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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2018 (25.01.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/017998 A1

(51) International Patent Classification:
C07K 14/20 (2006.01)

(21) International Application Number:
PCT/US2017/043366

(22) International Filing Date:
21 July 2017 (21.07.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/365,937 22 July 2016 (22.07.2016) US

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(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, ST, 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:
— *of inventorship (Rule 4.17(iv))*

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))*

(54) Title: RECOMBINANT BORRELIA PROTEINS AND METHODS OF USE THEREOF

(57) Abstract: Novel Borrelia burgdoferi recombinant proteins and methods of assessing a sample for the presence of antibodies to proteins of Borrelia burgdorferi are described, as are methods of diagnosing Lyme disease. Protein arrays comprising Borrelia burgdorferi proteins are also described.

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RECOMBINANT BORRELIA PROTEINS AND METHODS OF USE THEREOF

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 62/365,937, filed on July 22, 2016. The entire teachings of the above application are incorporated herein by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. CK000150 from Centers for Disease Control (CDC). The government has certain rights in the invention.

BACKGROUND

[0003] Lyme disease is the most common vector-borne disease in North America and Europe, and its range and incidence are increasing. Human Lyme disease is caused by several members of a group of closely related spirochetes belonging to the *Borrelia burgdorferi* sensu lato species complex. The spirochete is transmitted to humans via ticks of the genus *Ixodes* (Steere, A. C., N. Engl. J.Med. 1989; 321:586-96). It is a progressive multisystem disorder characterized by an initial cutaneous infection that can spread early in infection to secondary sites that include the nervous system, heart and joints (Masuzawa, T. *et al.,* Microbiol. Immunol. 1996; 40:539-45; Stanek, G., Infection 1991; 19:263-7). Accurate, reliable diagnostic assays for Lyme disease are critical to ensure successful treatment and recovery. Improved assays for accurate and reliable diagnosis of Lyme disease are needed to identify patients with Lyme disease.

SUMMARY

[0004] The present invention encompasses novel recombinant *Borrelia burgdorferi* proteins and their use in highly sensitive and specific methods of diagnosing Lyme disease in a subject. In one embodiment, the present invention is drawn to recombinant *Borrelia* fusion protein constructs comprising more than one (*e.g.,* two or three) single recombinant *Borrelia* protein antigens, such as those described herein. These constructs are also referred to herein as *Borrelia* chimeric proteins, or chimeric *Borrelia* proteins, or *Borrelia* chimeras, or recombinant *Borrelia burgdorferi* fusion protein constructs, or recombinant *Borrelia* fusion protein constructs, or

recombinant *Borrelia* fusion proteins, or recombinant *Borrelia* proteins, or fusion proteins, or chimeric proteins. Examples of recombinant *Borrelia* fusion protein constructs are described in Tables 2 and 3 herein. In other embodiments, the recombinant *Borrelia* fusion protein constructs of the present invention can have only one single recombinant *Borrelia* protein antigen described herein (*e.g.,* any one of the single recombinant *Borrelia* protein antigens listed in Table 1, Table 2 or Table 3).

[0005] The present invention is also drawn to methods of using one or more or all of these recombinant *Borrelia* fusion protein constructs in assays for assessing test samples for detecting the presence of antibodies to *Borrelia burgdorferi* in the test sample. For example, the *Borrelia* fusion protein constructs described herein (*e.g.,* Tables 2 and 3) can be used to prepare protein arrays (*e.g.,* microarrays, Q-Plex Arrays), for use in the detection methods, as well as in ELISA, other immunological assays, or any assay method capable of detecting antibody-antigen binding or antibody-antigen binding complexes. In addition to the use of the *Borrelia* fusion protein constructs of Tables 2 and 3 in these detection assays, one or more additional single recombinant *Borrelia* antigens can be included in such assays (*e.g.,* microarrays, Q-Plex Array), such as the single recombinant *Borrelia* antigens listed in Table 1 (see PCT Publication WO 2016/057562, the entire teachings of which are incorporated herein by reference). In one embodiment of the present invention, the microarray and/or Q-Plex Array comprises the 6 recombinant *Borrelia* fusion protein constructs selected from Table 2. In another embodiment, the microarray and/or Q-Plex Array comprises the 5 recombinant *Borrelia* fusion protein constructs selected from Table 3. In another embodiment, the microarray and/or Q-Plex Array comprises the 11 recombinant *Borrelia* fusion protein constructs of Tables 2 and 3 (e.g., SEQ ID NOS 6-16), and/or subsets thereof. In one embodiment of the present invention, the microarray and/or Q-Plex Array comprises 3 single recombinant *Borrelia* protein antigens described in Table 2 (e.g., Decorin binding protein A (dbpA), Decorin binding protein B (dbpB) and ABC transporter substrate binding protein (OppA)). In another embodiment of the present invention, the microarray and/or Q-Plex Array comprises recombinant *Borrelia* fusion protein constructs comprising the three single recombinant *Borrelia* protein antigens described in Table 2 (e.g., SEQ ID NOS 6-11). In one embodiment of the present invention, the microarray and/or Q-Plex Array comprises 8 single recombinant *Borrelia* protein antigens described in Table 3 (e.g., Outer surface protein A (OspA), purine-binding chemotaxis protein, VMP-like sequence protein VlsE(Vls), p83/100 antigen, surface lipoprotein P27, Outer surface protein C (OspC) typeB, Outer surface protein C (OspC) typeK and BapA protein). In another embodiment of the present invention, the microarray and/or Q-Plex Array comprises recombinant *Borrelia* fusion protein

constructs comprising the 8 single recombinant *Borrelia* protein antigens described in Table 3 (e.g., SEQ ID NOS 6-11).

[0006] Further encompassed by the present invention are methods of diagnosing Lyme disease in a subject (such as a mammal, including a human) by assessing a test sample obtained from the subject for antibodies reactive with one or more of the recombinant *Borrelia burgdorferi* fusion protein constructs selected from Tables 2 or 3, or, additionally one or more of the single recombinant *Borrelia* antigens listed in Table 1, wherein the detection of antibody-antigen reactions (such as antibody-antigen bound complexes, also referred to herein as antibody-antigen reaction products) is indicative of Lyme disease in the individual. Such assays to detect antibody-antigen complexes are well-known to those of skill in the art. In a particular embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise the recombinant *Borrelia* fusion protein constructs described herein as in Tables 2 and 3, and/or subsets thereof, and also one or more single recombinant *Borrelia* antigens described in Table 1. In one embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise the 6 recombinant *Borrelia* fusion protein constructs selected from Table 2. In another embodiment, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise 5 recombinant *Borrelia* fusion protein constructs selected from Table 3. In another embodiment, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise 11 recombinant *Borrelia* fusion protein constructs of Tables 2 and 3 (*e.g.,* SEQ ID NOS 6-16), and/or subsets thereof. In one embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise 3 single recombinant *Borrelia* protein antigens described in Table 2 (*e.g.,* Decorin binding protein A (dbpA), Decorin binding protein B (dbpB).and ABC transporter substrate binding protein (OppA)). In another embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise recombinant *Borrelia* fusion protein constructs comprising the 3 single recombinant *Borrelia* protein antigens described in Table 2 (*e.g.,* SEQ ID NOS 6-11). In one embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise 8 single recombinant *Borrelia* protein antigens described in Table 3 (e.g., Outer surface protein A (OspA), purine-binding chemotaxis protein, VMP-like sequence protein VlsE(Vls), p83/100

antigen, surface lipoprotein P27, Outer surface protein C (OspC) typeB, Outer surface protein C (OspC) typeK and BapA protein). In another embodiment of the present invention, microarrays and/or Q-Plex Arrays are used in the detection/diagnostic assays, wherein the microarrays and/or Q-Plex Arrays comprise recombinant *Borrelia* fusion protein constructs comprising the 8 single recombinant *Borrelia* protein antigens described in Table 3 (e.g., SEQ ID NOS 6-11).

