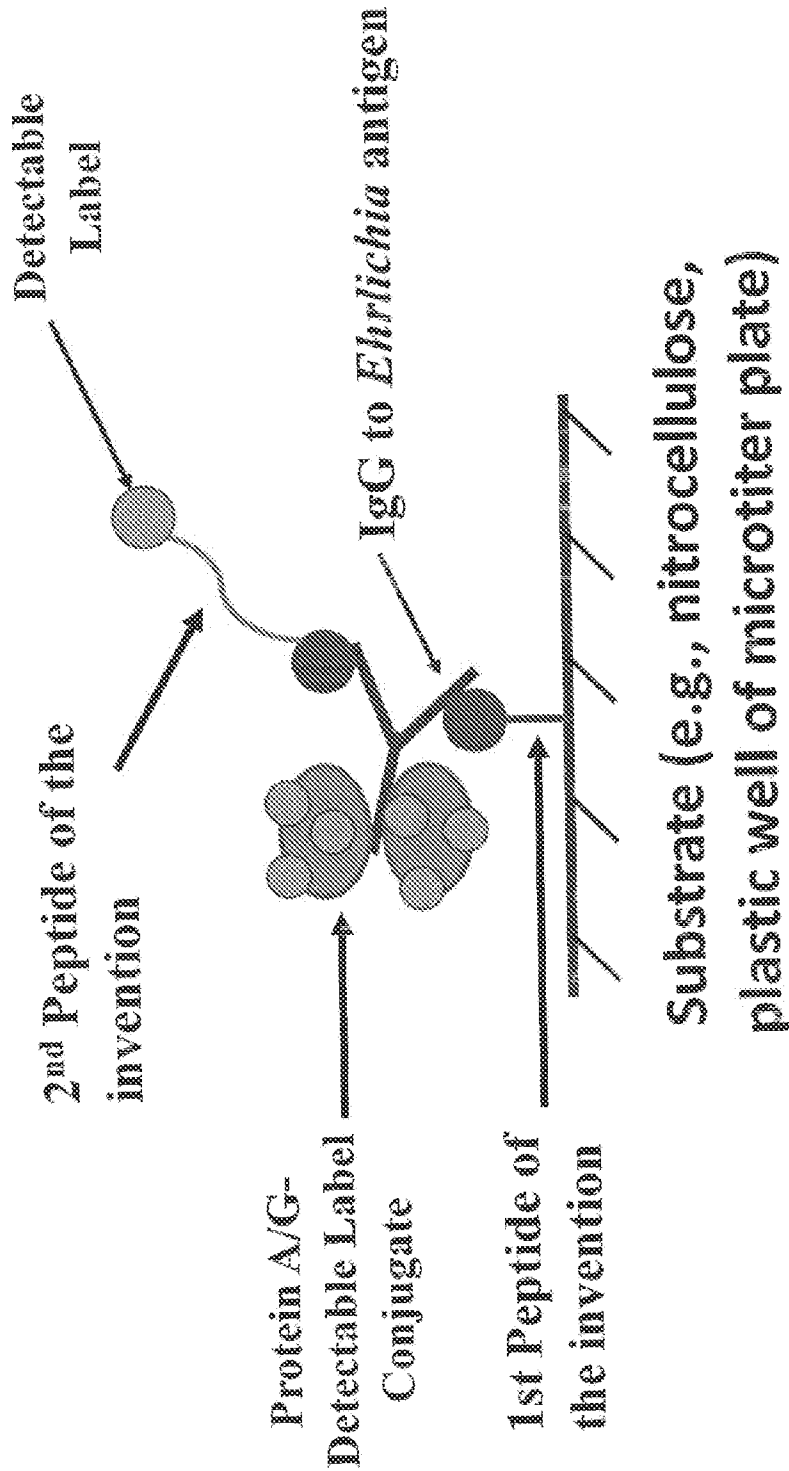
2. The population of isolated peptides of claim 1, wherein X<sub>39</sub> is K.
3. The population of isolated peptides of claim 1, wherein X<sub>44</sub> is an amino acid selected from the group consisting of K and R, and X<sub>49</sub> is an amino acid selected from the group consisting of E and D.
4. The population of isolated peptides of claim 1, wherein X<sub>56</sub> is an amino acid selected from the group consisting of K and Q, and X<sub>58</sub> is an amino acid selected from the group consisting of E and T.
5. The population of isolated peptides of claim 1, wherein said fragment comprises at least 20, 25, 30, 35, or 40 contiguous amino acids from SEQ ID NO: 3 or SEQ ID NO: 72.
6. The population of isolated peptides of claim 5, wherein said fragment comprises amino acids 33 to 71 of SEQ ID NO: 3 or SEQ ID NO: 72.
7. The population of isolated peptides of claim 1, wherein each isolated peptide comprises an additional N-terminal peptide sequence that is a native OMP-1 sequence or non-OMP-1 *Ehrlichia* antigen.
8. The population of isolated peptides of claim 1, wherein each isolated peptide comprises an additional C-terminal peptide sequence that is a native OMP-1 sequence or non-OMP-1 *Ehrlichia* antigen.
9. The population of isolated peptides of claim 1, wherein each isolated peptide comprises at least 70, 75, 80, or 85 amino acids.

