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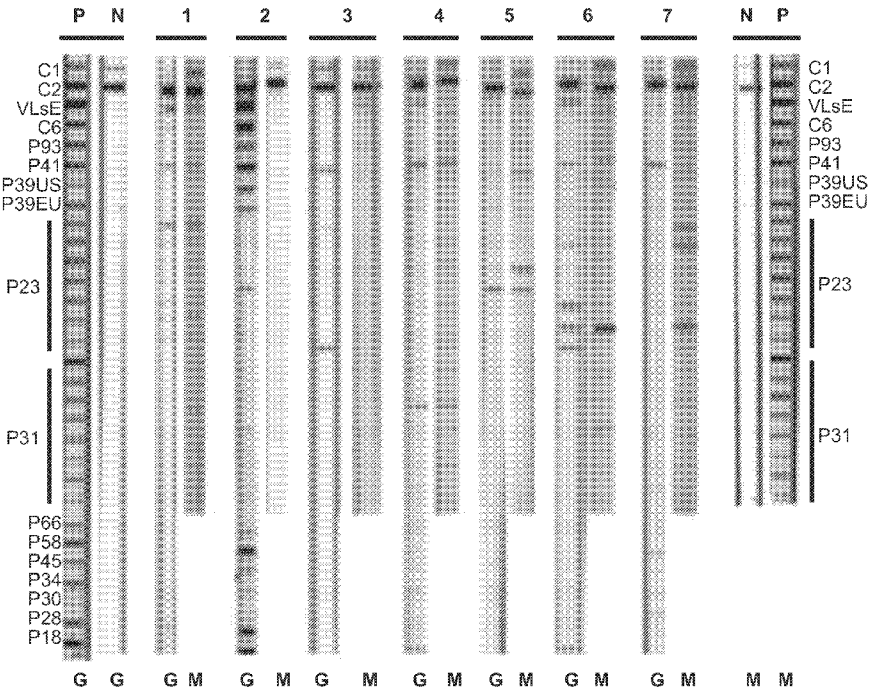
(54) Title: DETECTION OF LYME DISEASE

FIG. 3



(57) Abstract: The disclosure, in some aspects, provides antigen-specific amino acid sequences for Borrelia burgdorferi sensu iato species.

[Continued on next page]

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DETECTION OF LYME DISEASE

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional application serial number 63/009,216, filed April 13, 2020, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

Aspects of the present disclosure provide novel compositions and methods for identifying antibodies resulting from infection by diverse *Borrelia* species.

SEQUENCE LISTING

The instant application incorporates by reference the Sequence Listing in the ASCII text file filed April 13, 2021, entitled "ID-FISH 0153-2016WO01_ST25.txt", which file was created on April 13, 2021, the size of which file is 179,900 bytes.

BACKGROUND

With more than 300,000 new cases reported each year in the USA, Lyme disease is a major public health concern. *Borrelia burgdorferi* sensu stricto (Bbss) is considered the primary agent of Lyme disease in North America. The CDC states that approximately 30,000 cases of Lyme disease are reported in the USA each year using surveillance criteria featuring two-tier Bbss testing, but when tracked by other methods it is estimated that more than 300,000 people develop Lyme disease in the USA annually. The fact that CDC surveillance criteria featuring two-tier Bbss testing captures less than one out of every ten cases shows that Lyme disease is underreported.

SUMMARY

According to an aspect of the disclosure, a panel for detecting IgM- or IgG-class antibodies is provided, the panel including SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences include amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences

is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32, and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43. In some aspects, the labelled and/or tagged and/or bound amino acid sequences are bound to a substance selected from the group consisting of nitrocellulose, nylon, polyvinylidene difluoride (PVDF), magnetic beads, and agarose. In other aspects, the panel further includes one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant. In some aspects, each of the one or more amino acid sequences are tagged with an antibody with specificity for the amino acid sequence.

According to another aspect of the disclosure, a method for detecting IgM- or IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease is provided, the method including: (a) providing a screening panel including SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences include amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32, and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43; (b) providing the biological sample obtained

from the subject suspected of having Lyme disease; (c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and (d) detecting specific binding of IgM- or IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when: (1) a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i)–(iii), (2) a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii), (3) a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (iii) and (v), or (4) a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (vi), and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject. In some aspects, the binding of IgM-class antibodies is detected through the use of an anti-human IgM antibody linked to a detectable moiety. In some aspects, the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety. In some aspects, the detectable moiety is selected from the group consisting of chromophores, radioactive moieties, and enzymes. In other aspects, the detectable moiety includes alkaline phosphatase. In some aspects, the detectable moiety includes biotin. In some aspects, the one or more Bbsl species include *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297. In some aspects, the screening panel of step (a) further includes at least one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

According to yet another aspect of the disclosure, a method for detecting IgM-class and IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease is provided, the method including: (a) providing a screening panel including SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences include amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled

and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32, and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43; (b) providing the biological sample obtained from the subject suspected of having Lyme disease; (c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and (d) detecting specific binding of IgM-class and IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when a positive immunobinding reaction with IgM-class or IgG-class antibodies is detected for SEQ ID NO: 45 and: (1)(A) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i)-(iii), and (1)(B) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii); or (2)(A) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (v), and (2)(B) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi), and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject. In some aspects, the binding of IgM-class antibodies is detected through the use of an anti-human IgM antibody linked to a detectable moiety. In some aspects, the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety. In some aspects, the detectable moiety is selected from the group consisting of chromophores, radioactive moieties, and enzymes. In other aspects, the detectable moiety includes alkaline phosphatase. In some aspects, the detectable moiety includes biotin. In some aspects, the one or more Bbsl species include *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297. In some aspects, the screening panel of step (a) further includes

at least one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

According to another aspect of the disclosure, a method for detecting IgM- or IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease is provided, the method including: (a) providing a screening panel including a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences include amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32, and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43; (b) providing the biological sample obtained from the subject suspected of having Lyme disease; (c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and (d) detecting specific binding of IgM- or IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when: (1) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii), (2) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii), (3) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii) and (v), or (4) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi), and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject. In some aspects, the binding of IgM-class antibodies is

detected through the use of an anti-human IgM antibody linked to a detectable moiety. In some aspects, the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety. In some aspects, the detectable moiety is selected from the group consisting of chromophores, radioactive moieties, and enzymes. In other aspects, the detectable moiety includes alkaline phosphatase. In some aspects, the detectable moiety includes biotin. In some aspects, the one or more Bbsl species include *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297. In some aspects, the screening panel of step (a) further includes at least one or more of SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-C present schematics of amino acid sequence alignments of selected Bbsl antigenic polypeptides among multiple Bbsl species. Fig. 1A shows an alignment of the 23 kDa protein (P23, SEQ ID NOs: 47-56). Fig. 1B shows an alignment of the 31 kDa protein (P31, SEQ ID NOs: 57-66). Fig. 1C shows an alignment of 39 kDa (P39, SEQ ID NOs: 67-73).

Fig. 2 presents a photomicrographic image showing that Lyme ImmunoBlot strips were tested with rabbit anti-*Borrelia burgdorferi* serum samples and rabbit anti-TBRF Borrelia serum samples. Rabbit sera with antibodies to the following *B. burgdorferi* species were tested: *B. burgdorferi* sensu lato species *B. burgdorferi B31* (1) and *B. burgdorferi 297* (2); and *B. burgdorferi* sensu stricto species *B. californiensis* (3), *B. afzalii* (4), *B. garinii* (5), *B. spielmanii* (6), and *B. valensiana* (7). Rabbit sera with antibodies to the following TBRF Borrelia species were used as controls: *B. hermsii* (8) and *TBRF Borrelia* species *B. coriaceae* (9).

Fig. 3 presents a photomicrographic image showing immunoblot reactivity of patient serum samples for *Borrelia burgdorferi* sensu lato (Bbsl) species. Immunoblot strips with recombinant *Borrelia* antigens were incubated with representative patient serum samples. Lanes are identified as follows: P, positive control; N, negative control; 1, *B. burgdorferi* B31 positive IgM & IgG; 2, *B. mayonii* positive IgG; 3, *B. speilmanii* positive IgG; 4, *B. californiensis* positive IgM & IgG; 5, mixed infection *B. mayonii* and *B. californiensis* positive IgM; 6, *B. burgdorferi* European species (*B. burgdorferi sensu stricto, B. afzelli and B. garinii and b. speilmanii*) positive IgM & IgG; 7, Bbss and *B. burgdorferi* European

species positive IgM. G indicates a Bbsl Immunoblot IgG test; M indicates a Bbsl Immunoblot IgM test.

DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 – P23 *B. burgdorferi* B31

MFLFISCNNSGKDGNTSANSADESVKGPNLTEISKKITDSNAVLLAVKEVEALLSSID
EIAAKAIGKKIHQNNGLDTENNHNGSLLAGAYAISTLIKQKLDGLKNEGLKEKIDAA
KKCSETFTNKLKEKHTDLGKEGVTDADAKEAILKTNGTKTKGAEELGKLFESVEVLS
KAAKEMLANSVKELTSPVVAESPKKP.

SEQ ID NO: 2 – P23 *B. burgdorferi* 297

MFLFISCNNSGKDGNTSANSADESVKGPNLTEISKKITESNAVVLAVKEVETLLTSIDE
LAKAIGKKIKNDVSLDNEADHNGSLISGAYLISTLITKKISAIKDSGELKAEIEKAKKC
SEEFTAKLKGEHTDLGKEGVTDDNAKKAILKTNNDKTKGADELEKLFESVKNLSKA
AKEMLTNSVKELTSPVVAESPKNPGSVDKL.

SEQ ID NO: 3 – P23 *B. bissettii*

MFLFISCNNSGKDGNSASTNPADESAKGPNLTEISKKITDSNAIVLAVKEVETLLLSID
ELAKAIGKKINNNGLDVLQNFNASLLGGAHTISKLITEKLSKLNGSEELKEKIEAAKK
CSDDFTKKLQSSHAELGVAGGATTDENAKKAILKSNADKTKGADELGKLFESVESL
AKAAKEMLANSVKELTSPVVAETPKKP.

SEQ ID NO: 4 – P23 *B. californiensis*

MFLFISCNNSGKDGNSASTNPADESKGPNLTEISKKITDSNAVVLAVKEVETLLASID
ELAEKAIGKKIQQNNGLGAEANKNGSLLAGVYSISTLITEKLSAMKDSGGLKAEIEKA
KDCSEKFTKKLETSHAELGKNEATDDDAKKAILRTNGDKTKGAEELQKLFESVGGL
AKAAKEMLTNSVKELTSPVVAETPKKPGSVDKL.

SEQ ID NO: 5 – P23 *B. mayonii*

MFLFISCNNSGKDGNASNSADESAKGPNLTEISKKITDSNAVVLAVKEVEALVASIDE
LAKAIGKKIQQNNGLGNEAGKNGSLLSGIYTISTVITQKLGALNNEELKERIKEAKEC
SEAFTKKLETNHTDLGKHDASDDDAKKAILRTNGDKTKGAEELEKLFKAVESLSTEA
KGMLTNSVKQLTSPVVAETPKKP.

SEQ ID NO: 6 – P23 *B. garinii*

MFLFISCNNSGGDTASTNPDESVKGPNLTEISKKITDSNAFVLAVKEVEALISSIDELA
KAIGQRIQQNGLVADAGHNSALLAGAHEISILITQKLDGLKGLEGLKAEIAEAKKYSE

AFTKKLKDNHAQLGIQNGASLDDEAKKAILKTNVDKTKGAEELEKLFKSVESLSKA
AQEALTNSVKELTNPVVAETPKKP.

SEQ ID NO: 7 – P23 *B. afzelii*

MFLFISCNNSGKGGDIASTNPDESAKGPNLTEISKKITDSNAVVLAVKEVEALLSSIDE
LAKTIGKKIEANGLGNEADKNGSLLAGAYAISTLIKQKLDGLKGLEGLNKEIAEAKK
CSEAFTKKLQDSNADLGKHNATDADSKEAILKTNGTKTKGAKELEELFKSVESLSKA
AKEALSNSVKELTSPVVAESPKNPGSVDKL.

SEQ ID NO: 8 – P23 *B. spielmanii*

MFLFISCNNSGGDSTSTKPVDEPAKGPNLAEISKKITDSNTFVLAVKEVETLVSSIDEL
AKKAIGQKIDQNSGLGALQNQNGSLLAGVYAISTLITDKLSKLKNSEELKAEIAKAK
KCSEDFTNKLKLSHADLGAVNGATTDDHAKAAILKTNAPDDKGAKEFKGLFESVES
LSKAAKAALANSVKELTSPVAAEAPKKPGSVDKL.

SEQ ID NO: 9 – P23 *B. valaisiana*

MGDTASTNPVDESAKGPNLTEISKKITDSNAIVLAVKEVETLLASINEIANKGIGKKIN
QNGLDNLTDHNGSLIAGAYVISTLITEKLNNLKNSEGLKEKIKKVKECSDKFTKKLTT
SNGDLGKENVTDAHAQAAILKTNPTNDKGAKELGELFESVEILSKAAQEALTNSIAE
LTSPVVAENPKNP.