[0007] As a result of this discovery, highly sensitive and specific methods and microarrays and /or Q-Plex Arrays are now available for the assessment of a test sample for the presence of antibodies to proteins of *Borrelia*. The presence of such antibodies is diagnostic for Lyme disease. The present invention provides methods to identify potential antigenic candidates for use in diagnosis of Lyme disease.

DETAILED DESCRIPTION OF THE INVENTION

[0008] A description of example embodiments of the invention follows. Accurate diagnosis and treatment of Lyme disease depends on correlating objective clinical abnormalities with serological evidence of exposure to *B. burgdorferi*. However, serodiagnosis of the disease is particularly difficult due to the high level of genetic heterogeneity of *B. burgdorferi* isolates, even among those collected from a single location. Furthermore, current serodiagnostic tests for Lyme disease lack sensitivity and specificity for detecting *B. burgdorferi* infection in the early stages of the disease. In one embodiment of the present invention, a highly antigenic *Borrelia* protein, an ABC transporter substrate-binding protein (OppA) (Signorino G *et.al.,)* that is recognized by approximately 50% of all sera samples analyzed including sera from patients with early Lyme disease, was used to create recombinant *Borrelia* fusion protein constructs. OppA, along with the library of 48 other highly antigenic single recombinant *Borrelia* protein antigens shown in Table 1, was used to develop a set of recombinant *Borrelia* fusion protein constructs as shown in Tables 2 and 3 and a highly sensitive and specific recombinant protein-based assay containing the 11 recombinant *Borrelia* fusion protein constructs selected from Table 2 and/or 3 and/or subsets thereof, as described herein.

[0009] It has been discovered that antibodies to certain cell envelope proteins, lipoproteins and extra-cellular proteins are present in sera of individuals with disseminated Lyme disease. A microarray containing multiple cell envelope proteins, lipoproteins, extracellular proteins and their corresponding homologs was prepared. The microarray was exposed to sera from individuals previously diagnosed with disseminated Lyme disease. Results indicated that the sera of individuals with Lyme disease reacted with single recombinant Borrelia protein antigens, shown in Table 1. In particular, high numbers of the sera from the individuals reacted with a

specific subset of those proteins. The sensitivity and specificity of the Lyme disease assay are described in Tables 2 and 3 of the PCT Publication WO 2016/057562, the entire teachings of which are incorporated herein by reference.

[0010] The multiplex ELISA array measures multiple proteins simultaneously within a single tissue sample. The Q-Plex™ technology using the Q-Plex Array (Quansys BioScience, Logan, UT) is a multiplex ELISA platform that can be used to develop a simple yet accurate tool to quantitate a panel of diagnostic proteins in biologic specimens that can be readily implemented into a clinical laboratory setting. Q-Plex Arrays containing proteins encoded by recombinant Borrelia fusion protein constructs (for, example, those described in Tables 2 and 3) were prepared. The assay using Q-Plex technology and the use of Q-Plex Array for improving Lyme disease diagnosis are described in Example 3 of this application. The Q-Plex Array was exposed to sera from individuals previously diagnosed with disseminated Lyme disease. Results indicated that the sera of individuals with Lyme disease reacted with the highly immunogenic recombinant *Borrelia* fusion protein constructs, shown in Tables 2 and 3. The overall sensitivity and specificity of the assay using the Q-Plex Array are also described in Example 3 of this application.

[0011] In some embodiments, for example, methods and the assays (*e.g.*, protein arrays including microarrays, Q-Plex Arrays) of the invention, one or more single recombinant *Borrelia* protein antigens are used. Examples of single recombinant *Borrelia* protein antigens, including but not limited to, cell envelope protein antigens, recombinant lipoprotein antigens, extracellular protein antigens, recombinant antigens described herein (for, example, those described in Tables 1, 2 and 3) can be used. In some embodiments, a set of two or more (*e.g.*, three) single recombinant *Borrelia* protein antigens are used. In some examples, sets include the set of single recombinant *Borrelia* protein antigens shown in Table 1, Table 2 and/or Table 3. In certain embodiments, a set of two or more (*e.g.*, three) single recombinant Borrelia protein antigens are used. Other example sets of single recombinant *Borrelia* protein antigens include the set of all known and putative cell envelope proteins and /or lipoproteins of *B. burgdorferi*. Such a set can further include homologs and paralogs of cell envelope lipoproteins and extra-cellular proteins. Other sets include sets of two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or other groups of cell envelope proteins (*e.g.*, selected from those set forth in Table 2 and/or 3 and additional proteins in Table 1). In one particular embodiment, the set consists essentially of the single recombinant *Borrelia* protein antigens set forth in Table 1. In another embodiment, the set consists essentially of the single recombinant *Borrelia* protein antigens set forth in Table 2 (for, example, those described under column “gene locus” in Table

2). In another embodiment, the set consists essentially of the single recombinant *Borrelia* protein antigens set forth in Table 3 (for, example, those described under column "gene locus" in Table 3).

[0012] In some embodiments, for example, methods and assays (*e.g.,* protein arrays including microarrays, Q-Plex Arrays) of the invention, one or more recombinant *Borrelia* fusion protein constructs were used. Examples of recombinant *Borrelia* fusion protein constructs, including but not limited to, SEQ ID NOS: 6-16 described herein (*e.g.,* in Tables 2 and 3) can be used. Representative sets include the recombinant *Borrelia* fusion protein constructs shown in Table 2, and/or Table 3, and subsets thereof. In certain embodiments, only one recombinant *Borrelia* fusion protein construct was used. In other embodiments, two or more (*e.g.,* three) recombinant *Borrelia* fusion protein constructs are used. Other example sets of recombinant *Borrelia* fusion protein constructs include the set of all possible combinations of recombinant *Borrelia* fusion protein constructs shown in Table 2, and/or Table 3, and subsets thereof. Such a set can further include recombinant *Borrelia* fusion protein constructs further comprising one or more single recombinant *Borrelia* protein antigens shown in Table 1. Other sets include sets of two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or other groups of recombinant *Borrelia* fusion protein constructs (*e.g.,* selected from those set forth in Table 2 and/or 3 and optionally can include one or more single recombinant *Borrelia* protein antigens shown in Table 1). In another embodiment, the set consists essentially of the recombinant *Borrelia* fusion protein constructs set forth in Table 2. In another embodiment, the set consists essentially of the recombinant *Borrelia* fusion protein constructs set forth in Table 3.

[0013] In further methods and assays (*e.g.,* protein arrays including microarrays, Q-Plex Arrays) of the invention, one or more single recombinant *Borrelia* protein antigens and recombinant *Borrelia* fusion protein constructs as identified herein are used, and/or combinations of single recombinant *Borrelia* protein antigens and recombinant *Borrelia* fusion protein constructs are used (*e.g.,* combinations of proteins selected from any of Tables 1, 2 and/or 3). Such combinations include sets of two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, or other groups of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs (*e.g.,* with one or more selected from Table 1, Table 2 and/or Table 3).

[0014] In one embodiment of the invention, a test sample from an individual (*e.g.,* a human) is assessed for the presence of antibodies to one or more to one or more single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs (*e.g.,* selected

from Table 1, Table 2 and/or Table 3) of *B. burgdorferi*. The "test sample" can be a sample of blood, serum, cerebrospinal fluid, or other appropriate biological fluid from the individual. The test sample can be assessed for the presence of antibodies to one or more single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs using routine methods established in the art. In one r embodiment, the assessment is performed using a microarray of single recombinant *Borrelia* protein antigens. In another embodiment, the assessment is performed using a microarray of recombinant *Borrelia* fusion protein constructs. In certain examples, a microarray as described herein, or a protein or set of proteins as described herein, is exposed to the test sample from the individual, and any resultant binding of antibodies (if present in the test sample) to the single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs was determined. In one embodiment, the assessment is performed using an ELISA array, such as a multiples ELISA array (*e.g.*, Q-Plex Array) of single recombinant *Borrelia* protein antigens. In another embodiment, the assessment is performed using a Q-Plex Array of recombinant *Borrelia* fusion protein constructs. In certain examples, a Q-Plex Array as described herein, or a protein or set of proteins as described herein, is exposed to the test sample from the individual or multiple samples from the same and/or multiple individuals, and any resultant binding of antibodies (if present in the test sample (s)) to the single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs is determined. The presence of antibodies bound to (also referred to herein as "reactive with") one or more single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs is indicative of antibodies specific to those proteins of *B. burgdorferi*. The presence of such antibodies is diagnostic for Lyme disease in the individual from whom the test sample was obtained.