10. The population of isolated peptides of claim 1, wherein one or more of the isolated peptides is conjugated to a ligand or is biotinylated.
11. The population of isolated peptides of claim 1, wherein one or more of the isolated peptides is conjugated to avidin, streptavidin, neutravidin, serum albumin, keyhole limpet hemocyanin (KLH), an enzyme, or a metallic nanoparticle or nanoshell.
12. The population of isolated peptides of claim 1, wherein the population of peptides is immobilized to a solid support optionally through a metallic nanolayer.
13. The population of isolated peptides of claim 1, wherein the solid support is a plurality of beads, a flow path in a lateral flow immunoassay device, a well in a microtiter plate, or a flow path in a rotor.
14. The population of isolated peptides of claim 1, wherein the population further comprises one or more antigenic peptides from an *Ehrlichia* species.
15. The population of isolated peptides of claim 14, wherein said one or more antigenic peptides has a sequence of F-S-A-K-E-E-X<sub>7</sub>-A-E-T-R-X<sub>12</sub>-T-F-G-L-X<sub>17</sub>-K-Q-Y-D-G-A-X<sub>24</sub>-I-X<sub>26</sub>-E-N-Q-V-Q-N-K-F-T-I-S-N-C (SEQ ID NO: 71) or a fragment thereof, wherein X<sub>7</sub> is any amino acid, X<sub>12</sub> is any amino acid, X<sub>17</sub> is any amino acid, X<sub>24</sub> is any amino acid, and X<sub>26</sub> is any amino acid.
16. The population of isolated peptides of claim 15, wherein X<sub>7</sub> in SEQ ID NO: 71 is K.
17. The population of isolated peptides of claim 15, wherein X<sub>12</sub> in SEQ ID NO: 71 is an amino acid selected from the group consisting of K and R, and X<sub>17</sub> in SEQ ID NO: 71 is an amino acid selected from the group consisting of E and D.
18. The population of isolated peptides of claim 15, wherein X<sub>24</sub> in SEQ ID NO: 71 is an amino acid selected from the group consisting of K and Q, and X<sub>26</sub> in SEQ ID NO: 71 is an amino acid selected from the group consisting of E and T.

19. A method for detecting in a sample an antibody to an epitope of an *Ehrlichia* antigen, the method comprising:
- contacting a sample with the population of isolated peptides of claim 1; and
  - detecting formation of an antibody-peptide complex comprising said one or more peptides in the population,
- wherein formation of said complex is indicative of an antibody to an epitope of an *Ehrlichia* antigen being present in said sample.
20. The method of claim 19, wherein said *Ehrlichia* antigen is from an *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia canis*, or *Ehrlichia muris* species.
21. The method of claim 19, wherein the population of isolated peptides is immobilized to a solid support optionally through a metallic nanolayer.
22. The method of claim 21, wherein said solid support is a plurality of beads, a flow path in a lateral flow assay device, a well in a microtiter plate, or a flow path in a rotor.
23. The method of claim 19, wherein said detecting step comprises (i) performing an ELISA assay, (ii) running a lateral flow assay, (iii) performing an agglutination assay, (iv) performing a Western blot, a slot blot, or dot blot, (v) performing a wavelength shift assay, or (vi) running the sample through an analytical or centrifugal rotor.
24. The method of claim 19, wherein said sample is from a human, canine, or feline subject.
25. The method of claim 19, wherein said sample is a blood, serum, plasma, cerebral spinal fluid, tissue extract, urine, or saliva sample.
26. The method of claim 19, wherein the formation of a plurality of antibody-peptide complexes are detected, and wherein formation of said complexes is indicative of antibodies to epitopes of *Ehrlichia* antigens from multiple *Ehrlichia* species being present in said sample.