SEQ ID NO: 10 – P23 *B. bavariensis*

MFLFISCNNSGGDSASTNPDESAKGPNLTVISKKITDSNAFLLAVKEVEALLSSIDELS
KAIGKKIKNDGTLDNEANRNESLIAGAYEISKLITQKLSVLNSEELKEKIKEAKDCSEK
FTTKLKDSHAELGIQSVQDDNAKKAILKTHGTKDKGAKELEELFKSLESLSKAAQAA
LTNSVKELTNPVVAETPKKP.

SEQ ID NO: 11 – P31 *B. burgdorferi* B31

MAKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIATVDKLELKGTSDKN
NGSGVLEGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFN
EKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGT
VTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKEN
TITVQQYDSNGTKLEGSAVEITKLDEIKNALK.

SEQ ID NO: 12 – P31 *B. burgdorferi* 297

MAKQNVSSLDEKNSVSVDLPGEMNVLVSKEKNKDGKYDLIATVDKLELKGTSDKN
NGSGVLEGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKKVTSKDKSSTEEKFN
EKGEVSEKIITRADGTRLEYTEIKSDGSGKAKEVLKGYVLEGTLTAEKTTLVVKEGT
VTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVNSKKTKDLVFTKEN
TITVQQYDSNGTKLEGSAV.

SEQ ID NO: 13 – P31 *B. bissettii*

MKQNVSGLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMATVDKLELKGTSDKN
NGSGILEGVKADKSKVKLTVSEDLSTTTLEVLKEDGKTLVSKKTTSKDKSSTEEKFN
DKGELAEKTIVRANGTRLEYTEVKSDGSGKAKETLKDYALEGTLTAEKATLVVKEG
TVTLSKHISKSGEVTAELNDTDSAQATKKTGKWDAGTSTLTISVNSKKTKNLVFTKQ
DTITVQKYDSAGTNLEGTAVEIKTLDELKNALK.

SEQ ID NO: 14 – P31 *B. californiensis*

MAKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMATVDKLELKGTSDKN
NGSGVLEGVKDDKSKVKLTVSDDLSTTTLEVLKEDGKTLVSRKETSKDKSSTEEKFN
EKGELTEKIMERSNGTRLEYTEIKTDGSGKAKETLKDFVLEGTLTTEKAILTVKEGTV
TLNKNISKSGEVTVDLNDTSTTAATKKTGKWDSSTSTLTVSVNSKKTKDLVFTKQDT
ITVQKYDSAGTNLE GTAVEIKTLDEIKNALK.

SEQ ID NO: 15 – P31 *B. mayonii*

MAKQNVSSLDEKNSVSVDLPGEIKVLVSKEKDKDGKYSLMATVDKLELKGTSDKN
NGSGVLEGVKADKSKVKLTVSDDLSKTTLEVLKEDGKTLVSRKVTSKDKSSTEEKF
NEKGELAEKTMTRADETRLEYTEIKSDGSGKAKEVLKGYALEGTLTAEKTTLVVKE
GTVTLSKNISKSGEVTAELNDTDSAAATKKTGAWNSGTSTLTITANSKKTKDLVFTK
ENTITVQKYDTAGIKLEGSAVEIKTLDELKNALK.

SEQ ID NO: 16 – P31 *B. garinii*

MKQNVSSLDEKNSVSVDLPGGMQVLVSKEKDKDGKYSLMATVDKLELKGTSDKN
NGSGTLEGEKTDKSKAKLTIAEDLSKTTFEIFKEDGKTLVSKKVTLKDKSSTEEKFNA
KGEASEKTIVRANGTRLEYTDIKSDKTGKAKEVLKDFALEGTLAADGKTTLKVTEGT
VVLSKHISNSGEITVELNDSDTTQATKKTGTWDSKTSTLTISVNSRKTKNLVFTKEDT
ITVQKYDSAGTNLEGKAVEITTLKELKDALK.

SEQ ID NO: 17 – P31 *B. afzelii*

MAKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLKATVDKIELKGTSDKD
NGSGVLEGTKDDKSKAKLTIADDLSKTTFELFKEDGKTLVSRKVSSKDKTSTDEMFN
EKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEGKVANDKVTLEVKE
GTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTISVNSKKTTQLVFTK
QDTITVQKYDSAGTNLEGTAVEIKTLDELKNALK.

SEQ ID NO: 18 – P31 *B. spielmanii*

MAKQNVSGLDEKNSTSVDVPGELKVLVSKEKDKDGKYSLMATVDKLELKGTSDKN
DGSGVLEGVKADKSKVKLTISDHLSKTTFEVFKEDGKTLVSRNVNSKDKSSTKEKFN
EKGELSEKTLVRANGTKLEYTEIKSDGTGKAKEVLKDFTLEGTLANEKATLTVKEGT

VTLSKNIDKSGEVTVALNDTDSTAATKKTGAWDSKTSTLTITVNSKKTKDLVFTKQD
TITVQKYDSAGTNLEGSAVEIKTLDELKNALK.

SEQ ID NO: 19 – P31 *B. valaisiana*

MAKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLVATVDKVELKGTSDKN
NGSGTLEGVKDDKSKVKLTISDDLGETKLETFKEDGTLVSRKVNFKDKSFTEEKFNE
KGEVSEKILTRSNGTTLEYSQMTDAENATKAVETLKNGIKLPGNLVGGKTTLKITEG
TVTLSKHIAKSGEVTVEINDTSSTPNTKKTGKWDARNSTLTIIVDSKNKTKLVFTKQD
TITVQSYNPAGNKLEGTAVEIKTLQELKNALK.

SEQ ID NO: 20 – P31 *B. bavariensis*

MKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMATVDKLELKGTSDKSN
GSGTLEGEKSDKSKAKLTISEDLSKTTFEIFKEDGKTLVSKKVNSKDKSSIEEKFNAK
GELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLAADKTTLKVTEGTVV
LSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSKKTKNIVFTKEDTITV
QKYDSAGTNLEGNAVEIKTLDELKNALK.

SEQ ID NO: 21 – P39 *B. burgdorferi* B31

MKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFKIELVLKESSSNSYLSDLE
GLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYSNDPIPANLVGMTFRAQ
EGAFLTGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGAKYANKDIKISTQYIGSFA
DLEAGRSVATRMYSDEIDIIHHAASLGGIGAIEVPKELGSGHYIIGVDEDQAYLAPDN
VITSTTKDVGRALNIFTSNHLKTNTFEGGKLINYGLKEGVVGFVRNPKMISFELEKEI
DNLSSKIINKEIIVPSNKESYEKFLKEFI.

SEQ ID NO: 22 – P39 *B. burgdorferi* 297

MKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFKIELVLKESSSNSYLSDLE
GLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYSNDPIPANLVGMTFRAQ
EGAFLTGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGAKYANKDIKISTQYIGSFA
DLEAGRSVATRMYSDEIDIIHHAASLGGIGAIEVPKELGSGHYIIGVDEDQAYLAPDN
VITSTTKDVGRALNIFTSNHLKTNTSEGGKLINYGLKEGVVGFVRNPKMISFELEKEI
DNLSSKIINKEIIVPSNKESYEKFLKEFI.

SEQ ID NO: 23 – P39 *B. garinii*

MKGSLESEIPKVSLIIDGTFDDKSFNESALNGIKKVKEEFKIEPVLKESSINSYLSDLEG
LKDTGSNLIWLIGYKFSDVAKAVSLQNPEIKYAIIDPIYSDEPIPANLVGMTFRSQEGA
FLTGYIAAKVSKTGKIGFLGGIEGEIVDSFRYGYEAGAKYANKDIKISAYYIGSFADLE
AGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPNNIITS
ATKDVGRSLNIFTSNYLKTNTFEGGRLINYGLKEGVVGFVKNPKMIPFELEKEIDNLS
SKIINKEIIVPYNKESYEKFLKE.

SEQ ID NO: 24 – P39 *B. afzelii*

MKSGLESGIPKVSLVIDGTFDDKSFNESALNGVKKLKEEFEIELVLKESSTNSYLSDLE
GLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPVYSNEPIPANLVGMTFRAQ
EGAFLTGYIAAKVSKTGKIGFLGGIEGDIVDAFRYGYEAGAKYANKDIKIFSQYIGSFS
DLEAGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPNN
VITSTTKDVGRSLNLFTSNYLKTNTFEGGKLINYGLKEGVVGFVRNPKMIPFEVEKEI
DSLSSKIINKEVIVPYNKESYEKFLKEFI.

SEQ ID NO: 25 – P39 B. *spielmanii*

MKGGLENKIPKVSLIIDGTFDDKSFNESALNGVKKLKEEFEIDLVLKESSTNSYVSDL
EGLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPVYSSEPIPANLVGMTFRA
QEGAFLTGYIASKVSKTGKIGFLGGIEGDIVDAFRYGYEAGAKYANKDIKIFSQYIGSF
ADIEAGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPN
NVITSSTKDVGRSLNLFTSNYLKTNNFEGGKLINYGLKEGVVGFVRNPKMIPFEVEKE
IDSLSGKIINKEVIVPYNKESYEKFLKEFL.

SEQ ID NO: 26 – P39 *B. valaisiana*

MKGSLEGGIPKVSVIIDGTFDDKSFNESALNGIKKVKEEFKVEFVLKESSSNSYLSDLE
GLKDTGSNLIWLIGYRFSDVAKVVSLQNSEVKYAIIDPVYSNEPIPANLVGMTFRAQE
GAFLTGYIASKVSKTGKIGFLGGIKSEIVDAFRYGYEAGAKYANKDIKIFTHYIGSFAD
LEASRSIAIKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPDNVIT
SSTKDVGRALNIFTSNYLKTNTFEGGKLINYGLKEGVVGFVRNPKMIPFELEKEIDSIS
SKIINKEVIVPYNKGSYEKFLKEFI.

SEQ ID NO: 27 – P93 *B. burgdorferi* B31

MFLNGFPLNARKVDKEKLKDFVNMDLEFVNYKGPYDSTNTYEQIVGIGEFLARPLT
NSNSNSSYYGKYFINRFIDDQDKKASVDVFSISSKSELDSILNLRRILTGYIIKSFDYDR
SSAELIAKVITIYNAVYRGDLDYYKGFYIEPALKSLTKENAGLSRVYSQWAGKTQIFI
PLKKDILSGNIESDIDIDSLVTDKVIAALLSENEAGVNFARDITDIQGETHKADQDKID
TELDNIHESDSNITETIENLRDQLEKATDEEHKKEIESQVDAKKKEKEELDKKAINLD
KAQQKLDSAEDNLDVQRDTVREKIQEDINEINKEKNLPKPGDVSSPKVDKQLQIKES
LEDLQEQLKEAGDENQKREIEKQIEIKKRDEELLKSKDGKVSKDYEALDLDRELSKA
SSKEKSKVKEEEITKGKSRASLGDLNNDKNLMLPEDQKLPEDKKLDSKLDGKKEFKP
VSEVEKLDKISKSNNNEVGKLSPLDKPSYDDIDSKEEVDNKAINLQKIDPKVKDQTTS
LNEDLDKDLTTMSIDSSSPVFLEVIDPITNLGTLQLIDLNTGVRLKESTQQGIQRYGIY
EREKDLVVIKMDSGKAKLQILNKLENLKVVSESNFEINKNSSLYVDSKMILAAVRDK
DDSNAWRLAKFSPKNLDEFILSENKILPFTSFSVRKNFIYLQDELKNLVILDVNTLKK
VK.

SEQ ID NO: 28 – P93 *B. burgdorferi* 297

MFLNGFPVSAREVDREKLKDFVNMDLEFVNYKGPYDSTNTYEQIVGIGEFLARPLTN
SNSNSSYYGKYFINRFIDDQDKKASVDVFSIGSKSELDSILNLRRILTGYLIKSFDYDRS
SAELIAKVITIYNAVYRGDLDYYKGFYIEAALKSLSKENAGLSRVYSQWAGKTQIFIP
LKKDILSGNIESDIDIDSLVTDKVVAALLSENEAGVNFARDITDIQGETHKADQDKIDI
ELDNIHESDSNITETIENLRDQLEKATDEEHKKEIESQVDAKKKQKEELDKKAINLDK
AQQKLDSAEDNLDVQRNTVREKIQEDINEINKEKNLPKPGDVSSPKVDKQLQIKESL
EDLQEQLKETGDENQKREIEKQIEIKKSDEKLLKSKDDKASKDGKALDLDRELNSKA
SSKEKSKAKEEEITKGKSQKSLGDLNNDENLMMPEDQKLPEVKKLDSKKEFKPVSE
VDKLDKISKSNNNVGELSPLDKSSYKDIDSKEETVNKDVNLQKTKPQVKDQVTSLNE
DLTTMSIDSSSPVFLEVIDPITNLGTLQLIDLNTGVRLKESTQQGIQRYGIYEREKDLV
VIKMDSGKAKLQILDKLKNLKVVSESNFEINKNSSLYVDSKMILVAIRDKDSSNDWR
LAKFSPKNLDEFILSENKIMPFTSFSVRKNFIYLQDEFKSLVILDVNTLKKVK.