[0015] In some embodiments, the assay methods described herein use protein arrays of *B. burgdorferi* antigens. In one embodiment, the protein array comprises a subset of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs of *B. burgdorferi*, such as the set of proteins set forth in Tables 1, 2 or 3. In other embodiments, other protein arrays include various subsets of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs of *B. burgdorferi*, such as sets of two or more, four or more, six or more, eight or more, or other groups of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs as set forth in Tables 1, 2 or 3. In one embodiment, the microarray consists essentially of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 2, or consists of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 2. In another embodiment, the microarray consists

essentially of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 3, or consists of all of the recombinant *Borrelia* fusion protein constructs of Table 3. In further embodiments, other microarrays include various subsets of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs of *B. burgdorferi*, such as subsets of the proteins set forth in Table 1, 2 and 3 (e.g., sets of two or more, four or more, six or more, eight or more, or other combinations of proteins). In one embodiment, the Q-Plex Array consists essentially of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 2, or consists of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 2. In another embodiment, the Q-Plex Array consists essentially of all of the recombinant *Borrelia* fusion protein constructs set forth in Table 3, or consists of all of the recombinant *Borrelia* fusion protein constructs of Table 3. In further embodiments, other Q-Plex Arrays include various subsets of single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs of *B. burgdorferi*, such as subsets of the proteins set forth in Table 1, 2 and 3 (e.g., sets of two or more, four or more, six or more, eight or more, or other combinations of proteins).

[0016] Other embodiments of the present invention encompass methods for the evaluation/assessment of other or additional proteins of *B. burgdorferi* as potential candidates for the development of a diagnostic test for Lyme disease. In some embodiments, one or more proteins, for example, one or more recombinant *Borrelia* fusion protein constructs of *B. burgdorferi,* such as described herein in Table 2 or Table 3 (*e.g.,* in a microarray and /or Q-Plex Array as described above), are exposed to sera from one or more individuals known to have Lyme disease, and the proteins to which antibodies from the sera bind are then determined. For example, Cy5 intensity/Cy3 intensity ratio of fluorescence, as described in the Examples described herein, can be used. In one embodiment, the ratio of any proteins greater than the mean ratio of the reactivity of the Lyme sera to a negative control plus three times the standard deviation indicates significant interactions between antibodies present in the Lyme sera and the proteins (*e.g.,* single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs). The proteins identified by such methods (*e.g.,* single recombinant *Borrelia* protein antigens and/or recombinant *Borrelia* fusion protein constructs which have or are identified to have significant interactions with the antibodies present in the Lyme sera) are useful in diagnostic tests for Lyme disease (*e.g.,* in the methods described herein), as well as in protein arrays (for, *e.g.,* microarrays, Q-Plex Arrays) as described herein.

[0017] The present invention is also drawn to diagnostic kits which comprise the proteins described herein (*e.g.,* in a microarray and /or in a Q-Plex Array as described herein). The kit

optionally can include reagents for detecting antibody-antigen complexes that are formed between the protein and antibodies that are present in a sample, *e.g.,* a user-supplied host sample.

[0018] The specific embodiments of the present invention are described in the following examples, which are not to be interpreted as limiting in any way.

EXEMPLIFICATION

EXAMPLE 1: Discovery of 48 highly antigenic single recombinant *Borrelia* protein antigens.

[0019] **Protein arrays.** A total of 416 genes were identified in a *B. burgdorferi* genome comparison. Of these, 350 (84%) produced a product that was the correct size when PCR was performed, and all 350 were successfully cloned into the T7 expression vector pET28b. Sequence-confirmed plasmids were expressed using an overnight autoexpression system; expressed proteins were purified using IDA resin and printed onto nitrocellulose-coated FAST slides (see, for example, US Serial No. 12/784,584 corresponding to published US Application No. US 20100292096 A1and US Serial No. 12/989,003 corresponding to published US Application No. US20110105355 A1, the teachings of which are incorporated herein). In addition, representatives of several different Outer surface protein (OspC) types were amplified from human isolates. The OspC types included in this study were types A, B, C, D, E, F, G, H, I, J, K and U. This study also included the highly antigenic B31 cell envelope proteins that were identified in an earlier protein array study (Xu Y. et al., 2008). In total, the arrays contained approximately 400 samples including expressed *Borrelia* proteins, negative controls and blanks. Arrays were probed with sera from Lyme disease patients, and antibodies were visualized with Cy5-conjugated goat antihuman lgG or Cy5-conjugated goat anti-human lgM to determine antibody isotype (Xu Y et al., 2008).

[0020] **Microarray**. For protein array (*e.g.,* microarray), each of the individual 48 single recombinant *Borrelia* protein antigens (*e.g.,* in Table 1) were printed onto nitrocellulose-coated FAST glass slides. In addition, recombinant proteins OspB-OspC-Flagellin (B-C-Fia), OspA-p39-p93 (A-39-93) and an OspC dimer comprised of OspC Type B and Type H (OC2/9) from an early study were also printed (Gomes Solecki et al., 2000). Each slide in the arrays also contained 10 immobilized bovine serum albumin (BSA) spots for background determination. Proteome chips were probed with serum from patients with untreated early Lyme disease and sick non-Lyme patients using the Fast Pak protein array kit. Briefly, slides were first blocked overnight at 4°C in protein array-blocking buffer before incubation in primary antibody (human sera and mouse anti His-Tag for quantitation) for 2 h. Antibodies were visualized with Cy5-

conjugated goat anti-human lgG or Cy5-conjugated goat anti-human lgM (to detect bound human antibodies) and Cy3-conjugated goat anti mouse lgG (to quantify the amount of recombinant protein in each spot). After a 2 hr incubation, the slides were stringently washed and then scanned with an Axon GenePix 4200A microarray scanner. The raw data was captured and analyzed with Gene Pix Pro image analysis software. To minimize the variability among samples, the PMT gain was adjusted to equal 1.0 in all the arrays with power setting at 50%. A global background subtraction method was used to subtract the background from each spot using the average mean intensity value of BSA from each slide (Xu et al., 2008).

[0021] **Serum samples.** For earlier array studies, sera of patients with Lyme disease were obtained from the Centers for Disease Control (CDC). The CDC samples included sera from 31 patients collected upon initial presentation (Samples C =0 days) and at 10 days (Samples D), 20 days (Samples E), 30 days (Samples F), 60 days (Samples G), and 90 days (Samples H) post presentation. Fourteen late Lyme samples (Late) from patients who exhibited late clinical manifestations (Lyme arthritis or neuroborreliosis) obtained from the Lyme Disease Center at Stony Brook University were also analyzed. A total of 115 samples were analyzed that included 21 Sample C, 29 Sample D, 15 Sample E, 8 Sample F, 1 Sample G and 12 Sample H. Normal control sera (n=14) were obtained from healthy donors. The overall sensitivity and specificity of the Lyme disease assay are described in Tables 2 and 3 of the PCT Publication WO 2016/057562, the entire teachings of which are incorporated herein by reference).