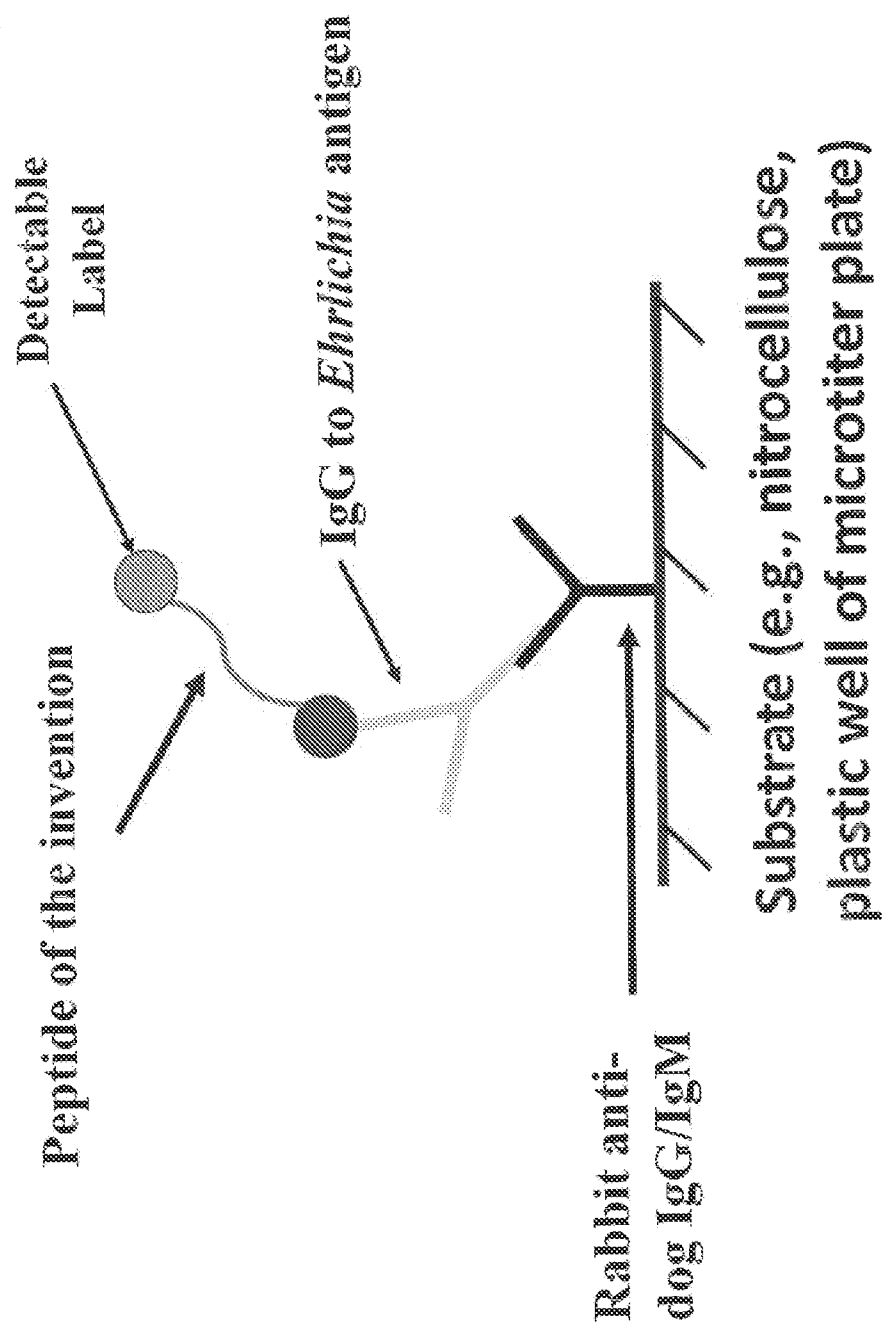
27. A method for diagnosing monocytic and/or granulocytic ehrlichiosis in a subject, the method comprising:
- contacting a sample from the subject with the population of isolated peptides of claim 1; and
  - detecting formation of an antibody-peptide complex comprising said one or more peptides in the population,
  - wherein formation of the complex is indicative the subject having monocytic and/or granulocytic ehrlichiosis.
28. A kit comprising the population of isolated peptides of claim 1 and a labeling reagent capable of binding to an antibody that recognizes an epitope of said one or more peptides in the population.
29. The kit of claim 28, wherein the population of isolated peptides is attached to or immobilized on a solid support optionally through a metallic nanolayer.
30. The kit of claim 28, wherein the solid support is a plurality of beads, a tube or a well, a lateral flow assay device, or an analytical or centrifugal rotor.
31. The kit of claim 28, wherein the labeling reagent is an anti-human, anti-canine, or anti-feline IgG or IgM antibody conjugated to a detectable label.
32. The kit of claim 31, wherein the detectable label is an enzyme, a metallic nanoparticle, metallic nanoshell, metallic nanolayer, fluorophore, or colored latex particle.
33. The kit of claim 28, wherein the labeling reagent is protein A, protein G, and/or a protein A/G fusion protein conjugated to a detectable label.
34. The kit of claim 33, wherein the detectable label is an enzyme, a metallic nanoparticle, metallic nanoshell, metallic nanolayer, fluorophore, or colored latex particle.