SEQ ID NO: 29 – P66 *B. burgdorferi* B31

MKEKDIFKINPWMPTFGFENTSEFRLDMDELVPGFENKSKITIKLKPFEANPELGKDD
PFSAYIKVEDLALKAEGKKGDQFKIDVGDITAQINMYDFFIKISTMTDFDFNKESLFSF
APMTGFKSTYYGFPSNDRAVRGTILARGTSKNIGTIQLGYKLPKLDLTFAIGGTGTGN
RNQENDKDTPYNKTYQGILYGIQATWKPIKNLLDQNEDTKSVIAETPFELNFGLSGA
YGNETFNNSSITYSLKDKSVVGNDLLSPTLSNSAILASFGAKYKLGLTKINDKNTYLIL
QMGTDFGIDPFASDFSIFGHISKAANFKKETPSDPNKKAEIFDPNGNALNFSKNTELGI
AFSTGASIGFAWNKDTGEKESWAIKGSDSYSTRLFGEQDKKSGVALGISYGQNLYRS
KDTEKRLKTISENAFQSLNVEISSYEDNKKGIINGLGWITSIGLYDILRQKSVENYPTTI
SSTTENNQTEQSSTSTKTTTPNLTFEDAMKLGLALYLDYAIPIASISTEAYVVPYIGAY
ILGPSNKLSSDATKIYLKTGLSLEKLIRFTTISLGWDSNNIIELANKNTNNAAIGSAFLQ
FKIAYSGS.

SEQ ID NO: 30 – P58 *B. burgdorferi* B31

MKERKEGVSFKISLGAEPSSLDPQLAEDNVASKMIDTMFRGIVTGDPNTGGNKPGLA
KGWDISSDGTVYTFNLREKITWSDGVAITAEGIRKSYLRILNKETGSKYVEMVKSVIK
NGQKYFDGQVTDSELGIRAIDEKTLEITLESPKPYFIDMLVHQSFIPVPVHVTEKYGQ
NWTSPENMVTSGPFKLKERIPNEKYVFEKNNKYYDSNEVELEEITFYTTNDSSTAYK
MYENEELDAIFGSIPPDLIKNLKLRSDYYSSAVNAIYFYAFNTHIKPLDNVKIRKALTL
AIDRETLTYKVLDNGTTPTRRATPNFSSYSYAKSLELFNPEIAKTLLAEAGYPNGNGF
PILKLKYNTNEANKKICEFIQNQWKKNLNIDVELENEEWTTYLNTKANGNYEIARAG
WIGDYADPLTFLSIFTQGYTQFSSHNYSNPEYNELIKKSDLELDPIKRQDILRQAEEIIIE
KDFPIAPIYIYGNSYLFRNDKWTGWNTNILERFDLSQLKLKNK.

SEQ ID NO: 31 – P45 *B. burgdorferi* B31

MRYEMKEESPGLFDKGNSILETSEESIKKPMNKKGKGKIARKKGKSKVSRKEPYIHS
LKRDSANKSNFLQKNVILEEESLKTELLKEQSETRKEKIQKQQDEYKGMTQGSLNSL
SGESGELEEPIESNEIDLTIDSDLRPKSSLQGIAGSNSISYTDEIEEEDYDQYYLDEYDE
EDEEEIRLSNRYQSYLEGVKYNVDSAIQTITKIYNTYTLFSTKLTQMYSTRLDNFAKA

KAKEEAAKFTKEDLEKNFKTLLNYIQVSVKTAANFVYINDTHAKRKLENIEAEIKTLI
AKIKEQSNLYEAYKAIVTSILLMRDSLKEVQGIIDKNGVWY.

SEQ ID NO: 32 – P41 *B. burgdorferi* B31

MRNNGINAANLSKTQEKLSSGYRINRASDDAAGMGVSGKINAQIRGLSQASRNTSK
AINFIQTTEGNLNEVEKVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQ
AQYNQMHMLSNKSASQNVRTAEELGMQPAKINTPASLSGSQASWTLRVHVGANQD
EAIAVNIYAANVANLFSGEGAQTAQAAPVQEGVQQEGAQQPAPATAPSQGGVNSPV
NVTTTVDANTSLAKIENAIRMISDQRANLGAFQNRLESIKNSTEYAIENLKASYAQIK
DATMTDEVVAATTNSILTQSAMAMIAQANQVPQYVLSLLR.

SEQ ID NO: 33 – P41 *B. mayonii*

MRNNGINAANLSKTQEKLSSGYRINRASDDAAGMGVSGKINAQIRGLSQASRNTSK
AINFIQTTEGNLNEVEKVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQ
AQYNQMHMLSNKSASQNVRTAEELGMQPAKINTPSSLSGSQASWTLRVHVGANQD
EAIAVNIYAANVANLFSGEGTQTAQVAPVQEGAQQEGAQQPAPATAPSQGGVNSPV
NVTTTVDANTSLAKIENAIRMISDQRANLGAFQNRLESIKNSTEYAIENLKASYAQIK
DATMTDEVVAATTNSILTQSAMAMIAQANQVPQYVLSLLR.

SEQ ID NO: 34 – P34 *B. burgdorferi* B31

MGSCAQKGAESIGSQKENDLNLEDSSKKSHQNAKQDLPAVTEDSVSLFNGNKIFVSK
EKNSSGKYDLRATIDQVELKGTSDKNNGSGTLEGSKPDKSKVKLTVSADLNTVTLE
AFDASNQKISSKVTKKQGSITEETLKANKLDSKKLTRSNGTTLEYSQITDADNATKA
VETLKNSIKLEGSLVGGKTTVEIKEGTVTLKREIEKDGKVKVFLNDTAGSNKKTGKW
EDSTSTLTISADSKKTKDLVFLTDGTITVQQYNTAGTSLEGSASEIKNLSELKNALK.

SEQ ID NO: 35 – P34 *B. burgdorferi* 297

MRLLIGFALALALIGCAQKGAESIGSQKENDLNLEDSSKKSHQNAKQDLPAVTEDSV
SLFNGNKIFVSKEKNSSGKYDLRATIDQVELKGTSDKNNGSGTLEGSKPDKSKVKLT
VSADLNTVTLEAFDASNQKISSKVTKKQGSITEETLKANKLDSKKLTRSNGTTLEYSQ
ITDADNATKAVETLKNSIKLEGSLVGGKTTVEIKEGTVTLKREIEKDGKVKVFLNDT
AGSNKKTGKWEDSTSTLTISADSTKTKDLVFLTDGTITVQQYNTAGTSLEGSASEIKN
LSELKNALK.

SEQ ID NO: 36 – P34 *B.valaisiana*

MRQYLIGFALVLALLACAQKGAEPKTQNSDREIMDSNKDSSKDSKQVLTTSTEKAV
SLFNGYTIFVSKEKNTSGKYDLRAVVDQFELKGTSDKDNGSGTLKGSKADKTKMTIS
ITEDLNSVTVETFDSGNKKVSSKVVKKHGLLTEENFKADKLDSQKLTRSNGTTLEYS
QMTDAENATKAVETLKNGIKLEGNLVGGKTTLKITVGTVTLTREIEKDGRIKLFLND

TDSSPTKKTAKWEDSTNTLTITSNRKKTKDLVFLIDGTITVQNYNSAGKLDGQASEIK
SLGELQGALK.

SEQ ID NO: 37 – P34 *B. spielmanii*

MRQQYLLVFALILALIACSQKGTEPKDDNYNDQEIASGDKEPKISKKELPRETETAVS
LFNGNEIFISKEKNSAGKYDLRARVDLVELKGTSDKNTGAGKLEGLKADKSKVTMTI
SDDLNTVTVETYDASNKKTGSEVVKKQGSVIKESYKANKLDSKKLTRSNDTTLEYS
QMTDEENATKAVETLKNGIKFEGNLVGGKTTVKITEGTVTLKREIDKDGKIKVFLDD
TATDNTKKTGKWNENNNTLTVTVDSKKTKDLVFSDDGTSTITVQKYNTAGTNLEGN
PSEIKDLAALKGALK.

SEQ ID NO: 38– P34 *B. garinii*

MKKYLLGFALVLALIACGQKGAEPKHNDQEVEDSKKDQKDASKKDLPLVTEDTVK
LFNDTEIFISKEKNNAGKYELRAMVDTVELKGFSEKNTGAGNLEGLKADKSKVTML
VSDDLNTITIETYNTSNKKVSSQVVKKQGLLTEESYKADKLDSKKLTRTNGTTLEYS
DMTDAANATKAVETLKNGIEFEGNLVGGKTTLNIKEGTVTLTREIDKDDKLKIYLND
TASSSKKTASWNDTDTLTISAEGKKTKDLVFRTDGTITVQNYDSASGTKLEGTATEIK
DLEALKAALK.

SEQ ID NO: 39 – P34 *B. afzelii*

MKQYLLVFALVLALIACSQKGTEPKSTSQDHNDQEIINSDNTPKDSKKDLTVLAEEN
SVPLFNGNKIFVSKEKNSAGKYELRATVDTVELKGVSDKNNGSGKLEGTKADKTKV
AMTIADDLNTITVETYDASNKKTGSEVVKKQGSVIKESYKANKLDSKKITRENETTL
EYSEMTDSSNATKAVETLKNGIKLEGSLVGGKTTVKLTEGTITLTREIEQDGKVKIYL
NDTTSGSTKKTATWNETTNTLTISADSKKTKDFVFLTDGTITVQAYDTAGTKLEGNS
SEIKDLAALKAALK.

SEQ ID NO: 40 – P30 *B. burgdorferi* B31

MFLLLSISCVHDKQELSSKSNLNNQKGYLDNEGANSNYESKKQSILSELNQLLKQTT
NSLKEAKNTTDNLNASNEANKVVEAVINAVNLISSAADQVKSATKNMHDLAQMAEI
DLEKIKNSSDKAIFASNLAKEAYSLTKAAEQNMQKLYKEQQKISESESESDYSDSAEI
KQAKEAVEIAWKATVEAKDKLIDVENTVKETLDKIKTETTNNTKLADIKEAAELVLQ
IAKNAKEIVQEVVALLNT.

SEQ ID NO: 41 – P28 B. *burgdorferi* B31

MTSKDLEGAVKDLESSEQNVKKTEQEIKKQVEGFLEILETKDLNTLDTKEIEKQIQEL
KNKIEKLDSKKTSIETYSGYEEKINKIKEKLNGKGLEDKLNELSESLKKRKEERKKAL
QEAKKKFEEYKNQAESATGVTHGSQVQRQGGVGLQAWQCANSLGFKNMTSGNNT
SDMTNEVITNSLKKIEEELKNIGETVEGKKE.

SEQ ID NO: 42 – P28 *B. burgdorferi* 297

MTSKDLEGVVQDLESSEQNVKKTEQEIKKQVEGFLEILETKDLNTLDTKEIEKQIQEL
KDTINKLEAKKTSLKTYSEYEEKLKQIKEKLKDKKELEDKLKGLEDSLKKKKEDRKK
ALEDAKKKFEEFKGQVGSATGVTTGHRAGNQGSIGAQAWQCANSLGLGVSYSSSTG
TDSNELANKVIDDSIKKIDEELKNTIENNGKVKKE.

SEQ ID NO: 43 – P18 *B. burgdorferi* B31

MKIRLERSAKDITDEIDAIKKDAALKGVNFDAFKDKKTGSGVSENPFILEAKVRATTV
AEKFVIAIEEEATKLKETGSSGEFSAMYDLMFEVSKPLQKLGIQEMTKTVSDAAEENP
PTTAQGVLEIAKKMREKLQRVHTKNYCTLKKKENSTFTDEKCKNN.

SEQ ID NO: 44 – VlsE *B. burgdorferi* B31

MDPKKSDVKTYFTTVAAKLEKTKTDLNSLPKEKSDISSTTGKPDSTGSVGTAVEGAI
KEVSELLDKLVKAVKTAEGASSGTAAIGEVVADADAAKVADKASVKGIAKGIKEIV
EAAGGSEKLKAVAAAKGENNKGAGKLFGKAGAAAHGDSEAASKAAGAVSAVSGE
QILSAIVTAADAAEQDGKKPEEAKNPIAAAIGDKDGGAEFGQDEMKKDDQIAAAIAL
RGMAKDGKFAVKDGEKEKAEGAIKGAAESAVRKVLGAITGLIGDAVSSGLRKVGDS
VKAASKETPPALNK.

SEQ ID NO: 45 – C6 chimera

MKKDDQIAAAIALRGMAKDGKFAVKDGGGMKKNDQIAAAIVLRGMAKDGEFALK
NELGSMKKNDQIAAAMVLRGMAKDGQFALTGGGGMKKDDQIAAAIALRGMAKDG
KFAVKDGGGMKKDAQIAAAIVLRGMAKDGKFAVKK.

SEQ ID NO: 46 – PepC10 chimera

MPVVAESPKKPGSPVVAETPKKPGSPVVAESPKKPGSPIVAESPKNPGSPVVAESPKK
PGSPVAAESPKKPGSPVVAESPKKPGSPVVAETPKKPGSPVVAESPKKPGSW.

SEQ ID NO: 47 – P23 *B. spielmanii*

MKKNTLSAILMTLFLFISCNNSGGDSTSTKPVDESAKGPNLTEISKKITDSNTFVLAVK
EVETLLLSIDELAKAIGKKIENNGLGTEASHNTSLLAGAYTISSLITQKLNALKNSGEL
KAEIEKAKNCSEAFTKKLKEKHQDLGTAGGNATDDHAKAAILKTNATDDKGAKEL
KELFESVESLSKAAKAALANSVKELTSPVVAETPKKP.