[0022] **Results.** Approximately 140 of the arrayed proteins detectably elicited an antibody response in humans with natural infections using both secondary antibodies (IgG and IgM). Of these 140 antigens, 80 proteins were recognized by at least half of the sera samples from each time point. Importantly, the majority of sera samples recognized at least one antigen from a subset of 48 highly antigenic single recombinant *Borrelia* protein antigens using lgM and lgG secondary antibodies (Table 1). This high positivity included all sera that were collected when the patients were first seen for their erythema migrans. The Cy5/Cy3 ratios of these 48 single recombinant *Borrelia* protein antigens were among the highest observed in the arrays, averaging greater than five times the standard deviation above the mean ratio of the reactivity of the Lyme sera to the negative control. In addition, a BLASTx search with highly immunogenic *Borrelia* proteins against the NCBI non-redundant protein database excluding all recorded *Borrelia* sequences revealed no significant matches to other organisms. Interestingly, 40 of the 48 single recombinant *Borrelia* protein antigens are plasmid encoded and the majority of encoded proteins are outer surface proteins, lipoproteins or putative outer membrane proteins. Over 90% of plasmid gene sequences have no significant homologs to non-*B. burgdorferi* genes. The

uniqueness of plasmid sequences suggests that they serve specialized adaptive roles for *Borrelia's* survival and propagation in nature and in a wide variety of hosts. Furthermore, the high numbers of outer surface lipoproteins encoded by the plasmids in conjunction with the variability of plasmid sequences supports the view that these sequences are directly involved in parasite-host interactions, and, therefore, are more than likely to be key immunogens (Casjens et al., 2000).

Table 1. 48 highly antigenic single recombinant *Borrelia* protein antigens.

Locus	Protein name		Locus	Protein name
BB_0024	hypothetical protein		BB_I38	hypothetical protein
BB_0067a	hypothetical protein		BB_J01	hypothetical protein
BB_0312	chemotaxis protein CheW		BB_J23	hypothetical protein
BB_0337	enolase		BB_K07	hypothetical protein
BB_0364	methylglyoxal synthase		BB_N26	hypothetical protein
BB_0546	hypothetical protein		BB_R22	hypothetical protein
BB_0744	antigen, p83/100		BB_S42	BapA protein
BB_0858	hypothetical protein		BbuJD1_F28	VLS-like protein
BB_A14	hypothetical protein		BbuJD1_F31	VLS-like protein
BB_A16	outer surface protein B (OspB)		BbuJD1_S13	hypothetical protein
BB_A60	surface lipoprotein P27		BbuN40_Q42	Erp26 protein
BB_A65	lipoprotein		BbuN40_V37	Erp25 protein
BB_A66	outer surface protein		BbuN40_X36	Erp27 protein
BB_A73	putative antigen P35		Bbu297_B19	outer surface protein C (OspC)
BB_A74	outer membrane porin OMS28		Bbu297_F32	VLS-like protein
BB_A70	hypothetical protein		Bbu297_F33	VLS-like protein
BB_B07	alpha3-beta1 integrin-binding protein		Bbu297_J03	hypothetical protein
BB_C08	hypothetical protein		Bbu297_J03a	hypothetical protein
BB_E09	protein p23		Bbu297_J05	hypothetical protein
BB_E19	hypothetical protein		Bbu297_M38	bbK2.10 protein precursor
BB_G01	hypothetical protein		Bbu297_P39	Erp42 protein
BB_H18	hypothetical protein		Bbu297_R41	MlpL (multicopy

				lipoproteins)
BB_H37	hypothetical protein		Bbu297_W44	hypothetical protein
BB_I16	repetitive antigen A		Bbu297_Y03	hypothetical protein

[0023] The overall sensitivity and specificity of the Lyme disease assay are described in Tables 2 and 3 of the PCT Publication WO 2016/057562, the entire teachings of which are incorporated herein by reference). The sensitivity of the assay described in the above-referenced PCT Publication WO 2016/057562 far exceeds that of commercially available Lyme disease assays in patients in the early stages of the disease.

EXAMPLE 2: Recombinant *Borrelia* fusion protein constructs.

[0024] **Overview**. A library of recombinant *Borrelia* fusion protein constructs using the 48 highly antigenic single recombinant *Borrelia* protein antigens shown in Table 1 was designed for use in developing a recombinant chimera-based diagnostic assay for Lyme disease. Additionally, a representative lipoprotein, OppA (ABC transporter substrate-binding protein), was amplified from human isolates and included in this study. Construction of recombinant Borrelia fusion protein constructs containing genes from several genospecies allows one to generate one protein that confers antigenicity to multiple strains, revealing the great potential and adaptability of this technique. In addition, the use of pure protein preparations as antigens offers greater flexibility in adapting the test to different assay formats. It is reasonable to believe that an assay of recombinant *Borrelia* fusion protein constructs will present superior sensitivity in detecting antibodies against *B. burgdorferi* for the early stages of the disease, and equivalent sensitivity for the late stages of the disease, when compared to the currently available whole-cell assays.

[0025] **Recombinant *Borrelia* fusion protein constructs**. From the library of 48 highly antigenic single recombinant *Borrelia* protein antigens and a representative lipoprotein, OppA, 11 sets of recombinant *Borrelia* fusion protein constructs each containing at least two of the *Borrelia* antigens were constructed (Tables 2 and 3). Chimeras of at least two (*e.g.*, three) single recombinant *Borrelia* protein antigens in most constructs were chosen to keep the molecular mass of the fusion protein at or less than (below) about 100 kDa to optimize protein yield, which can be significantly affected by protein size.

[0026] **Table 2. Structural Characterization of recombinant *Borrelia* fusion protein constructs.**

Item	Protein ID	gene locus	gene name	SEQ ID NOS.
	BB_A24-His	BB_A24	Decorin binding protein A (dbpA)	3

	BB_A25-His	BB_A25	Decorin binding protein B (dbpB)	4
	BB_0329-His	BB_0329	ABC transporter substrate-binding protein (OppA)	5
1	Tri-435-His	BB_A24	Decorin binding protein A (dbpA)	6
		BB_0329	ABC transporter substrate-binding protein (OppA)	
		BB_A25	Decorin binding protein B (dbpB)	
2	Tri-453-His	BB_A24	Decorin binding protein A (dbpA)	7
		BB_A25	Decorin binding protein B (dbpB)	
		BB_0329	ABC transporter substrate-binding protein (OppA)	
3	Tri-543-His	BB_A25	Decorin binding protein B (dbpB)	8
		BB_A24	Decorin binding protein A (dbpA)	
		BB_0329	ABC transporter substrate-binding protein (OppA)	
4	Tri-534-His	BB_A25	Decorin binding protein B (dbpB)	9
		BB_0329	ABC transporter substrate-binding protein (OppA)	
		BB_A24	Decorin binding protein A (dbpA)	
5	Tri-345-His	BB_0329	ABC transporter substrate-binding protein (OppA)	10
		BB_A24	Decorin binding protein A (dbpA)	
		BB_A25	Decorin binding protein B (dbpB)	
6	Tri-354_His	BB_0329	ABC transporter substrate-binding protein (OppA)	11
		BB_A25	Decorin binding protein B (dbpB)	
		BB_A24	Decorin binding protein A (dbpA)	

[0027] Table 3. Structural Characterization of recombinant *Borrelia* fusion protein constructs.

Item	Protein ID	gene locus	gene name	SEQ ID NOS.
1	**A-312-93**	BB_B15	outer surface protein A (OspA)	12
		BB_0312	purine-binding chemotaxis protein	

		BB_0744	p83/100 antigen	
2	**V-93**	Bbu297_F33	VMP-like sequence protein VlsE (Vls) ,C-terminal	13
		BB_0744	p83/100 antigen	
3	**A-V-93**	BB_B15	outer surface protein A (OspA)	14
		Bbu297_F33	VMP-like sequence protein VlsE (Vls)	
		BB_0744	p83/100 antigen	
4	**A60-OC2/9-S42**	BB_A60	surface lipoprotein P27	15
		BB_B19 type B	outer surface protein C (OspC) type B	
		BB_B19 type K	outer surface protein C (OspC) type K	
		BB_S42	BapA protein	
5	**S42-OC2/9-A60**	BB_S42	BapA protein	16
		BB_B19 type B	outer surface protein C (OspC) type B	
		BB_B19 type K	outer surface protein C (OspC) type K	
		BB_A60	surface lipoprotein P27	