**Fig. 1**

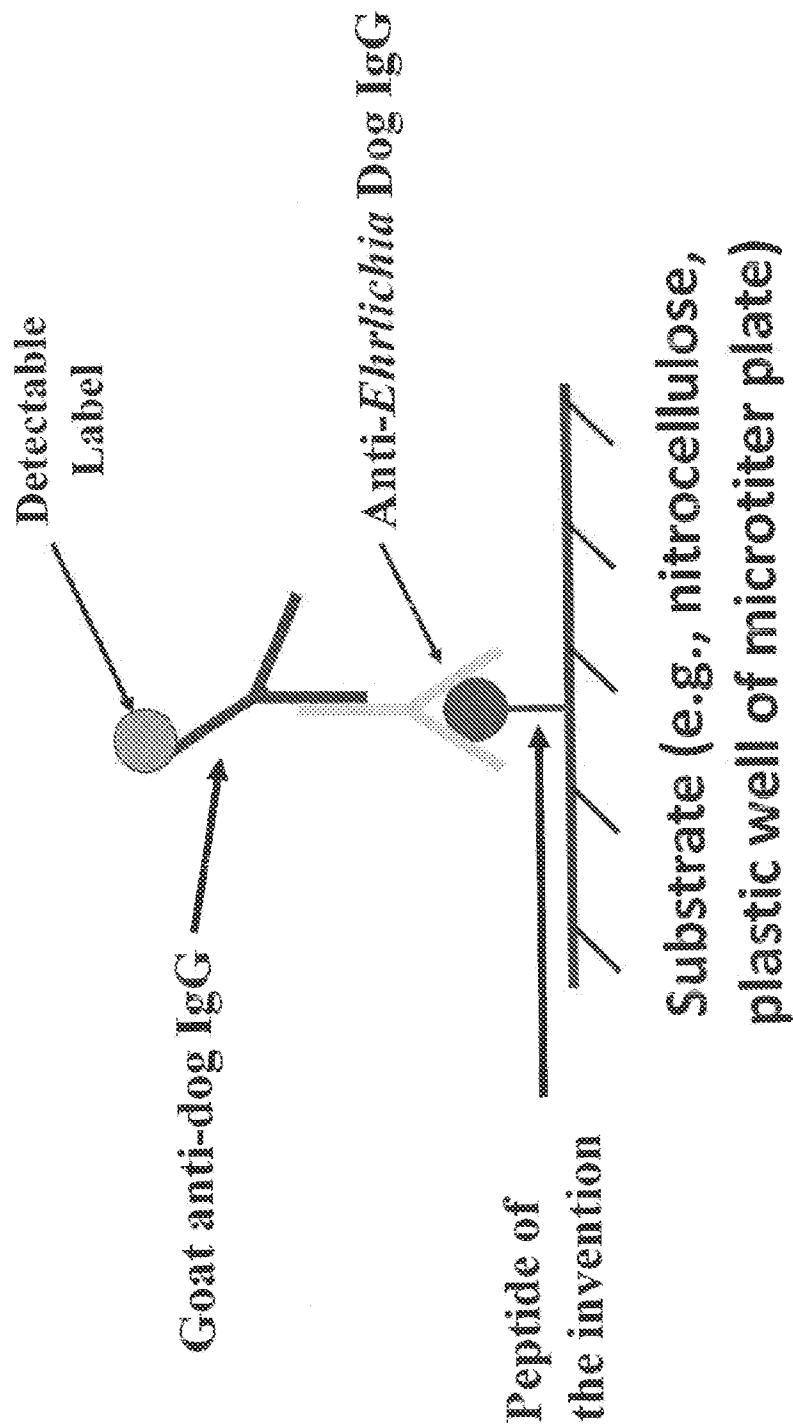


2/5

**Fig. 2**



**Fig. 3**



4/5