SEQ ID NO: 48 – P23 *B. bissettii*

MKKNTLSAILMTLFLFISCNNSGKDGNSASTNPADESAKGPNLTEISKKITDSNAIVLA
VKEVETLLLSIDELAKAIGKKINNNGLDVLQNFNASLLGGAHTISKLITEKLSKLNGSE

ELKEKIEAAKKCSDDFTKKLQSSHAELGVAGGATTDENAKKAILKSNADKTKGADE
LGKLFESVESLAKAAKEMLANSVKELTSPVVAETPKKP.

SEQ ID NO: 49 – P23 *B. valaisiana*

MKKNTLSAILMTLFLFISCNNSGKDVTTSTDSVDESAKGPNLVEISKKITDSNAIVLAV
KEVETLLSSIDELANKAIGKKIQQNGSLANEADHNGSLLAGTYAISTLITQKLGKLKIS
EELKEKIEDAKKCSEDFARKLSDNHNDLGKEGVTDDDAKKAILKTHGTKDKGAAEF
EKLFKSVESLVKAAQETLVNSIKELTSPVAAESPKKP.

SEQ ID NO: 50 – P23 *B. burgdorferi* B31
MKKNTLSAILMTLFLFISCNNSGKDGNTSANSADESVKGPNLTEISKKITDSNAVLLA
VKEVEALLSSIDEIAAKAIGKKIHQNNGLDTENNHNGSLLAGAYAISTLIKQKLDGLK
NEGLKEKIDAAKKCSETFTNKLKEKHTDLGKEGVTDADAKEAILKTNGTKTKGAEE
LGKLFESVEVLSKAAKEMLANSVKELTSPVVAESPKKP.

SEQ ID NO: 51 – P23 *B. burgdorferi* 297
MKKNTLSAILMTLFLFISCNNSGKDGNTSANSADESVKGPNLTEISKKITESNAVVLA
VKEIETLLASIDELATKAIGKKIQQNGGLAVEAGHNGTLLAGAYTISKLITQKLDGLK
NSEKLKEKIENAKKCSEDFTKKLEGEHAQLGIENVTDENAKKAILITDAAKDKGAAE
LEKLFKAVENLAKAAKEMLANSVKELTSPIVAESPKNP.

SEQ ID NO: 52 – P23 *B. mayonii*
MKKNTLSAILMTLFLFISCNNSGKDGNASNSADESAKGPNLTEISKKITDSNAVVLAV
KEVEALVASIDELAKAIGKKIQQNNGLGNEAGKNGSLLSGIYTISTVITQKLGALNNE
ELKERIKEAKECSEAFTKKLETNHTDLGKHDASDDDAKKAILRTNGDKTKGAEELEK
LFKAVESLSTEAKGMLTNSVKQLTSPVVAETPKKP.

SEQ ID NO: 53 – P23 *B. garinii*
MKKNTLSAILMTLFLFISCNNSGGDTASTNPDESAKGPDLTVISKKITDSNAVVLVVK
EVEALLSSIDELSKAIGKKIRNDGTLDNEANRNESLIAGAYEISKLITQKLSVLNSEELK
EKIKEAKDCSEKFTTKLRDSHAELGIQNVQDDNAKRAILKTHGNKDKGAKELKELSE
SLEKLSKAAQAALANSVKELTSPVVAESPKKP.

SEQ ID NO: 54 – P23 *B. bavariensis*

MKKNTLSAILMTLFLFISCNNSGGDSASTNPDESAKGPNLTVISKKITDSNAFLLAVK
EVEALLSSIDELSKAIGKKIKNDGTLDNEANRNESLIAGAYEISKLITQKLSVLNSEEL
KEKIKEAKDCSEKFTTKLKDSHAELGIQSVQDDNAKKAILKTHGTKDKGAKELEELF
KSLESLSKAAQAALTNSVKELTNPVVAETPKKP.

SEQ ID NO: 55 – P23 *B. afzelii*
MKKNTLSAILMTLFLFISCNNSGKGGDSTSTNPADESAKGPNLTEISKKITDSNAFVL
AVKEVETLVASIDELATKAIGKKIKNDGTLDNEANHNGSLLAGAYAISTLITQKLSVL
NSEELKAEIVKAKKCSEDFTKKLKDKHTELGKQDANDDDAKKAILKTNGDKTLGAA
ELEKLSESVTSLSKAAKESLTNSVKELTSPVVAESPKKP.

SEQ ID NO: 56 – P23 *B. californiensis*
MKKNTLSAILMTLFLFISCNNSGKDGNSASTNPADESKGPNLTEISKKITDSNAVVLA
VKEVETLLASIDELAEKAIGKKIQQNNGLGAEANKNGSLLAGVYSISTLITEKLSAMK
DSGGLKAEIEKAKDCSEKFTKKLETSHAELGKNEATDDDAKKAILRTNGDKTKGAE
ELQKLFESVGGLAKAAKEMLTNSVKELTSPVVAETPKKP.

SEQ ID NO: 57 – P31 *B. bissettii*
MKKYLLGIGLILALIACKQNVSGLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMA
TVDKLELKGTSDKNNGSGILEGVKADKSKVKLTVSEDLSTTTLEVLKEDGKTLVSKK
TTSKDKSSTEEKFNDKGELAEKTIVRANGTRLEYTEVKSDGSGKAKETLKDYALEGT
LTAEKATLVVKEGTVTLSKHISKSGEVTAELNDTDSAQATKKTGKWDAGTSTLTISV
NSKKTKNLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELKNALK.

SEQ ID NO: 58 – P31 *B. mayonii*

MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEIKVLVSKEKDKDGKYSLMAT
VDKLELKGTSDKNNGSGVLEGVKADKSKVKLTVSDDLSKTTLEVLKEDGKTLVSRK
VTSKDKSSTEEKFNEKGELAEKTMTRADETRLEYTEIKSDGSGKAKEVLKGYALEGT
LTAEKTTLVVKEGTVTLSKNISKSGEVTAELNDTDSAAATKKTGAWNSGTSTLTITA
NSKKTKDLVFTKENTITVQKYDTAGIKLEGSAVEIKTLDELKNALK.

SEQ ID NO: 59 – P31 *B. burgdorferi* B31

MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMKVLVSKEKNKDGKYDLIAT
VDKLELKGTSDKNNGSGVLEGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKK
VTSKDKSSTEEKFNEKGEVSEKIITRADGTRLEYTGIKSDGSGKAKEVLKGYVLEGTL
TAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVN
SKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIKNALK.

SEQ ID NO: 60 – P31 *B. burgdorferi* 297

MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMNVLVSKEKNKDGKYDLIAT
VDKLELKGTSDKNNGSGVLEGVKADKSKVKLTISDDLGQTTLEVFKEDGKTLVSKK
VTSKDKSSTEEKFNEKGEVSEKIITRADGTRLEYTEIKSDGSGKAKEVLKGYVLEGTL
TAEKTTLVVKEGTVTLSKNISKSGEVSVELNDTDSSAATKKTAAWNSGTSTLTITVN
SKKTKDLVFTKENTITVQQYDSNGTKLEGSAVEITKLDEIKNALK.

SEQ ID NO: 61 – P31 *B. californiensis*

MKKYLLGIGLILALIAKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMAT
VDKLELKGTSDKNNGSGVLEGVKDDKSKVKLTVSDDLSTTTLEVLKEDGKTLVSRK
ETSKDKSSTEEKFNEKGELTEKIMERSNGTRLEYTEIKTDGSGKAKETLKDFVLEGTL
TTEKAILTVKEGTVTLNKNISKSGEVTVDLNDTSTTAATKKTGKWDSSTSTLTVSVN
SKKTKDLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDEIKNALK.

SEQ ID NO: 62 – P31 *B. spielmanii*

MKKYLLGIGLILALIACKQNVSGLDEKNSTSVDVPGELKVLVSKEKDKDGKYSLMA
TVDKLELKGTSDKNDGSGVLEGVKADKSKVKLTISDHLSKTTFEVFKEDGKTLVSR
NVNSKDKSSTKEKFNEKGELSEKTLVRANGTKLEYTEIKSDGTGKAKEVLKDFTLEG
TLANEKATLTVKEGTVTLSKNIDKSGEVTVALNDTDSTAATKKTGAWDSKTSTLTIT
VNSKKTKDLVFTKQDTITVQKYDSAGTTLEGSAVEIKTLDELKNALK.

SEQ ID NO: 63 – P31 *B. valaisiana*

MKKYLLGIGLILALIACAKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLM
ATVDKVELKGTSDKNNGSGTLEGVKDDKSKVKLTISDDLNKTTFETFKEDGKTLVS
RKVNSKDKSSTVEKFNEKGELSEKTITRENGTRLEYTEIKSDGTGKAKEVLKDFTLEG

TLAADKTTLEVKEGTVTLSKHIPNSGEVTVEINDTSTTQATKKTGKWDAKTSTLTIA
VNNKNTKSLVFTKEDTITVQNYDSAGTNLEGTAVEIKTLDELKNALK.

SEQ ID NO: 64 – P31 *B. afzelii*

MKKYLLGIGLILALIACKQNVSSLDEKNSASVDLPGEMKVLVSKEKDKDGKYSLKA
TVDKIELKGTSDKDNGSGVLEGTKDDKSKAKLTIADDLSKTTFELFKEDAKTLVSRK
VSSKDKTSTDEMFNEKGELSAKTMTRENGTKLEYTEMKSDGTGKAKEVLKNFTLEG
KVANDKVTLEVKEGTVTLSKEIAKSGEVTVALNDTNTTQATKKTGAWDSKTSTLTIS
VNSKKTTQLVFTKQDTITVQKYDSAGTNLEGTAVEIKTLDELKNALK.

SEQ ID NO: 65 – P31 *B. garinii*

MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGGMTVLVSKEKDKDGKYSLEAT
VDKLELKGTSDKNNGSGTLEGEKTDKSKVKLTIADDLSQTKFEIFKEDGKTLVSKKV
TLKDKSSTEEKFNEKGETSEKTIVRANGTRLEYTDIKSDGSGKAKEVLKDFTLEGTLA
ADGKTTLKVTEGTVVLSKNILKSGEITVALDDSDTTQATKKTGKWDSKTSTLTISVN
SQKTKNLVFTKEDTITVQKYDSAGTNLEGKAVEITTLKELKDALK.

SEQ ID NO: 66 – P31 *B. bavariensis*

MKKYLLGIGLILALIACKQNVSSLDEKNSVSVDLPGEMKVLVSKEKDKDGKYSLMA
TVDKLELKGTSDKSNGSGTLEGEKSDKSKAKLTISEDLSKTTFEIFKEDGKTLVSKKV
NSKDKSSIEEKFNAKGELSEKTILRANGTRLEYTEIKSDGTGKAKEVLKDFALEGTLA
ADKTTLKVTEGTVVLSKHIPNSGEITVELNDSNSTQATKKTGKWDSNTSTLTISVNSK
KTKNIVFTKEDTITVQKYDSAGTNLEGNAVEIKTLDELKNALK.

SEQ ID NO: 67 – P39 *B. burgdorferi* B31

MNKILLLILLESIVFLSCSGKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFK
IELVLKESSSNSYLSDLEGLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYS
NDPIPANLVGMTFRAQEGAFLTGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGA
KYANKDIKISTQYIGSFADLEAGRSVATRMYSDEIDIIHHAAGLGGIGAIEVAKELGSG
HYIIGVDEDQAYLAPDNVITSTTKDVGRALNIFTSNHLKTNTFEGGKLINYGLKEGVV
GFVRNPKMISFELEKEIDNLSSKIINKEIIVPSNKESYEKFLKEFI.

SEQ ID NO: 68 – P39 *B. burgdorferi* 297

MNKILLLILLESIVFLSCSGKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFK
IELVLKESSSNSYLSDLEGLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYS
NDPIPANLVGMTFRAQEGAFLTGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGA
KYANKDIKISTQYIGSFADLEAGRSVATRMYSDEIDIIHHAAGLGGIGAIEVAKELGSG
HYIIGVDEDQAYLAPDNVITSTTKDVGRALNIFTSNHLKTNTSEGGKLINYGLKEGVV
GFVRNPKMISFELEKEIDNLSSKIINKEIIVPSNKESYEKFLKEFI.

SEQ ID NO: 69 – P39 *B. afzelii*

MNKLLLLILFEGVIFLSCSGKSGLESGIPKVSLVIDGTFDDKSFNESALNGVKKLKEEF
EIELVLKESSTNSYLSDLEGLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPV
YSNEPIPANLVGMTFRAQEGAFLTGYIAAKVSKTGKIGFLGGIEGDIVDAFRYGYEAG
AKYANKDIKIFSQYIGSFSDLEAGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGS
GHYIIGVDEDQSYLAPNNVITSTTKDVGRSLNLFTSNYLKTNTFEGGKLINYGLKEGV
VGFVRNPKMIPFEVEKEIDSLSSKIINKEVIVPYNKESYEKFLKEFI.