[0028] Cloning of recombinant fusion proteins. *Borrelia* genes encoding the components (*e.g.,* single recombinant *Borrelia* protein antigens) of the 11 recombinant *Borrelia* fusion protein constructs were amplified using a unique gene-specific primer pair that was specifically designed for chimeric protein construction. The primers were designed from the genomic sequence of *B. burgdorferi* strains and each contains a novel DNA cassette coding for the recognition sequence for three restriction endonucleases that are in-frame with the gene coding sequence. The 5' primer (5'-AATTGGTACCCCA**GGATCC**CATATG + 15mer ORF specific sequence) (SEQ ID NO:1) contains Kpnl (underlined), BamHI (bold) and Ndel (italics) recognition sequence. The 3' primer (5'GCG**GGATCC**GGTACCGTCGAC +15mer ORF specific sequence) (SEQ ID NO:2) contains BamHI, Kpnl and Sall (dashed underline) recognition sequences. **GGATCC** is bolded in the 5' and 3' primers described above. For amplification, ten ng of genomic DNA was used as a template in a 50-µI PCR reaction containing two ORF-specific primer pairs (SEQ ID NOS: 1&2). To increase the solubility properties of expressed cell envelope proteins, the primer sets were designed to amplify coding regions without a membrane anchoring signal sequence (Dunn et al., 1990). PCR amplification was performed under stringent conditions with Platinum Taq DNA polymerase High Fidelity (Invitrogen) using conditions previously described (Xu et al., 2003). The PCR products were

visualized using agarose gel electrophoresis. For quantification, the products were purified (PCR purification kit, Qiagen) and quantified by UV absorbance.

[0029] To create the library of recombinant *Borrelia* fusion protein constructs comprised of two gene fragments, amplified products were cleaved with NdeI/BamHI (for 1st position fragment), or BamHI/KpnI (for 2nd position fragment) or KpnI/SalI (for 3rd position fragment). To create the library of recombinant *Borrelia* fusion protein constructs comprised of three gene fragments, amplified products were cleaved with NdeI/BamHI (for 1'1 position fragment), or BamHI/KpnI (for 2nd position fragment) or KpnI/SalI (for 3rd position fragment). The 1st position fragments were directionally cloned into the unique NdeI and BamHI sites of the T7-based expression vector pET-28 (Novagen). This vector provides an N-terminal poly (His) affinity tag fused to the expressed proteins to aid in purification on nickel-Sepharose columns. Ligation reactions were transformed into *E. coli* GC5 competent cells and plasmids were purified using Eppendorf Perfectprep Plasmid 96 VAC Direct Bind Kit and verified by sequencing across the inserts. Plasmids containing fragment 1 now served as vectors for all subsequent cloning. For directional cloning of fragments two and three, vectors were digested with BamHI/XhoII, and restriction digested amplicons of fragment two and three were ligated simultaneously into the digested vector. Following transformation, plasmids were purified and sequenced.

[0030] **Protein expression and purification**. Purified plasmids were transformed into *E. coli* BL21/DE3 competent cells for expression. Recombinant *Borrelia* fusion protein constructs containing an N-terminal poly (His) affinity tag were expressed using the Overnight Express Autoinduction protocol. Induced cells were harvested by centrifugation and resuspended in 8M urea. Aliquots were run on SDS-PAGE. N-terminal poly His-tagged proteins were purified on nickel-Sepharose columns under denaturing conditions using Ni-NTA Spin Kit (Qiagen). The kit is designed for rapid screening and purification of His Tag fusion proteins. Protein concentration was determined by the measurement of the absorbance shift when Coomassie brilliant blue G-250 reacted with protein (Bio-Rad). Protein purity was checked by SDS-PAGE.

EXAMPLE 3: Protein arrays using recombinant *Borrelia* fusion protein constructs and Diagnostic Test for Lyme Disease.

[0031] Overview. Using genomic and proteomic approaches, protein arrays (*e.g.*, microarrays and /or Q-Plex Arrays) were fabricated based on multiple genotypes of *Borrelia burgdorferi sensu stricto* to identify antigens that may be useful in the development of a highly sensitive and specific single-tier assay that will have a wide range of specificity in detecting Lyme disease. The assay containing 11 recombinant *Borrelia* fusion protein constructs described above (*e.g.*, SEQ ID NOS: 6-16 in Tables 2 and 3) is very accurate and sensitive.

[0032] **Serum samples**. The antigenicity of the recombinant *Borrelia* fusion protein constructs was tested using CDC sera samples, as described above in Example 1. For Quansys Array studies, sera from individuals representing all stages of Lyme disease including 41 samples with early localized Lyme disease, 40 with early disseminated infection, and 16 late Lyme disease samples, were used. Late Lyme samples included sera from Lyme arthritis, neurologic Lyme and cardiac Lyme patients. In addition, for specificity studies, 242 sera were obtained from Bioreclamation LLC, Westbury, NY and the CDC from patients with diseases associated with serological responses that are known to produce cross-reactivity in Lyme disease test including syphilis, fibromyalgia, rheumatoid arthritis, mononucleosis, multiple sclerosis, fibromyalgia, psoriasis, osteoarthritis, and EBV. One hundred twenty eight normal control sera from the same sources were obtained from healthy donors from an area of endemicity and healthy donors from non-endemic areas. All of the Lyme sera and a number of the control sera were used in previous studies described in Example 1 and the array studies (*e.g.*, Q-Plex Arrays) described below. All serological assays involving human serum were performed on coded specimens with all investigators blinded to clinical information pertaining to individual samples. No patient identifying information was available to the investigators or technician testing the samples.

[0033] **Q-Plex technology**. The multiplex assay using Q-Plex technology involves the micro-spotting of individual groups of capture proteins on the bottom of a 96-well plate with each spot being its own 'micro ELISA' assay. Standard ELISA incubation steps including sample incubation, washing, and secondary antibody incubation were employed. The labeling and reporting system used in the Q-Plex Array was chemiluminescent. The binding of secondary antibody was measured via the chemiluminescence produced by streptavidin horseradish peroxidase in the presence of a luminol-based substrate. Chemiluminescent ELISAs have been shown to be more sensitive than colorimetric detection systems. The intensity of chemiluminescence from each array was measured using the Quansys Q-View chemiluminescent imager. Data were then analyzed using Quansys Q-View software.

[0034] **Q-Plex Arrays**. Protein arrays (*e.g.*, Q-Plex Arrays) comprising the 11 highly antigenic recombinant *Borrelia* proteins described in Table 2 and 3, were constructed. The sensitivity of Q-Plex Array was tested by screening arrays with sera from patients with early and late Lyme disease using buffers and methods provided by Quansys Biosciences. In addition, specificity was determined using sera from patients with diseases associated with serological responses that are known to produce cross-reactivity in Lyme disease tests. The results of Q-

Plex arrays were compared with the results obtained with the standard two-tier assay for the same set of 11 recombinant *Borrelia* fusion protein constructs.

[0035] **Results**. The sensitivity of the Q-Plex Arrays was determined using sera from 97 Lyme disease patients. Of 41 acute-phase sera from patients presenting with erythema migrans, 83% (34/41) were positive with the Q-Plex Arrays while 37% (15/41) were positive by the two-tier test. Testing 40 serum samples from EM patients, convalescent at 3 weeks post-presentation, 90% (36/40) were positive by the Q-Plex Arrays while 78% (31/40) were positive by the two-tier assay. Assaying 16 late Lyme serum samples, 100 % (16/16) were positive by the Q-Plex Arrays while 94% (15/16) were positive by two-tier testing. Overall, the sensitivity of the Q-Plex Arrays was 89% (86/97) compared to 63% (60/97) for the two-tier test.

[0036] The specificity of the Q-Plex Arrays was tested using sera from individuals with diseases associated with serological responses that are known to produce cross-reactivity in currently used tests and sera from healthy individuals from both endemic and non-endemic regions. Testing 242 sera from individuals with 11 other disease conditions (sick non-Lyme) resulted in a specificity of 97% with both the Q-Plex Arrays and with two-tier testing. Specificity was 100% for both the Q-Plex Arrays and the two-tier assay in a population of healthy blood donors from regions of the USA nonendemic for Lyme disease and donors from regions endemic for Lyme disease. Overall, the specificity of the Q-Plex Arrays was 99% (5/370) compared to 99% (5/370) for the two-tier assay.