**Fig. 4**

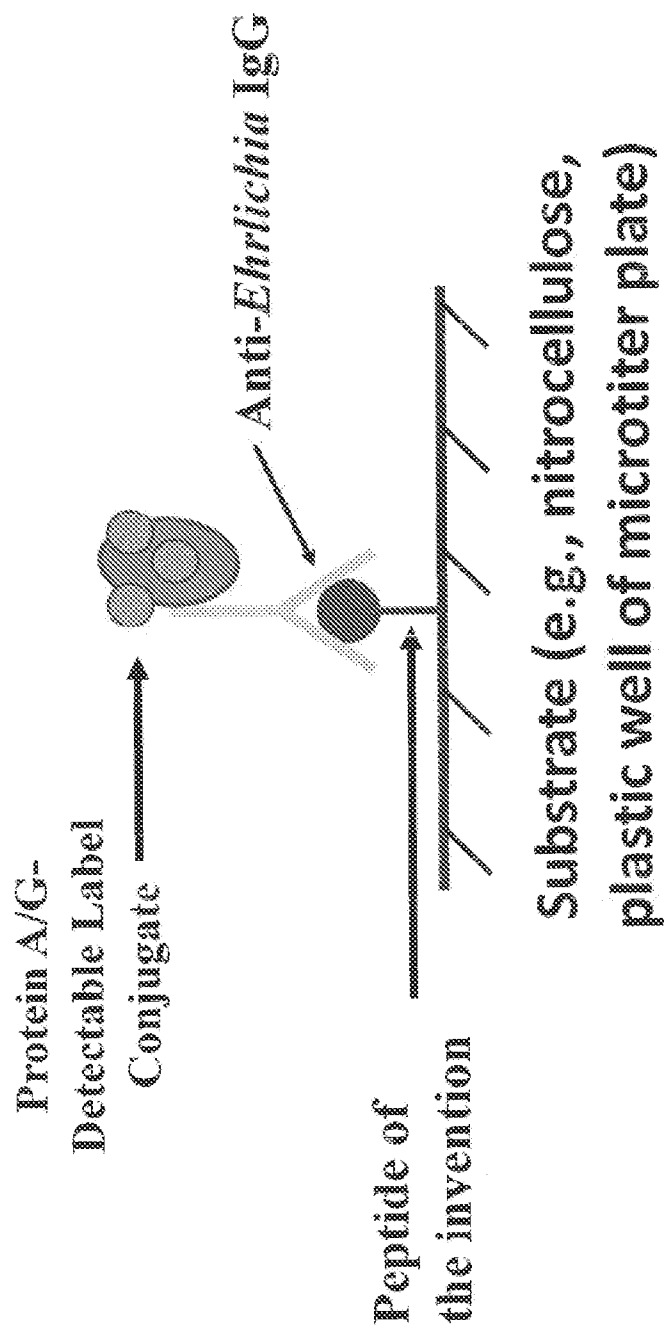
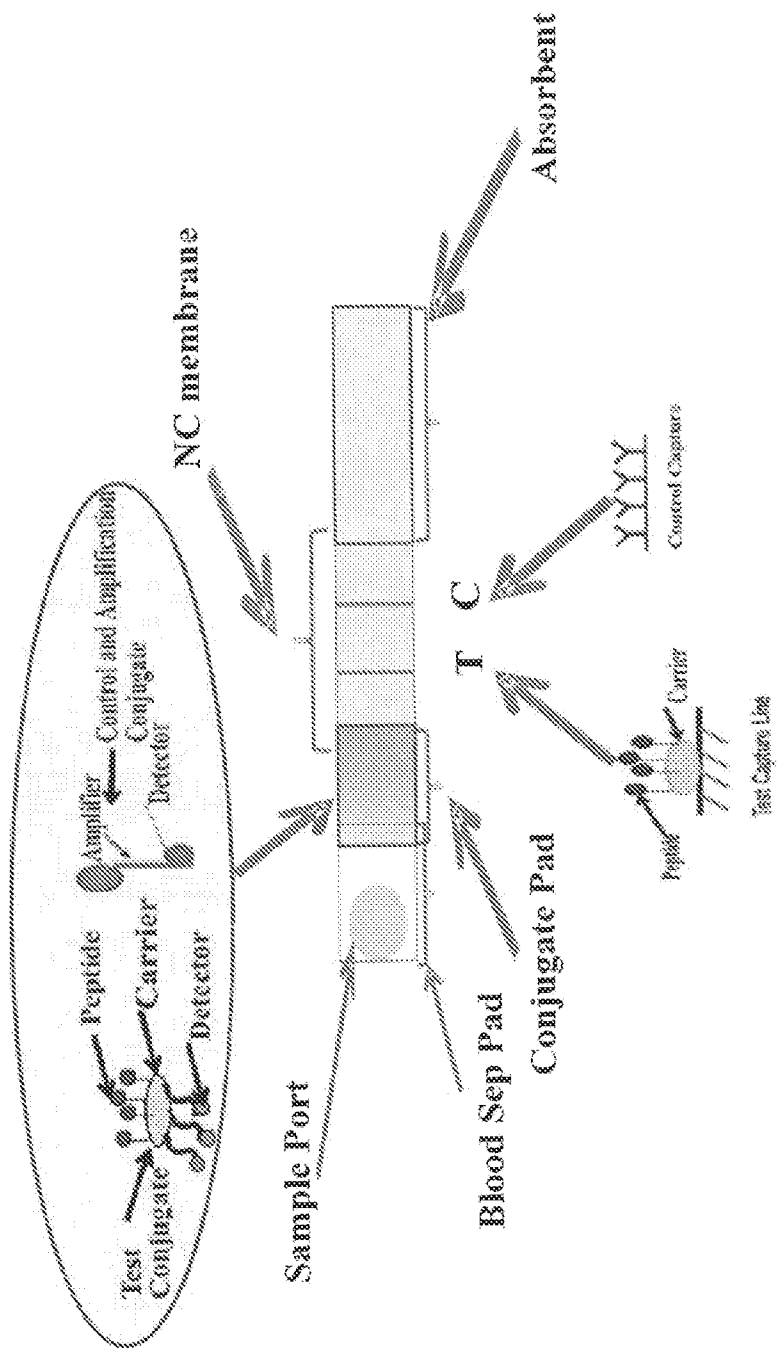