SEQ ID NO: 70 – P39 B. *spielmanii*

MNKLLLFILLEGIIFLSCSDKGGLENKIPKVSLIIDGTFDDKSFNESALNGVKKLKEEFE
IDLVLKESSTNSYVSDLEGLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPVY
SSEPIPANLVGMTFRAQEGAFLTGYIASKVSKTGKIGFLGGIEGDIVDAFRYGYEAGA
KYANKDIKIFSQYIGSFADIEAGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGSG
HYIIGVDEDQSYLAPNNVITSSTKDVGRSLNLFTSNYLKTNNFEGGKLINYGLKEGVV
GFVRNPKMIPFEVEKEIDSLSGKIINKEVIVPYNKESYEKFLKEFL.

SEQ ID NO: 71 – P39 *B. valaisiana*

MSKLLLLILFESIIFLSCSGKGSLEGGIPKVSVIIDGTFDDKSFNESALNGIKKVKEEFKV
EFVLKESSSNSYLSDLEGLKDTGSNLIWLIGYRFSDVAKVVSLQNSEVKYAIIDPVYSS
EPIPANLVGMTFRAQEGAFLTGYIASKVSKTGKIGFLGGIKSEIVDAFRYGYEAGAKY
ANKDIKIFTHYIGSFADLEASRSIAIKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYII
GVDEDQSYLAPDNVITSSTKDVGRALNIFTSNYLKTNTFEGGKLINYGLKEGVVGFV
RNPKMIPFELEKEIDSISSKIINKEVIVPYNKESYEKFLKEFI.

SEQ ID NO: 72 – P39 *B. garinii*

MNKSLLLILFECIIFLSCGGKGSLESEIPKVSLIIDGTFDDKSFNESALNGIKKVKEEFKI
EPVLKESSINSYLSDLEGLKDTGSNLIWLIGYKFSDVAKAVSLQNPEIKYAIIDPIYSDE
PIPANLVGMTFRSQEGAFLTGYIAAKVSKTGKIGFLGGIEGEIVDSFRYGYEAGAKYA
NKDIKISAYYIGSFADLEAGRSVATKMYSDGIDIIHHAAGLGGIGAIEVAKELGSGHYI
IGVDEDQSYLAPNNIITSATKDVGRSLNIFTSNYLKTNTFEGGRLINYGLKEGVVGFV
KNPKMIPFELEKEIDNLSSKIINKEIIVPYNKESYEKFLKE.

SEQ ID NO: 73 – consensus

MNKLLLLILFEXXXFLSCSGKGSLESXIPKVSLXIDGTFDDKSFNESALNGXKKVKEE
FKXXLVLKESSXNSYLSDLEGLKDAGSXLIWLIGYKFSDVAKAVSLQNSXMKYAIID
PXYSNXPIPANLVGMTFRAQEGAFLTGYIAAKVSKTGKIGFLGGIEGXIVDAFRYGYE
AGAKYANKDIKIFXQYIGSFADLEAGRSXATKMYSDGIDIIHHAAGLGGIGAIEVAKE
LGSGHYIIGVDEDQSYLAPXNXITSXTKDVGRSLNIFTSNYLKTNTFEGGKLINYGLK
EGVVGFVRNPKMIPFELEKEIDSLSSKIINKEXIVPYNKESYEKFLKEFI.

DETAILED DESCRIPTION

The present disclosure provides novel compositions and methods for diagnosing, and treating Lyme disease (sometimes referred to as LD herein) resulting from infection by diverse *Borrelia burgdorferi* species. As discussed above, the current Centers for Disease Control and Prevention (CDC) surveillance criteria features two-tier *Borrelia burgdorferi* sensu stricto (Bbss) testing and has been estimated to capture less than one out of every 10 cases of Lyme disease. Lyme disease prevalence is clearly underreported, and the experiments described in the Exemplification section which follows shows that a percentage of people exhibiting Lyme disease symptoms, who have failed to meet CDC surveillance criteria, are infected by members of the *Borrelia burgdorferi* sensu lato (Bbsl) complex.

The Bbsl complex comprises genetically diverse bacteria that are distributed worldwide primarily throughout the Northern hemisphere and are vectored by ixodid ticks. In the USA, LD is currently the largest vector-borne illness and causes an array of symptoms including musculoskeletal, neuropsychiatric and cardiac problems and on rare occasions even death. At least 11 Bbsl genospecies have been identified in North America including: Bbss, *B. americana*, *B. andersoni*, *B. bissettii*, *B. californiensis*, *B. carolinensis*, *B. garinii*, *B. kurtenbachii*, *B. laneii*, *B. mayonii*, and *B. spielmanii*.

To assess the impact of testing limitations and to determine levels of exposure to Bbsl, a recently developed modified Western Blot procedure was employed. The procedure, termed the line immunoblot, uses recombinant antigens from common strains and species of the Bbsl complex for the serological diagnosis of LD. As discussed in greater detail below, testing was conducted on patients with suspected tickborne disease. Data presented in the Exemplification section below confirm that the serotype makeup of spirochetal exposure appears to be more complex than has been acknowledged previously.

Using the new immunoblot test, the study of patients who met the chronic Lyme disease (CLD) case definition revealed that all had exposure to Bbsl species. Positive immunoblots were further characterized at the species level for the following Bbsl species: *B. californiensis*, *B. spielmanii*, *B. afzelii/B. garinii*, and *B. mayonii*.

Spirochetes falling into the Bbsl complex are distributed worldwide, with most cases of LD reported in the USA, Europe, and Asia. The CDC states that approximately 30,000 cases of LD are reported in the USA each year using surveillance criteria featuring two-tier Bbss testing, but when tracked by other methods it is estimated that more than 300,000 people develop LD in the USA annually. The fact that CDC surveillance criteria featuring two-tier Bbss testing captures less than one out of every ten cases shows that LD is underreported, and the results of studies described elsewhere herein suggest that some people with suspected LD have failed to meet surveillance criteria.

The immunoblot testing used in the Exemplification section below, enabled differentiation of Bbsl into five specific categories: *B. californiensis*, *B. spielmanii*, *B. mayonii*, the European species *B. afzelii/B. garinii*, and undifferentiated Bbsl species. Based on surveillance reporting in the USA, the distribution pattern of Bbss is characterized by human cases reported in all 50 states with the majority reported in the Northeast, upper Midwest and northern California. However, other Bbsl species are not detected by commercial testing in the USA. Until recently, Bbss, *B. garinii* and *B. afzelii* were considered to be the only etiologic agents of LD. Currently, nine species are said to have pathogenic potential: *B. afzelii*, *B. bavariensis*, *B. bissettii*, *Bbss*, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. spielmanii*, and *B. valaisiana*. Nine other species including *B. californiensis* have not been isolated from humans. *B. afzelii*, *B. garinii*, and *B. spielmanii* are considered to be *Borrelia* species primarily found in Eurasia, while *B. californiensis* is considered to be a North American species. Understanding of the pathogenicity of *Borrelia* species is evolving, and some species that have not been isolated from humans and are not considered to be pathogenic may be capable of causing illness.

Aspects of the instant disclosure provide a compositions and methods for quickly, easily, and accurately detecting Bbsl antibodies in a biological sample from a subject suspected of having LD, thereby satisfying the need for such a test. Because multiple Bbsl species have pathogenic potential for Lyme disease, and because geographic ranges of Bbsl species may overlap, tests for Bbsl species need to be inclusive—that is, a test needs to be able to detect antibodies to multiple species concurrently. The present disclosure provides for antigenic amino acid sequences specific for various *Borrelia* species. The amino acid sequences of the present disclosure encode antigenic peptides that have high specificity and/or sensitivity for the indicated species. The inclusion of antigenic peptides that exhibit cross-reactivity across *Borrelia* species boundaries is also important with respect to the development of inclusive serological, or other immunologically-based assays, wherein the goal is to detect infection, not necessarily to identify a particular species responsible for infection. For example, the disclosure includes panel immunoassays wherein, in the context of a single test screen, a full spectrum of Bbsl species are detectable.

Aspects of the present disclosure provide novel compositions and methods for diagnosing infection by Bbsl species. The disclosure is based, in part, on the discovery of species-specific amino acid sequences encoding antigenic peptides (which may also be referred to in the art as peptide antigens or antigens), as described herein. Aspects of the present disclosure provide antigen-specific amino acid sequences for Bbsl species. These novel amino acid sequences may be used in assays to identify Bbsl species in samples from subjects suspected of having Lyme disease, including but not limited to Bbsl species comprising *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297. With the amino acid sequences of the present disclosure, identification of Bbsl species in subject samples is performed with greater speed, sensitivity, and specificity than other current methods. The amino acid sequences of the present disclosure may be used in diagnostic and scientific assays. Non-limiting examples of suitable assays include immunoblots, line immunoblots, ELISA (enzyme-linked immunosorbent assay), etc. The amino acid sequences of the present disclosure may be used for the detection of Bbsl specific T-cells, for example, with the IgXSPOT test (IGeneX, Milpitas, CA).

In one aspect, the present disclosure is a panel for detecting IgM- or IgG-class antibodies, the panel comprising an amino acid sequence having at least 90%, 95%, 98%, 99%, 99.5%, or 100% homology to SEQ ID NO: 45, and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or

bound amino acid sequences comprise amino acid sequences having at least 90%, 95%, 98%, 99%, 99.5%, or 100% homology to the corresponding amino acid sequence and retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32 and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43. In some aspects, the panel may further comprise one or more of amino acid sequences having at least 90%, 95%, 98%, 99%, 99.5%, or 100% homology to SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant. In some aspects, the panel may not comprise an amino acid sequence having at least 90%, 95%, 98%, 99%, 99.5%, or 100% homology to SEQ ID NO: 45 or variants thereof which retain the immunological binding profile of the corresponding non-variant.

Sequences with less than 100% homology may be modified with one or more substitutions, deletions, insertions, or other modifications with respect to the amino acid sequences provided herein. Exemplary modifications include, but are not limited to conservative amino acid substitutions, which will produce molecules having functional characteristics similar to those of the molecule from which such modifications are made. Conservative amino acid substitutions are substitutions that do not result in a significant change in the activity or tertiary structure of a selected polypeptide or protein. Such substitutions typically involve replacing a selected amino acid residue with a different residue having similar physico-chemical properties. For example, substitution of Glu for Asp is considered a conservative substitution because both are similarly-sized negatively-charged amino acids. Groupings of amino acids by physico-chemical properties are known to those of skill in the art. The following groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan

(W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). One of ordinary skill in the art can determine if sequences with less than 100% homology can bind naturally- or non-naturally-occurring Bbsl-related antibodies, as well as the sensitivity and specificity of the antibody to the modified sequences. One of ordinary skill in the art will be able to identify sequences with significant homology to SEQ ID NOs: 1–46 of the present disclosure that give acceptable or equivalent responses in the methods of the present disclosure without undue experimentation, in view of the teachings of this specification.

The present disclosure, in one aspect, is a panel for detecting IgM- or IgG-class antibodies, the panel comprising SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32 and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43. In some aspects, the panel may further comprise one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant. In some aspects, the panel may not comprise SEQ ID NO: 45 or variants thereof which retain the immunological binding profile of the corresponding non-variant. Non-limiting examples of panels of the present disclosure include: a panel comprising SEQ ID NO: 45 and groups (i)-(xii); a panel comprising SEQ ID NO: 45, groups (i)-(xii), and SEQ ID NOs: 44 and 46; a panel comprising SEQ ID NO: 45, groups (i)-(iv) and (vii)-(xii); a panel comprising SEQ ID NO: 45 and groups (i)-(vi); and a panel comprising groups (i)-(vi). Variants of amino acid sequences SEQ ID NOs: 1-46 which retain the immunological binding profile of the corresponding non-variant may have conservative amino acid substitutions in conserved or non-conserved regions. The expression

"variants" encompasses any modification(s) of a specified amino acid sequence (e.g., SEQ ID NOs: 1–46) which retain(s) the immunological binding profile of the corresponding non-variant. Such modifications may include insertions and deletions (internal or from the N- or C- terminus, or both).

Alignment data is provided in the drawings showing amino acid sequence comparisons across species boundaries. One skilled in the art, using no more than routine experimentation, could design and produce antigenic peptides carrying conservative amino acid substitutions in non-conserved regions, or even at non-conserved amino acid positions as identified by alignment comparisons.

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

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

patent literature. One of ordinary skill in the art will be able to determine suitable conditions for determining the homology of the nucleic acid sequences encoding the antigenic peptides of the present disclosure.

Homologous nucleic acid sequences may be determined based on the nature of a nucleotide substitution in the nucleic acid sequence. For example, synonymous nucleotide substitutions, that is, nucleotide changes within a nucleic acid sequence that do not alter the encoded amino acid sequence, will be better tolerated and, therefore, may be more numerous in a particular nucleic acid sequence than non-synonymous nucleotide substitutions. One of ordinary skill in the art will be able to determine the suitable number and location of substitutions that may be allowed in a nucleic acid sequence that encodes an amino acid sequence of the present disclosure without adversely affecting the antigenicity of the encoded antigenic peptide, without undue experimentation.

In another aspect, the present disclosure is a panel for detecting IgM- or IgG-class antibodies, the panel comprising SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences consist of the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10; (ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; (iii) SEQ ID NO: 32 and SEQ ID NO: 33; (iv) SEQ ID NO: 27 and SEQ ID NO: 28; (v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20; (vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; (vii) SEQ ID NO: 29; (viii) SEQ ID NO: 30; (ix) SEQ ID NO: 31; (x) SEQ ID NO: 40; (xi) SEQ ID NOs: 41 and 42; and (xii) SEQ ID NO: 43. In some aspects, the panel may further consist of one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant. In some aspects, the panel may not consist of SEQ ID NO: 45 or variants thereof which retain the immunological binding profile of the corresponding non-variant. As used herein, "consist of" or "consisting of", when used as a claim transition referring to an amino acid sequence, refers to amino acid sequences having 100% homology to the specified amino acid sequence (i.e., SEQ ID NOs: 1–46).