[0037] Results described above demonstrate that Q-Plex Arrays offer superior sensitivity and specificity in detecting antibodies against *B. burgdorferi* for not only early stages of the disease, but show equivalent or better sensitivity for the late stages of the disease as well. The recombinant *Borrelia* fusion protein constructs described herein (*e.g.,* SEQ ID NOS: 6-16 in Tables 2 and 3) represent strong candidates for the development of diagnostic tests. These variants, as well as variants of the other recombinant *Borrelia* fusion protein constructs described herein, can be used to develop a standardized sensitive and specific single-tier Lyme disease assay of recombinant *Borrelia* fusion protein constructs that will have a wide range of coverage and specificity against Lyme disease. Furthermore, these assays will have significant potential for the development of a next-generation rapid, single-tier point of care assay.

[0038] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety including the references below.

Sequences

BB_A24-His: SEQ ID NO:3
MGNKTFNNLLKLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKT
GSGVSENPFILEAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQ
EMTKTVSDAAEENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVD
KLAAALEHHHHHH

BB_A25-His: SEQ ID NO:4
MGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSGGLALREAKVQA
IVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVLEESKNNPINTA
ERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVDKLAAALEHHHHHH

BB_0329-His: SEQ ID NO:5
MGNKERKEGVSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLAKG
WDISSDGTVYTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNGQKYFD
GQVTDSELGIRAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPENMVTSG
PFKLKERIPNEKYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIPPDLIKN
LKLRSDYYSSAVNAIYFYAFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRATPNFSS
YSYAKSLELFNPEIAKTLLAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNLNIDVEL
ENEEWTTYLNTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSD
LELDPIKRQDILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVD
KLAAALEHHHHHH

Tri-435-His: SEQ ID NO:6
MGNKTFNNLLKLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKT
GSGVSENPFILEAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQ
EMTKTVSDAAEENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVD
GTGSHMGNKERKEGVSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKP
GLAKGWDISSDGTVYTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNG
QKYFDGQVTDSELGIRAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPEN
MVTSGPFKLKERIPNEKYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIP
PDLIKNLKLRSDYYSSAVNAIYFYAFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRA
TPNFSSYSYAKSLELFNPEIAKTLLAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNL
NIDVELENEEWTTYLNTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYN
ELIKKSDLELDPIKRQDILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLK
LKNKVDGTPGSHMGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTS
GGLALREAKVQAIVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKAR
VLEESKNNPINTAERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVEHHHHHH

Tri-453-His: SEQ ID NO:7

MGNKTFNNLLKLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKT
GSGVSENPFILEAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQ
EMTKTVSDAAEENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVD
GTGSHMGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSGGLALRE
AKVQAIVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVLEESKN
NPINTAERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVDGTPGSHMGNKERKEG
VSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLAKGWDISSDGTV
YTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNGQKYFDGQVTDSELGI
RAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPENMVTSGPFKLKERIPNE
KYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIPPDLIKNLKLRSDYYSS
AVNAIYFYAFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRATPNFSSYSYAKSLELF
NPEIAKTLLAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNLNIDVELENEEWTTYL
NTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSDLELDPIKRQD
ILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVDVEHHHHHH

Tri-543-His: SEQ ID NO:8

MGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSGGLALREAKVQA
IVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVLEESKNNPINTA
ERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVDGTGSHMGNKTFNNLLKLTILVN
LLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKTGSGVSENPFILEAKVRA
TTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQEMTKTVSDAAEENPPT
TAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVDGTPGSHMGNKERKEG
VSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLAKGWDISSDGTV
YTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNGQKYFDGQVTDSELGI
RAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPENMVTSGPFKLKERIPNE
KYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIPPDLIKNLKLRSDYYSS
AVNAIYFYAFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRATPNFSSYSYAKSLELF
NPEIAKTLLAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNLNIDVELENEEWTTYL
NTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSDLELDPIKRQD
ILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVEHHHHHH

Tri-534-His: SEQ ID NO:9

MGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSGGLALREAKVQA
IVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVLEESKNNPINTA
ERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVDGTGSHMGNKERKEGVSFKISLG
AEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLAKGWDISSDGTVYTFNLREK
ITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNGQKYFDGQVTDSELGIRAIDEKTL
EITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPENMVTSGPFKLKERIPNEKYVFEKN
NKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIPPDLIKNLKLRSDYYSSAVNAIYFY

AFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRATPNFSSYSYAKSLELFNPEIAKTL
LAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNLNIDVELENEEWTTYLNTKANGN
YEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSDLELDPIKRQDILRQAEEII
IEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVDGTPGSHMGNKTFNNLL
KLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKTGSGVSENPFIL
EAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQEMTKTVSDAA
EENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVEHHHHHH

Tri-345-His: SEQ ID NO:10
MGNKERKEGVSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLAKG
WDISSDGTVYTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIKNGQKYFD
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ENEEWTTYLNTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSD
LELDPIKRQDILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVD
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KDKKTGSGVSENPFILEAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQ
KLGIQEMTKTASDAAEENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCK
NNVDGTPGSHMGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSG
GLALREAKVQAIVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVL
EESKNNPINTAERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVEHHHHHH

Tri-354-His: SEQ ID NO:11
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GQVTDSELGIRAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQNWTSPENMVTSG
PFKLKERIPNEKYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYKMYENEELDAIFGSIPPDLIKN
LKLRSDYYSSAVNAIYFYAFNTHIKPLDNVKIRKALTLAIDRETLTYKVLDNGTTPTRRATPNFSS
YSYAKSLELFNPEIAKTLLAEAGYPNGNGFPILKLKYNTNEANKKICEFIQNQWKKNLNIDVEL
ENEEWTTYLNTKANGNYEIARAGWIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSD
LELDPIKRQDILRQAEEIIIEKDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNKVD
GTGSHMGIGLVERTNAALESSSKDLKNKILKIKKEATGKGVLFEAFTGLKTGSKVTSGGLALRE
AKVQAIVETGKFLKIIEEEALKLKETGNSGQFLAMFDLMLEVVESLEDVGIIGLKARVLEESKN
NPINTAERLLAAKAQIENQLKVVKEKQNIENGGEKKNNKSKKKKVDGTPGSHMGNKTFNNL
LKLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKTGSGVSENPFI
LEAKVRATTVAEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQEMTKTVSDA
AEENPPTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNNVEHHHHHH

A-312-93: SEQ ID NO:12

MAKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDKNNGSGVL
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GTRLEYTGIKSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTD
SSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIK
NALKGHPMARKESSKDSRSQLQVAGFKIGKESYGVSIEHIREIIKVPSEGVYAIPNVPEYIIGIYN
LRGSIIPLINLNIKFGVPSISVTEEDMLLTGYLIVKIKNKLLGIFVDRVLKVISFDDSRVQEPPATL
QTLDRKYISGVVKLDEADNLESEYLVLIDIAKIFDKCEFDDIPYKDQYEEAMDEKLLKSKDDKAS
KDGKALDLDRGLNSKASSKEKSKAKEEEITKGKSQKSLGDLNNDENLMMPEDQKLPEVKKLD
SKEEFKPVSEVEKLDKIFKSNNNVGELSPLDKSSYKDIDSKEETVNKDVNLQKTKPQVKDQVT
SLNEDLTTMSIDSSSPVFLEVIDPITNLGTLQLIDLNTGVRLKESTQQGIQRYGIHEREKDLVVIK
MDSGKAKLQILDKLENLKVVSESNFEINKNSSLYVDSKMILVAVRDKDSSNDWRLAKFSPKN
LDEFILSENKIMPFTSFSVRKNFIYLQDEFKSLVILDVNTLKKVKGHHHHHHHNM