Fig. 5



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/64536

**Box No. I**      **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/64536

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/554, 33/567, 33/53 (2014.01)

USPC - 435/7.32, 7.2, 7.1, 4; 424/133.1, 130.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): G01N 33/554, 33/567, 33/53 (2014.01)

USPC: 435/7.32, 7.2, 7.1, 4; 424/133.1, 130.1

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

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

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; PubMed; 'isolated peptides,' 'attached,' 'immobilized,' 'solid support,' 'metallic nanolayer,' 'rickettsia, antibody; 'plurality of antigens,' polypeptides, 'antigen- antibody complexes'

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2011/0124125 A1 (MEHRA, RK et al.) May 26, 2011; paragraphs [0013], [0021], [0022], [0053], [0055], [0063], [0069], [0076], [0081]-[0083], [0098], [0113]; Claims 17, 22, 23, 27, 28, 30-35; SEQ ID NOs: 77, 79	1, 3, 4, 7, 8-25, 27-34 ----- 2, 26 ----- 5, 6
Y --- A	GUSA, A et al. Identification Of A p28 Gene In Ehrlichia ewingii: Evaluation Of Gene For Use As A Target For A Species-Specific PCR Diagnostic Assay. Journal of Clinical Microbiology. November 2001, Vol. 39, No. 11, ppages 3871-3876; page 3874, figure 2, line C-1/ OK-2.	2 ----- 5, 6
Y	US 6593147 B1 (BARBET, AF et al.) July 15, 2003; column 6, lines 53-57; Claim 5	26

☐ Further documents are listed in the continuation of Box C.

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

Date of the actual completion of the international search

21 January 2014 (21.01.2014)

Date of mailing of the international search report

06 FEB 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774