With regard to the present disclosure, the phrase "wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences consist of the following groups" encompasses a composition having the one or more of the recited sequences and, for example, buffers, labels, etc. In other words, the sequence is limited to the sequence or sequences given but the composition is not limited. The definition specifically excludes amino acids naturally contiguous with a recited sequence being used as a label or tag, such as an oligonucleotide mass tag (OMT) for detection with mass spectrophotometry, as an element of the "composition comprising."

Labels and Tags

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

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

Further, in some aspects, the amino acid sequences of the present disclosure may be "bound." A "bound" amino acid sequence is an amino acid sequence that has been immobilized in order to permit the use of the amino acid sequence in a biological test such as, for example, immunoassays. In the context of the present disclosure, a "bound" amino acid sequence is an amino acid sequence attached (*e.g.*, covalently or non-covalently bound, etc.)

directly or indirectly to a non-natural surface or substance. Further still, the "bound" amino acid sequences of the present disclosure may be attached, directly or indirectly, to a natural surface or substance, either of which is not naturally associated with the amino acid sequence. Non-limiting examples of substances to which the amino acid sequences of the present disclosure may be bound are nitrocellulose, nylon, polyvinylidene difluoride (PVDF), plastics, metals, magnetic beads and agarose (*e.g.*, beads). Linking agents known to those of ordinary skill in the art may be used to aid or enhance binding of the amino acid sequences of the present disclosure to a surface or substance.

Production of amino acid sequences

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

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. In addition to including a nucleic acid sequence encoding an amino acid sequence of the disclosure (e.g., SEQ ID NOs: 1-46) or variants thereof which retain the immunological binding profile of the corresponding non-variants, vectors of the present disclosure also include a heterologous nucleic acid sequence. As used herein, heterologous refers to a nucleic acid sequence that does not naturally occur in the organism from which the Markush group sequences are derived. The term "vector" may also refer to a virus or organism that is capable of transporting the nucleic acid molecule. One type of vector is a plasmid, a small, circular, double-stranded, extrachromosomal DNA molecule that is physically separate from and can self-replicate independently from chromosomal DNA. Some useful vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of nucleic acids to

which they are operatively linked are referred to herein as "expression vectors." Other useful vectors, include, but are not limited to bacterial plasmids and bacterial artificial chromosomes (BACs), cosmids, and viruses such as lentiviruses, retroviruses, adenoviruses, and phages.

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

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

Assays and methods of detection

Amino acid sequences of the present disclosure may be tagged with an antibody with specificity for any of said amino acid sequences. Specificity for said amino acid sequence, *i.e.*, antibody specificity, is the property of antibodies which enables them to react preferentially with some antigenic determinants and not with others. Specificity is dependent on chemical composition, physical forces and molecular structure at the binding site. Sensitivity is how strongly the antibody binds to the antigenic determinant. One of ordinary skill in the art can easily determine specificity and sensitivity of an antibody for a particular amino acid sequence using standard affinity assays, such as immunoblotting, Ouchterlony assays, titer assays, etc.

In another aspect, the present disclosure provides methods of quickly and accurately detecting Bbsl antibodies in a sample from a subject suspected of having Lyme disease. Methods of the present disclosure for detecting Bbsl antibodies in a sample from a subject suspected of having Lyme disease, may comprise, for example, providing a biological sample (including but not limited to blood, saliva) obtained from a subject suspected of having Lyme disease, mixing the biological sample with one or more of the labeled and/or bound amino acid sequences of the present disclosure and detecting a positive immunobinding reaction which indicates the presence of Lyme disease causing bacteria specific antibodies in the sample. The antibodies may be detected by, for example, immunoblotting, Elispot, ELISA, Western blotting, lateral flow assay, or any other appropriate immunoassay known to one of ordinary skill in the art. These techniques are known to one of ordinary skill in the art and procedures can be found in common technical references. While similar, each of these techniques has its advantages and disadvantages. Other suitable techniques may be known to those of skill in the art and are incorporated herein.

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

Western blotting can involve separating proteins by electrophoresis and then transferring to nitrocellulose or other solid media (*e.g.*, polyvinylidene fluoride or PVDF-membrane and nylon membrane), and is described in more detail below. Immunoblotting can also involve applying proteins to a solid media manually or by machine. Preferably, the proteins are applied in straight lines or spots and dried, binding them to the solid support medium, *e.g.*, nitrocellulose. The proteins used in an immunoblot can be isolated from biological samples or produced by recombinant technology, as is well known by those of ordinary skill in the art. The bound proteins are then exposed to a sample or samples suspected of having antibodies specific for the target proteins. With this procedure, a known antibody can be used to determine if a protein is present in a sample, such as when the proteins of lysed cells are separated by electrophoresis and transferred to the solid medium. Western blotting allows for the identification of proteins by size as well as by specificity for a specific antibody.

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

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

For a specific example of an ELISPOT test, each well of the plate is coated with a purified cytokine-specific antibody specific for the test or cell being detected. Subject's (*i.e.*, a subject suspected of having Lyme disease) T cells are isolated and cultured in each well and stimulated with recombinant antigens of one or more sequences of the present disclosure. Lyme-positive patient cells secrete cytokine in response to stimuli, which is captured by the antibody coated in the well and further detected by ELISA.

ELISA assays are also used to detect antigens. The ELISA assay can permit the quantification of a specific protein in a mix of proteins (for example, a lysate) or determine if a peptide is present in a sample. Likewise, ELISA assays can be used to determine if a specific antibody is present by using a specific antigen as a target. As used with the present

disclosure, target amino acid sequence(s) are attached to a surface. Then, if present in the sample being tested, the reactive antibody can bind to the antigen. A secondary antibody linked to an enzyme is added, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

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

In one aspect of a method of the present disclosure, a positive result for infection by one or more Bbsl species is indicated when a biological sample obtained from a subject suspected of having Lyme disease is contacted with a screening panel of the disclosure under conditions appropriate for specific antibody binding to an epitope, and specific binding of IgM- or IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel is detected, wherein a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i)–(iii) (wherein groups (i)–(iii) are as described elsewhere herein). In another aspect of the disclosure, a positive result is

indicated when a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii) (wherein groups (i) – (iv) and (vii) – (xii) are as described elsewhere herein). In yet another aspect of the disclosure, a positive result for infection by one or more Bbsl species is indicated when a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (iii) and (v) (wherein groups (i) – (iii) and (v) are as described elsewhere herein). In another aspect, a positive result is indicated when a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (vi) (wherein groups (i) – (vi) are as described elsewhere herein).

In another aspect of a method of the present disclosure, a positive result for infection by one or more Bbsl species is indicated when a biological sample obtained from a subject suspected of having Lyme disease is contacted with a screening panel of the disclosure under conditions appropriate for specific antibody binding to an epitope, and specific binding of IgM- and IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel is detected, wherein a positive immunobinding reaction with IgM-class or IgG-class antibodies is detected for SEQ ID NO: 45, and a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i)-(iii) (wherein groups (i)–(iii) are as described elsewhere herein) and a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii) (wherein groups (i) – (iv) and (vii) – (xii) are as described elsewhere herein). In another aspect, a positive result is indicated when a positive immunobinding reaction with IgM-class or IgG-class antibodies is detected for SEQ ID NO: 45, and a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (v) (wherein groups (i) – (v) are as described elsewhere herein), and a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi) (wherein groups (i) – (vi) are as described elsewhere herein).

In yet another aspect of a method of the present disclosure, a positive result for infection by one or more Bbsl species is indicated when a biological sample obtained from a subject suspected of having Lyme disease is contacted with a screening panel of the disclosure under conditions appropriate for specific antibody binding to an epitope, and

specific binding of IgM- or IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel is detected, wherein a positive immunobinding reaction IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii) (wherein groups (i)–(iii) are as described elsewhere herein). In one aspect, a positive result is indicated when a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii) (wherein groups (i) – (iv) and (vii) – (xii) are as described elsewhere herein). In another aspect, a positive result is indicated when a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii) and (v) (wherein groups (i) – (iii) and (v) are as described elsewhere herein). In yet another aspect, a positive result is indicated when a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi) (wherein groups (i) – (vi) are as described elsewhere herein).

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

In another aspect of the disclosure, a method is provided for detecting and distinguishing various species of *Borrelia* in a sample. The sample may be from a subject suspected of having Lyme disease. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having Lyme disease and mixing or contacting the biological sample with a panel of the present disclosure. Amino acids may be labeled to confirm their presence if positive results are not obtained in the assay. In an aspect of the disclosure, a sample is considered positive in a multi-species panel assay for *Borrelia* if at least one amino acid sequence is detected from at least two groups of the panel. A sample is considered positive for a specific species of *Borrelia* if at least two amino acid sequences identified with the specific species are detected.

Subjects and cells

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

EXEMPLIFICATION

Example 1. Lyme ImmunoBlot antigen specificity

The Lyme ImmunoBlot IgG and IgM Tests are qualitative immunoblot assays that detect IgG and IgM antibodies directed against *B. burgdorferi* sensu lato species in serum samples suspected of having Lyme disease. Recombinant *B. burgdorferi* sensu lato antigens are sprayed as straight lines onto nitrocellulose strips, which are then used in the Lyme ImmunoBlot Test. Experiments were undertaken to determine the specificity of the Lyme ImmunoBlot Test.

Methods

Antigen preparation

Recombinant target proteins were obtained by cloning hybrid gene constructs or portions of genes into pET vectors, expressing the gene products in *Escherichia coli* (GenScript, Piscataway, NJ, USA), then isolating the proteins to > 90% purity, as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99; Shah et al., *Healthcare* (2019) 7:121]. Bbsl recombinant proteins were derived from several US and European species of Bbsl including Bbss strains B31 and 297 for the detection of the following targets: P23 (OspC), P31 (OspA), P34 (OspB), P39 (BmpA), P41, P93, the variable surface antigen of Bbss (VlsE), and C6 (a hybrid protein containing the immunodominant region of VlsE from different Bbsl species) for IgM ImmunoBlot and all the above, plus P66, P58, P45, P30, P28, P18, for IgG as previously described. The targeted Bbsl species were Bbss (*B. burgdorferi* B31 and *B. burgdorferi* 297), *B. afzelii*, *B. garinii*, *B. californiensis*, *B .mayonii*, *B. spielmanii*, and *B. valaisiana*. For specificity controls, recombinant proteins from several

tick-borne relapsing fever (TBRF) *Borrelia* species (*B. hermsii*, *B. miyamotoi*, *B. turicatae* and *B. turcica*) were derived for the detection of four target antigens: BipA, GlpQ, fHbp and FlaB, as previously described [Shah et al., *Healthcare* (2019) 7:121].

Preparation of antigen strips

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

Detection of antibody reactivity

Serological immunoblot testing was performed at IGeneX Reference Laboratory, a high-complexity testing facility with Clinical Laboratory Improvement Amendments (CLIA) certification. Lyme ImmunoBlot strips were tested with alkaline phosphatase (AP)-conjugated anti-rabbit antibodies rabbit anti-*Borrelia burgdorferi* serum samples and rabbit anti-TBRF Borrelia serum samples. Rabbit sera with antibodies to the following *B. burgdorferi* species were tested: *B. burgdorferi* sensu lato species *B. burgdorferi* B31 and *B. burgdorferi* 297; and *B. burgdorferi* sensu stricto species *B. californiensis*, *B. afzalii*, *B. garinii*, *B. spielmanii*, and *B. valaisiana*. Rabbit sera with antibodies to the TBRF *Borrelia* species described above were used as controls.

Reactivity between *Borrelia*-specific antibodies from test sera and *Borrelia* antigens on immunoblots was detected as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99; Shah et al., *Healthcare* (2019) 7:121]. In brief, strips were labeled, soaked in diluent (100 mM Tris, 0.9% NaCl, 0.1% Tween-20 and 1% non-fat dry milk) for 5 min in a trough, then a 10 µL aliquot of the test or control serum was added to the strip. Strips with sera were incubated at room temperature for one hour, washed 3 times with wash buffer (KPL, Gaithersburg, MD, USA) at room temperature, and the final wash solution was then aspirated. To detect IgG and IgM reactivity, strips were incubated with alkaline phosphatase-conjugated goat anti-human IgG at 1:10,000 dilution or IgM at 1:6000 dilution respectively (KPL, Gaithersburg, MD, USA) for one hour, then were washed 3 times. To visualize bands of antibody/antigen reactivity, the strips were reacted with a chromogenic substrate, 5-bromo-4-chloro-3-indolylphosphatenitro-blue tetrazolium (BCIP/NBT, KPL, Gaithersburg, MD,

USA) and the reaction was terminated by washing with distilled water after the calibration control produced a visible band at 39 kDa. Bands demonstrating an intensity lower than that of the calibration control were reported as negative.