V-93: SEQ ID NO:13

MKKNDQIAAAIVLRGMAKDGEFALKNDANNAEKGLKSTVESAVNKTVVDGQKKWAMDE
KLLKSKDDKASKDGKALDLDRGLNSKASSKEKSKAKEEEITKGKSQKSLGDLNNDENLMMPE
DQKLPEVKKLDSKEEFKPVSEVEKLDKIFKSNNNVGELSPLDKSSYKDIDSKEETVNKDVNLQK
TKPQVKDQVTSLNEDLTTMSIDSSSPVFLEVIDPITNLGTLQLIDLNTGVRLKESTQQGIQRYGI
HEREKDLVVIKMDSGKAKLQILDKLENLKVVSESNFEINKNSSLYVDSKMILVAVRDKDSSND
WRLAKFSPKNLDEFILSENKIMPFTSFSVRKNFIYLQDEFKSLVILDVNTLKKVKGHHHHHHHN
M

A-V-93: SEQ ID NO:14

MAKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDKNNGSGVL
EGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFNEKGEVSEKIITRAD
GTRLEYTGIKSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTD
SSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIK
NALKGHPMAGKVVKVNAAGAAAKGGEETSVNGIASGIKGIVTAAEKAGEEGKLKSEAAGD
GEANEDAGKLFAKKNDAGGDAKDAEKAAAAVSAVSGKQILKAIVDAAGKEEKGVVADVKD
AKNPIEAAIGSTGEQNAAAFSHMKKNDQIAAAIVLRGMAKDGEFALKNDANNAEKGLKSTV
ESAVNKTVVDGQKKWAMDEKLLKSKDDKASKDGKALDLDRGLNSKASSKEKSKAKEEEITK
GKSQKSLGDLNNDENLMMPEDQKLPEVKKLDSKEEFKPVSEVEKLDKIFKSNNNVGELSPLD
KSSYKDIDSKEETVNKDVNLQKTKPQVKDQVTSLNEDLTTMSIDSSSPVFLEVIDPITNLGTLQ
LIDLNTGVRLKESTQQGIQRYGIHEREKDLVVIKMDSGKAKLQILDKLENLKVVSESNFEINKN
SSLYVDSKMILVAVRDKDSSNDWRLAKFSPKNLDEFILSENKIMPFTSFSVRKNFIYLQDEFKS
LVILDVNTLKKVKGHHHHHHHNM

A60-OC2/9-S42: SEQ ID NO:15

MSINKEQKTKEKTSEKQESEKQNIEKQEPEKQKQNAAKIIPTVSIQTVEIRESNQIPKSIEKYYK
QAYPIQTFTLDFSITREKEFLKPEDKILPTQGKVESLSILINKKLLDFKAPENPKSSTLKNFKEIKNI
ENFFQNQDLLFVLTLKDKNNNNTINIMLNPPNDIQKPKDYILKDLKDTIKKGTGEKYLNPIYRF
QIKNKKDYHSIDYNKVTISEKTIELDLLPHEQVFQMNKNFTKILDTITDLNNLKLVIQKELVVDG
TGSHMACNNSGKDGNTSANSADESVKGPNLTEISKKITDSNAVLLAVKEVEALLSSIDELAKA
IGKKIKNDGSLDNAANRNEALLAGAATISTLITQKLSKLNGSEGLKEKIAAAKKCSEEFSTKLKD
NHAQLGIQGVTDENAKKAILKANAAGKDKGVEELERLSGSLESLSKAAKEMLANSVKELTSP
VVVESPKKPAMACNNSGKDGNASANSADESVKGPNLTEISKKITESNAVVLAVKEVETLLASI
NQLAKAIGKKIDQNGTLGDAGGQNGALLAGAAAISTVIIEKLSTLKNVEELKEKITKAKDCSEK
FAGKLKNEHASLGKKDATDDDAKKAILKTHGNTDKGAKELKDLSDSVESLVKAAKEMLTNSV
KELTSPVVAESPKKPVDGTPGSHMAEENYTETKRAFSKEDFNLINKRLDNYDFKNEYEKSHVF
SDAPRIRGDLRKIGIKEKSVFLDALEAIEYLIKIKISTDSIFLSEDMIRLIGSYPDSIFNYLIQLNSDKI
DYAEKYGDNARNNFKKDYSEDKANTVKQILKQILADLPKDVEHHHHHH

S42-OC2/9-A60: SEQ ID NO:16

MAEENYTETKRAFSKEDFNLINKRLDNYDFKNEYEKSHVFSDAPRIRGDLRKIGIKEKSVFLDA
LEAIEYLIKIKISTDSIFLSEDMIRLIGSYPDSIFNYLIQLNSDKIDYAEKYGDNARNNFKKDYSEDK
ANTVKQILKQILADLPKDVDGTGSHMACNNSGKDGNTSANSADESVKGPNLTEISKKITDSN
AVLLAVKEVEALLSSIDELAKAIGKKIKNDGSLDNAANRNEALLAGAATISTLITQKLSKLNGSE
GLKEKIAAAKKCSEEFSTKLKDNHAQLGIQGVTDENAKKAILKANAAGKDKGVEELERLSGSL
ESLSKAAKEMLANSVKELTSPVVVESPKKPAMACNNSGKDGNASANSADESVKGPNLTEISK
KITESNAVVLAVKEVETLLASINQLAKAIGKKIDQNGTLGDAGGQNGALLAGAAAISTVIIEKL
STLKNVEELKEKITKAKDCSEKFAGKLKNEHASLGKKDATDDDAKKAILKTHGNTDKGAKELK
DLSDSVESLVKAAKEMLTNSVKELTSPVVAESPKKPVDGTPGSHMSINKEQKTKEKTSEKQES
EKQNIEKQEPEKQKQNAAKIIPTVSIQTVEIRESNQIPKSIEKYYKQAYPIQTFTLDFSITREKEFL
KPEDKILPTQGKVESLSILISKKLLDFKAPENPKSSTLKNFKEIKNIENFFQNQDLLFVLTLKDKN
NNNTINIMLNPPNDIQKPKDYILKDLKDTIKKGTGEKYLNPIYRFQIKNKKDYHSIDYNKVTISE
KTIELDLLPHEQVFQMNKNFTKILDTITDLNNLKLVIQKELVVEHHHHHH

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[0045] While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A recombinant *Borrelia* fusion protein construct comprising dbpA, dbpB and OppA.

2. A recombinant *Borrelia* fusion protein construct selected from a group of constructs selected from Table 2 or Table 3.

3. The recombinant *Borrelia* fusion protein construct of claim 1 wherein the molecular mass of the recombinant *Borrelia* fusion protein construct is about 100 kDa or less.

4. The recombinant *Borrelia* fusion protein construct of claim 2 wherein the molecular mass of the recombinant *Borrelia* fusion protein construct is about 100 kDa or less.

5. The recombinant *Borrelia* fusion protein construct of claim 1 wherein the single recombinant *Borrelia* protein antigens were amplified by PCR with primers comprising SEQ ID NO:1 and SEQ ID NO:2.

6. The recombinant *Borrelia* fusion protein construct of claim 2 wherein the single recombinant *Borrelia* protein antigens were amplified by PCR with primers comprising SEQ ID NO:1 and SEQ ID NO:2.

7. The recombinant *Borrelia* fusion protein construct of claim 1 further comprising one or more single recombinant *Borrelia* protein antigens selected from Table 1.

8. The recombinant *Borrelia* fusion protein construct of claim 2 further comprising one or more single recombinant *Borrelia* protein antigens selected from Table 1.

9. A vector comprising the recombinant *Borrelia* fusion protein construct of claim 1.

10. A vector comprising the recombinant *Borrelia* fusion protein construct of claim 2.

11. A protein array comprising one or more recombinant *Borrelia* fusion protein constructs wherein the one or more recombinant *Borrelia* fusion protein constructs comprise single recombinant *Borrelia* protein antigens selected from a group of dbpA, dbpB,OppA, OspA, purine-binding chemotaxis protein, p83/100 antigen, VMP-like sequence protein VlsE, surface lipoprotein P27, OspC type B, OspC type K, and BapA protein.

12. The protein array of claim 11 wherein the one or more recombinant *Borrelia* fusion protein constructs comprise one or more single recombinant *Borrelia* protein antigens selected from the group consisting of dbpA, dbpB and OppA.

13. The protein array of claim 11 wherein the one or more recombinant *Borrelia* fusion protein constructs comprise one or more single recombinant *Borrelia* protein antigens selected from the group consisting of purine-binding chemotaxis protein, p83/100 antigen, VMP-like sequence protein VlsE, surface lipoprotein P27, OspC type B, OspC type K, and BapA protein.

14. The protein array of claim 11 further comprising one or more single recombinant *Borrelia* protein antigens selected from Table 1.

15. A protein array comprising one or more recombinant *Borrelia* fusion protein constructs selected from Table 2.

16. A protein array comprising one or more recombinant *Borrelia* fusion protein constructs selected from Table 3.

17. The protein array of claim 15 further comprising one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

18. The protein array of claim 16 further comprising one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

19. A protein array comprising all of the recombinant *Borrelia* fusion proteins constructs selected from Table 2 and Table 3.

20. The protein array of claim 19 further comprising one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

21. A method of diagnosing Lyme disease, said method comprising:

A. contacting a test sample obtained from an individual suspected of having Lyme disease with a protein array, said protein array comprising one or more recombinant *Borrelia* fusion protein constructs wherein the one or more recombinant *Borrelia* fusion protein constructs comprise single recombinant *Borrelia* protein antigens selected from a group of dbpA, dbpB,OppA, OspA, purine-binding chemotaxis protein, p83/100 antigen, VMP-like sequence protein VlsE, surface lipoprotein P27, OspC type B, OspC type K, and BapA protein; and

B. detecting the presence of antibodies in the sample reactive to the single recombinant *Borrelia* protein antigens, wherein detecting the presence of antibodies to one or more single recombinant *Borrelia* protein antigens indicates the presence of Lyme disease in the individual.

22. The method of claim 21 wherein the protein array consists of all of the single recombinant *Borrelia* protein antigens dbpA, dbpB and OppA.

23. The method of claim 21 wherein the protein array comprises two or more of the single recombinant *Borrelia* protein antigens purine-binding chemotaxis protein, p83/100 antigen, VMP-like sequence protein VlsE, surface lipoprotein P27, OspC type B, OspC type K, and BapA protein.

24. The method of claim 21 wherein the protein array further comprises one or more single recombinant *Borrelia* protein antigens selected from Table 1.

25. A method of diagnosing Lyme disease, said method comprising:

A. contacting a test sample obtained from an individual suspected of having Lyme disease with a protein array, said protein array comprising one or more recombinant *Borrelia* fusion protein constructs selected from Table 2; and

B. detecting the presence of antibodies in the sample reactive to the recombinant *Borrelia* fusion protein constructs, wherein detecting the presence of antibodies to one or more recombinant *Borrelia* fusion protein constructs indicates the presence of Lyme disease in the individual.

26. A method of diagnosing Lyme disease, said method comprising:

A. contacting a test sample obtained from an individual suspected of having Lyme disease with a protein array, said protein array comprising one or more recombinant *Borrelia* fusion protein constructs selected from Table 3; and

B. detecting the presence of antibodies in the sample reactive to the recombinant *Borrelia* fusion protein constructs, wherein detecting the presence of antibodies to one or more recombinant *Borrelia* fusion protein constructs indicates the presence of Lyme disease in the individual.

27. The method of claim 25 wherein the protein array consists of all of the recombinant *Borrelia* fusion proteins constructs of Table 2.

28. The method of claim 26 wherein the protein array consists of all of the recombinant *Borrelia* fusion proteins constructs of Table 3.

29. The method of claim 25 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

30. The method of claim 26 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

31. A method of diagnosing Lyme disease, said method comprising:

A. contacting a test sample obtained from an individual suspected of having Lyme disease with a protein array, said protein array comprising all of the recombinant *Borrelia* fusion proteins constructs selected from Table 2 and Table 3; and

B. detecting the presence of antibodies in the sample reactive to the recombinant *Borrelia* fusion protein constructs, wherein detecting the presence of antibodies to one or more recombinant *Borrelia* fusion protein constructs indicates the presence of Lyme disease in the individual.

32. The method of claim 31 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

33. A method for detecting antibodies to *Borrelia burgdoferi* in a test sample comprising contacting the sample with a protein array of claim 11, under conditions sufficient for the antibodies in the sample to react with one or more single recombinant *Borrelia* protein antigens and detecting an antibody-antibody reaction.

34. The method of claim 33 wherein the protein array consists of all of the single recombinant *Borrelia* protein antigens dbpA, dbpB and OppA.

35. The method of claim 33 wherein the protein array comprises two or more of the single recombinant *Borrelia* protein antigens selected from the group consisting of purine-binding chemotaxis protein, p83/100 antigen, VMP-like sequence protein VlsE, surface lipoprotein P27, OspC type B, OspC type K, and BapA protein.

36. The method of claim 33 wherein the protein array further comprises one or more single recombinant *Borrelia* protein antigens selected from Table 1.

37. A method for detecting antibodies to *Borrelia burgdoferi* in a test sample comprising contacting the sample with a protein array of claim 15, under conditions sufficient for the antibodies in the sample to react with one or more recombinant *Borrelia* fusion protein constructs and detecting an antibody-antibody reaction.

38. A method for detecting antibodies to *Borrelia burgdoferi* in a test sample comprising contacting the sample with a protein array of claim 16, under conditions sufficient for the antibodies in the sample to react with one or more recombinant *Borrelia* fusion protein constructs and detecting an antibody-antibody reaction.

39. The method of claim 37 wherein the protein array consists of all of the recombinant *Borrelia* fusion proteins constructs of Table 2.

40. The method of claim 38 wherein the protein array consists of all of the recombinant *Borrelia* fusion proteins constructs of Table 3.

41. The method of claim 37 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

42. The method of claim 38 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

43. A method for detecting antibodies to *Borrelia burgdoferi* in a test sample comprising contacting the sample with a protein array of claim 19, under conditions sufficient for the antibodies in the sample to react with one or more recombinant *Borrelia* fusion protein constructs and detecting an antibody-antibody reaction.

44. The method of claim 43 wherein the protein array further comprises one or more recombinant *Borrelia* fusion proteins selected from the group consisting of OspB-OspC-Flagellin (B-C-Fla), OspA-p39-p93 (A-39-93), and OspC dimer consisting of OspC Type B and Type H (OC2/9).

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/043366

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/20
ADD.

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)
C07K G01N

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)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/057562 A1 (UNIV NEW YORK STATE RES FOUND [US]) 14 April 2016 (2016-04-14) claims 1-20; tables 1,4-6 -----	11,13, 14,21, 23,24, 33,35-37
X	WO 2015/054319 A1 (UNIV CENTRAL FLORIDA RES FOUND [US]) 16 April 2015 (2015-04-16) claims 6,12,16 ----- -/--	11,13, 21,23,33

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2017	10/11/2017

Name and mailing address of the ISA/	Authorized officer
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1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/043366

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:

   a. [ ] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	XU Y ET AL: "Profiling the humoral immune response to Borrelia burgdorferi infection with protein microarrays", MICROBIAL PATHOGENESIS, ACADEMIC PRESS LIMITED, NEW YORK, NY, US, vol. 45, no. 5-6, 1 November 2008 (2008-11-01), pages 403-407, XP025693260, ISSN: 0882-4010, DOI: 10.1016/J.MICPATH.2008.09.006 [retrieved on 2008-10-11] abstract; figure 1; table 1	1-44
A	WO 2011/023909 A1 (BIOMERIEUX SA [FR]; LEVET LIONEL [FR]; MEJAN-LETOURNEUR ODILE [FR]) 3 March 2011 (2011-03-03) claims 1-11	1-44
A	EMBERS MONICA E ET AL: "Five-Antigen Fluorescent Bead-Based Assay for Diagnosis of Lyme Disease", CLINICAL AND VACCINE IMMUNOLOGY, vol. 23, no. 4, April 2016 (2016-04), pages 294-303, XP002775066, abstract; figure 1	1-8,15, 25
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/043366

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2963643 A1	14-04-2016
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		US 2017205407 A1	20-07-2017
		WO 2011023909 A1	03-03-2011