Scoring of Immunoblots

For Bbsl immunoblots, the following bands (in kDa) were scored for IgG reactivity: 18 (group (xii) SEQ ID NO: 43), 23 (OspC; group (i) SEQ ID NOs: 1-10), 28 (group (xi) SEQ ID NOs: 41 and 42), 30 (group (x) SEQ ID NO: 40), 31 (OspA; group (v) SEQ ID NOs: 11-20), 34 (OspB; group (vi) SEQ ID NOs: 34-39), 39 (BmpA; group (ii) SEQ ID NOs: 21-26), 41 (FlaB; group (iii) SEQ ID NOs: 32 and 33), 45 (group (ix) SEQ ID NO: 31), 58 (group (viii) SEQ ID NO: 30), 66 (group (vii) SEQ ID NO: 29), and 83-93 (group (iv) SEQ ID NOs: 27 and 28). The following bands (in kDa) were scored for IgM reactivity: 23 (OspC; group (i) SEQ ID NOs: 1-10), 31 (Osp A; group (v) SEQ ID NOs: 11-20), 34 (Osp B; group (vi) SEQ ID NOs: 34-39), 39 (BmpA; group (ii) SEQ ID NOs: 21-26), 41 (FlaB; group (iii) SEQ ID NOs: 32 and 33) and 93 (group (iv) SEQ ID NOs: 27 and 28). Interpretation of immunoblots was determined by two different criteria: CDC criteria, and in-house criteria, as previously described [Liu et al., *Healthcare* (2018) 6(3) pii: E99]. By CDC criteria, IgM reactivity was interpreted as positive if two of the three antigen bands 23, 39, and 41 kDa were positive, and IgG reactivity was interpreted as positive if five of the ten antigen bands 18, 23, 28, 30, 39, 41, 45, 58, 66, and 83-93 were positive. By in-house criteria, IgM reactivity was considered positive if two of the four bands of 23, 31, 39, and 41 kDa were present, and IgG reactivity was considered positive if two out of the six bands of 23, 31, 34, 39, 41, and 83-93 kDa were present.

Results

As shown in Fig. 2, antibodies to *B. burgdorferi* species were detected, indicating that the Lyme ImmunoBlot is specific for the detection of *B. burgdorferi* group antibodies. A low signal to only 41kDa was detected with rabbit anti-TBRF Borrelia specific serum samples.

Example 2. Lyme ImmunoBlots validation study for clinical sensitivity and specificity

A total of 178 patient samples were tested as per Lyme ImmunoBlot IgM and IgG protocols to determine clinical sensitivity and specificity. The following patient samples

were tested as per Lyme Immunoblot IgM and IgG test protocols. The ImmunoBlots were read by in-house criteria and CDC criteria.

Methods

Antigen preparation

Antigens were prepared substantially as described in Example 1. The targeted Bbsl species were were Bbss (*B. burgdorferi* B31 and *B. burgdorferi* 297), *B. californiensis*, *B. spielmanii*, *B. afzelii*, *B. garinii*, and *B. mayonii*.

Preparation of antigen strips

Antigen strips were prepared substantially as described in Example 1. Protein L (Sigma, St. Louis, MO, USA) and mixed human IgM and IgG (Sigma, St. Louis, MO, USA) were used as control proteins for detecting the addition of human serum and for detecting the addition of alkaline phosphatase conjugated anti-human antibodies.

Detection of antibody reactivity

Serological immunoblot testing and detection of antibody reactivity were performed substantially as described in Example 1. Human sera from patients with confirmed *Borrelia* infection were used as positive controls and sera from uninfected persons were used as negative controls. All immunoblot testing of patient samples was performed with simultaneous testing of positive and negative control serum samples.

Scoring of Immunoblots

Bbsl immunoblots were scored substantially as described in Example 1. Immunoblot reactivity for Bbsl in representative patient serum samples is shown in Fig. 3.

Patients and Data Collection

The patient cohort was recruited from a medical practice located in San Francisco, CA, specializing in the diagnosis and treatment of tick-borne diseases. The Western Institutional Review Board (WIRB), Puyallup, WA approved the anonymous retrospective data collection protocol and consent form. Patients of either sex qualified for the study provided they were at least 18 years of age, had a medical history of musculoskeletal, neuropsychiatric and/or cardiac symptoms consistent with LD, and gave written informed consent for data collection. Subjects were included in the study if they met the case definition of untreated or previously treated chronic LD with symptoms lasting more than six

months, as described in detail elsewhere [Cameron et al., *Expert Rev Anti Infect Ther*. (2014) 12:1103–1135; Stricker et al., *Am J Infect Dis* (2018) 14:1-44]. Patients were not required to have had a documented tick bite or erythema migrans rash for participation in the study because serological testing was used to detect exposure rather than active infection. De-identified patient samples were coded according to the patient's place of residence. Blood was drawn and serum was separated at independent laboratories including BioReference®, LabCorp®, and AnyLabTestNow®, and serum samples were transported to the reference laboratory for immunoblot testing.

A total of 175 human sera expected to be negative for Bbsl and TBRF were obtained from the Centers for Disease Control and Prevention (CDC), College of American Pathologists, New York State Department of Health, New York Biologics (Southampton, NY, USA) and IGeneX Reference Laboratory (Milpitas, CA, USA). The IGeneX samples were leftover sera received for routine testing for tick-borne diseases that would otherwise be discarded. Testing of control sera was performed by laboratory personnel in a blinded fashion in the same manner as testing of clinical samples from Bbsl and TBRF patients. Results are reported in Table 1, Table 2A-C, and Table 3.

Chronic Lyme Disease cohort

To assess the ability of the Lyme Immunoblot to identify *Borrelia* seroreactivity in patients with chronic Lyme disease (CLD), an additional study was performed with serum samples from a cohort of 90 patients who met the clinical case definition of CLD, as recently described [Stricker et al., *Am J Infect Dis* (2018) 14:1-44]. Immunoblot testing was performed as described previously herein. Results are reported in Table 4.

Results

Table 1. Patient Samples Tested

			Expected Result	
Source	**Samples**	**n**	Positives	Negatives
CDC	CDC - Set 1	10	5	5
CDC	CDC - Set 2	32	12	20
Proficiency Samples	PT Samples	20	9	11

Proficiency Samples	Autoimmune(22 Reumatoid arthritis)	42	0	42
New York Biologics	Viruses	46	0	46
New York Biologics	RPR (+)	28	0	28
Total Samples		178	26	152

Table 2A. Determination of Clinical Sensitivity

Samples	**Positives**	Lyme WB (in-house)			Lyme WB (CDC)			Lyme IB (in-house)			Lyme IB (CDC)		
		IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M
CDC - Set 1	5	2	4	4	2	3	4	2	5	5	2	4	4
CDC - Set 2	12	7	8	9	7	5	9	10	7	10	9	5	9
PT Samples	9	9	6	9	9	6	9	9	6	9	9	6	9

Total Positives	**26**	18	18	22	18	14	22		21	18	24	20	15	22
Sensitivity		**69.2%**	**69.2%**	**84.6%**	**69.2%**	**53.8%**	**84.6%**		**80.8%**	**69.2%**	**92.3%**	**76.9%**	**57.7%**	**84.6%**

Table 2B. Detailed Information on Lyme Positive Patients Samples (CDC Samples Panel 1 and 2 combined)

CDC Samples	**Positives**	Lyme WB (in-house)			Lyme WB (CDC)				Lyme IB (In-house)			Lyme IB (CDC)		
		IgM	IgG	G+M	IgM	IgG	G+M		IgM	IgG	G+M	IgM	IgG	G+M
Early Lyme	10	6	5	6	5	1	6		7	5	8	6	3	6
Lyme Arthritis	4	0	4	4	0	4	4		3	4	4	2	4	4
Neurologic Lyme	3	3	3	3	3	3	3		3	3	3	3	2	3
Total Positives	17	9	12	13	8	8	13		13	12	15	11	9	13
Sensitivity		**52.9%**	**70.6%**	**76.5%**	**47.1%**	**47.1%**	**76.5%**		**76.5%**	**70.6%**	**88.2%**	**64.7%**	**52.9%**	**76.5%**

Table 3A. Determination of Clinical Specificity

Samples	**Negatives**	Lyme WB (in-house)			Lyme WB CDC data (CDC)			Lyme IB (in-house)			Lyme IB (CDC)		
		IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M

CDC - Set 1*	5	0	1	1	0	0	0	0	0	0	0	0	0
CDC - Set 2*	20	0	1	1	0	0	0	3	1	4	0	0	0
PT Samples	11	0	0	0	0	0	0	0	0	0	0	0	0
Auto-immune (22 RA)	42	0	0	0	0	0	0	0	0	0	0	0	0
Viruses (11 CMV)	46	10	5	15	2	0	2	0	0	0	0	0	0
RPR (+)	28	6	3	7	1	0	1	0	1	1	0	0	0
False Positives		16	10	24	3	0	3	3	2	5	0	0	0
True Negatives	152	136	142	128	149	152	149	149	150	147	152	152	152
Specificity		**89.5%**	**93.4%**	**84.2%**	**98.0%**	**100.0%**	**98.0%**	**98.0%**	**98.7%**	**96.7%**	**100.0%**	**100.0%**	**100.0%**

* Western blot results provided by CDC

Table 3B: Detailed Summary of viral antibody positive samples

Antibodies to	n	Lyme WB (in-house)			Lyme WB (CDC)			Lyme IB (in-house)			Lyme IB (CDC)		
		IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M
EBV	24	6	4	10	2	0	2	0	0	0	0	0	0
HSV	7	2	0	2	0	0	0	0	0	0	0	0	0
CMV	11	0	1	1	0	0	0	0	0	0	0	0	0
HCV	4	2	0	2	0	0	0	0	0	0	0	0	0
Viruses	46	10	5	15	2	0	2	0	0	0	0	0	0
Specificity		**78.3%**	**89.1%**	**67.4%**	**95.7%**	**100.0%**	**95.7%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Table 3C: CDC Specificity Samples – Summary

CDC Samples	**Negatives**	Lyme WB (in-house)			Lyme WB (CDC)			Lyme IB (in-house)			Lyme IB (CDC)		
		IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M	IgM	IgG	G+M
Fibromyalgia	2	0	0	0	0	0	0	0	0	0	0	0	0
Healthy endemic	7	0	1	1	0	0	0	0	1	0	0	0	0
Healthy non-endemic	6	0	1	1	0	0	0	0	0	1	0	0	0
Mononucelosis	2	0	0	0	0	0	0	1	0	1	0	0	0
MS	2	0	0	0	0	0	0	0	0	0	0	0	0
RA	2	0	0	0	0	0	0	1	0	1	0	0	0
Severe Peridontitis	2	0	0	0	0	0	0	1	0	1	0	0	0

Syphilis	2	0	0	0	0	0	0	0	0	0	0	0	0
False Positives		0	2	2	0	0	0	3	1	4	0	0	0
True Negatives	25	25	23	23	0	0	0	22	2	21	0	0	0
Specificity		100.0%	92.0%	92.0%	0.0%	0.0%	0.0%	88.0%	8.0%	84.0%	0.0%	0.0%	0.0%

Western blot results provided by CDC

As shown in Table 1, 14/42 patients (33%) in the Bbsl group had antibodies to Bbss based on reactivity with Bbss-specific antigens derived from the B31 and/or 297 strains. Four had antibodies to Bbss only, while the remaining ten patients reacted with Bbss and other Bbsl species. In five samples (8, 12, 51, 86, and 88) the signal intensity with multiple species including Bbss was strong. In the remaining five samples (22, 31, 52, 63, and 64) the signal with other Bbsl species was much stronger than Bbss.

The results obtained with the 175 control sera that were expected to be negative for Bbsl and TBRF yielded a false positive rate of 2.3% (4/175 samples) for the Bbsl immunoblot and 2.7% (5/175 samples) for the TBRF immunoblot (Table 3). False positive tests for Bbsl were seen with a healthy endemic serum (one control), viral infection (one control) and syphilis (two controls). False positive tests for TBRF were seen with an allergy patient serum (one control), multiple sclerosis (one control), viral infection (one control) and syphilis (two controls).

Based on the data shown in Table 1, Table 2A-B, and Tables 3A-C, the sensitivity of the Lyme ImmunoBlot is 92.3%, and the specificity is 98% for IgM and 98.7% for IgG. Interestingly, all 3 patients with false positive IgM results—one with mononucleosis, one with rheumatoid arthritis, and one with severe periodontitis—had antibodies to Osp C (23 kDa) and Osp A (31 kDa), whereas none of the 11 patients positive for antibodies to CMV or the 22 patients with rheumatoid arthritis were negative on the Lyme Immunoblot IgM. Thus it is possible that these three patients could have been exposed to *B. burgdorferi* but did not have active disease. Based on the data obtained in this study, Lyme ImmunoBlots can be used in place of Lyme Western Blots.

Fig. 3 shows ImmunoBlots of representative patient serum samples and interpreted according to in-house criteria. These results illustrate how detection of antigens from multiple Bbsl species identifies patients that would otherwise be missed by only testing for Bbss antigens.

5

Table 4. Summary of seroreactivity for CLD cohort subjects in Group 1 (Bbsl), Group 2 (TBRF) or both

ImmunoBlot	**Total**
Group 1 (*B. burgdorferi* sensu lato Positive)	**42**
Group 2 (Tick-Borne Relapsing Fever *Borrelia* Positive)	**56**
Dual Group 1 and 2 Positive	**8**
Group 1 Bbsl Positive Samples	**34 (38%)**
B. bugdorferi sensu lato	8
BB sensu stricto (B31 and/or B297)	4
B. afzelii/garinii	6
B. californiensis	6
B. spielmanii	6
B. mayonii + B. speilmanii	2
B. spielmanii + B. afzelii/garinii	1
B. afzelii/garinii, B. californiensis, B. mayonii	1
Dual Group 1 and 2	**8 (9%)**
B. burgdorferi sensu lato + Tick-Borne Relapsing Fever borrelia	2
B. burgdorferi sensu lato + B. hermsii	1
B. californiensis + Tick-Borne Relapsing Fever borrelia	2
B. spielmanii + Tick-Borne Relapsing Fever borrelia	1

B. afzelii/garinii + Tick-Borne Relapsing Fever borrelia	1
B. afzelii/garinii + B. turicatae	1

In the CLD cohort (Table 4), ImmunoBlot testing revealed that out of the 90 subjects with suspected LD, a total of 42 patients (47%) were seropositive for Bbsl (Group 1). Seroreactivity within Group 1 was as follows: Bbss (14), *B. californiensis* (8), *B. spielmanii* (10), *B. afzelii/B. garinii* (10), and mixed infections that included *B. mayonii* (3). Thirty-four patients (38%) were seropositive for Bbsl alone, 48 patients (53%) were seropositive for TBRF alone, and 8 patients (9%) were positive for both Bbsl and TBRF. Sera from 4 patients in Group 1 were seropositive for 2 or more species of Bbsl. Seroreactivity to the exact *Borrelia* species in the remaining Group 1 and Group 2 patients could not be defined using the immunoblot technique.

Forty-eight patients were positive for *B. burgdorferi*-specific antibodies. In contrast, if only *B. burgdorferi* sensu stricto (B31 and 297) were used as antigens, only 14 patients would be identified as positive. This data demonstrates that antigens from multiple species are required for an inclusive test.

Discussion

Using the Lyme ImmunoBlot test described Examples 1 and 2, this study of patients who met the CLD case definition revealed that all had exposure to either Bb or TBRF species. Positive immunoblots were further characterized at the species level for the following Bbsl: *B. californiensis*, *B. spielmanii*, *B. afzelii/B. garinii*, and *B. mayonii*. Most sera were reactive to either Bb species alone (38%) or to TBRF species alone (53%), with few seropositive to both Bb species and TBRF species (9%). The lack of widespread dual reactivity suggests that cross-reactivity in our immunoblots between TBRF and Bb species is unlikely. Immunoblot testing of control sera demonstrated excellent specificities of 97.7% for the Bbsl assay and 97.1% for the TBRF assay (Table 3).

Spirochetes falling into the Bbsl complex are distributed worldwide, with most LD cases reported in the USA, Europe, and Asia. The CDC states that approximately 30,000 cases of LD are reported in the USA each year using surveillance criteria featuring two-tier Bbss testing, but when tracked by other methods it is estimated that more than 300,000 people develop LD in the USA annually. The fact that CDC surveillance criteria featuring two-tier Bbss testing captures less than one out of every ten cases shows that LD is

underreported, and the results of our study suggest that some people with suspected LD who have failed to meet surveillance criteria may be infected by Bbsl that are not crossreactive with Bbss on two-tier testing.

The immunoblot testing used in this study enabled differentiation of Bbsl into 5 specific categories: *B. californiensis*, *B. spielmanii*, *B. mayonii*, the European species *B. afzelii*/*B. garinii*, and undifferentiated Bb species Based on surveillance reporting in the USA, the distribution pattern of Bbss is characterized by human cases reported in all 50 states with the majority reported in the Northeast, upper Midwest and northern California. Importantly, however, other Bbsl species are not detected by commercial testing in the USA. Until recently, Bbss, *B. garinii* and *B. afzelii* were considered to be the only etiologic agents of LD. Currently, nine species are said to have pathogenic potential: *B. afzelii*, *B. bavariensis*, *B. bissettii*, *Bbss*, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. spielmanii* and *B. valaisiana*. Nine other species including *B. californiensis* have not been isolated from humans. *B. afzelii*, *B. garinii* and *B. spielmanii* are considered to be *Borrelia* species primarily found in Eurasia, while *B. californiensis* is considered to be a North American species. The understanding in the art of the pathogenicity of *Borrelia* species is evolving, and some species that have not been isolated from humans and are not considered to be pathogenic may be capable of causing illness.

The genetic diversity of *Borrelia* spirochetes, and the symptoms of infection that are as diverse as the organisms causing them, makes it challenging to diagnose *Borrelia*-associated disease. It is important to recognize that classification is a human concept and the organisms encompassing the genus *Borrelia* fall within a continuous spectrum of organisms rather than into well-defined genetically-distinct groups that are easily categorized.

This study highlights the dire need for a diagnostic approach that acknowledges the complexity and genetic diversity of *Borrelia* spirochetes. Commercially available serological testing kits, as endorsed by the CDC, are highly specific for Bbss, and have poor sensitivity. The CDC case definition for LD is narrowly drawn and the laboratory criteria needed to qualify as a positive case are rigid. Commercial serological tests are based on antigens of just one Bbss strain, B31, and this test protocol is therefore not capable of detecting antibody reactivity to *Borrelia* species and strains that lack crossreactivity with B31 – a limitation that excludes detection of many *Borrelia* pathogens.

Microscopy is nonspecific: spirochetes are only visible when there are high bacterial loads in the blood, and artifacts such as pseudospirochetes (filaments derived from erythrocytes) can be mistaken for spirochetes. Ideally, a microscopic diagnosis should be

confirmed by testing using other methodologies such as serological assays. In contrast, the immunoblot testing described herein detected exposure to a variety of Bbsl species.

Bb-seropositive patients tended to be more frequently IgM positive than IgG or dual IgM/IgG positive. Prolonged IgM reactivity has been demonstrated in patients with late or longstanding LD. Furthermore, IgM reactivity has been demonstrated in human subjects with persistent Bb infection despite treatment with antibiotics. The cohort studied herein met the case description for CLD and had symptoms consistent with LD and other tickborne co-infections, such as musculoskeletal, neuropsychiatric and/or cardiac manifestations. The instant study corroborates the findings of previous studies showing that prolonged IgM reactivity is associated with Bb infections and suggests that these infections may be persistent. The fact that IgM reactivity in *Borrelia* infections is likely to persist long after the onset of symptoms should be recognized when designing testing protocols for diagnosis.

In summary, exposure to Bbsl is a cause for concern, and Bbsl may explain LD symptoms in patients who are seronegative for Bbss. Some patients may demonstrate dual exposure to both Bb and TBRF species, further complicating diagnosis and treatment. Immunoblot testing for Bbsl has allowed the detection of a diverse group of *Borrelia* serotypes and has provided a greater understanding of human exposure to pathogenic *Borrelia*.

Example 3. Lyme IGXSpot Test

IGXSpot is an enzyme-linked immunosorbent spot (ELISPOT) based assay used for monitoring cellular immune responses. ELISPOT assay is highly sensitive and accurate in detecting rare antigen-specific T cells [Jin et al., *Cells* (2013) 2:607--620].

Methods

IGXSpot Lyme Disease (LD) test uses the following *B. burgdorferi* sensu lato species- specific antigens (mixture of Osp A, Osp B, Osp C, P39, P41, P93, C6 (SEQ ID NO: 45), and VslE (SEQ ID NO: 44)) to stimulate T cells isolated from patient whole blood that are cultured inside each well of the 96-well plates. If the patient has recently been infected by *B. burgdorferi*, isolated patient T cells will respond to the stimulation by secreting Interferon gamma (IFNγ). IFNγ is captured at the bottom of the well and detected by biotinylated IFNγ antibody bound to streptavidin conjugated alkaline phosphatase (AP). When the substrate of AP is added to each well, bright blue dots will appear if IFNγ is present. Any patient sample with 3 or more blue dots is considered positive. The number of

blue dots therefore represents the likelihood of the patient being infected by *B. burgdorferi* sensu lato.

Patient sample collection and preparation

A set of 97 blood samples from patients with Lyme-like symptoms were collected in sodium heparin tubes. All the blood samples were processed as per Lyme IGXSpot procedure [Jin et al., *Cells* (2013) 2:607–620]. In addition, serum samples from all patients were tested by IgM and IgG Western blots. Detailed results are presented in Tables 5 and 6.

Results

Table 5. IgXSpot Lyme Test Data Summary

Critiera	**Lyme IGXSpot**	**Lyme Positive WB/PCR/IFA**	**Lyme WB**	
			IgM	**IgG**
IGXSpot Pos.	22	10	6	1
IGXSpot Neg.	75	45	21	14
WB/PCR/IFA & IGXSpot Neg. (Total 30 Samples)	0	0	0	0
14 All tests Negative Samples (Normal Samples)	0	0	0	0
Sensitivity		18.2%		
Specificity		100.0%		

Table 6. 22 IGXSpot Positives

	n	**PCR (+)**	**WB (+)**	**Band 31**	**Band 23**	**Band 23, 31**
WB - IgM	6	1	6	5	2	2
WB - IgG	1	0	1	1	0	0
PCR	3	3	0	2	1	0
WB & PCR (-)	12	0	0	3	2	0
Total	22	4	7	11	5	2

As shown in Table 5, 22/97 samples were positive by IGXSpot. This included one positive by Western blot and PCR (Table 5), 6 positive by Western blot only, and 3 positive by PCR. The remaining 12 samples were negative by both Western blot and PCR. Interestingly, Band 31 was present in 11/22 patients tested. This included 6 Western blot positive samples, 1 Western blot and PCR positive sample, 2 PCR positive samples and 3 Western blot and PCR negative patients samples. Five samples were positive for band 23; 2 also had band 31 present.

Of the remaining 44 Lyme negative samples, 9 samples had antibodies to other tick-borne pathogens. This included 5 with antibodies to *B. duncani*; 2 with antibodies to *Analplasma phagocytophilum*; 1 with antibodies to *Ehrlichia chaffeenssis* and 1 with antibodies to *Rickettssia.*

Based on these data, of the 55 patents positive by other tests, 10 patients (18.2%) were positive by IGXSpot. The remaining 12 patients positive by IGXSpot were negative by all other tests. Of those 12 patients, 3 had band 31 kDa present on their Western blot and 2 had band 23 kDA present on the Western blot. If these are considered true positives, then IGXSpot sensitivity is 23.8%. An additional 7 patients were positive by IgXSpot. All 44 patients negative for Lyme disease were negative by IGXSpot test. Thus the specificity was 100%. These data suggest that IGXSpot is positive in patients with late disease, who produce very low antibody levels or no antibodies. Therefore, the IGXSpot may identify patients missed by other tests.

Example 4. Lyme ImmunoBLOT Validation Study with blinded CDC Samples

A set of 280 blinded serum samples were provided by CDC. Of these 90 were from patients confirmed positive for infection with *Borrelai burgdorferi* group. The remaining 190 were from non-Lyme patients. All samples were tested by Lyme ImmunoBlots IgM and IgG to determine clinical sensitivity and specificity.

Methods

Recombinant proteins derived from several US and European species of Bbsl were used to prepare antigen strips for Lyme IBs. The recombinant proteins selected included all the proteins used in scoring WBs by the CDC and in-house criteria described below. P23 (OspC) and P31 (OspA) proteins from several different BBsl species were used as target antigens in the Lyme IB. Separate P39 (BmpA) antigens derived from European and US

Bbsl species were included in the panel of test antigens. Additionally, a hybrid protein containing the immunodominant region of VslE from different Bbsl species referred to elsewhere herein as C6 (SEQ ID NO: 45) and also termed "Tier 1 antigen" was used in the Lyme IBs as a target antigen. Recombinant antigens were prepared by cloning the hybrid gene constructs or portions of the selected genes into pET vectors, and then expressing the proteins in *Escherichia coli* (GenScript, Piscataway, NJ, USA). The *E. coli*-produced recombinant Bbsl proteins were then purified using metal affinity chromatography followed by gel filtration. All the recombinant proteins were >90% pure by Coomassie blue staining after SDS PAGE.

Preparation of Lyme ImmunoBlot Strips

Purified proteins and two control proteins, diluted to yield 7–19 ng of protein as a line in each 3 mm strip of membrane were sprayed in straight lines onto nitrocellulose membrane (Amersham Protran, GE Healthcare Life Science) using a BioDot liquid dispenser (BioDot, Irvine, CA). The two control proteins were Protein L (Sigma, St. Louis, MO) for detecting the addition of human serum (termed serum control), and a mixture of human IgM and IgG (Sigma, St. Louis, MO) for detecting the addition of alkaline phosphatase conjugated anti-human antibodies (termed conjugate control). The membranes were then blocked with 5% non-fat dry milk and sliced into 3 mm wide strips.

Procedure for Detection of Borrelia Specific Antibodies on Lyme Immunoblots and Western Blots with Test Sera

Prior to use, each strip was labeled and then soaked in 1 mL of diluent (100 mM Tris, 0.9% NaCl, 0.1% Tween-20 and 1% non-fat dry milk) for 5 min in a trough. A 10 µL aliquot of the test or control serum was added to a corresponding IB or WB strip in the trough. The strips were then incubated at room temperature for one hour with serum, followed by three washes with wash buffer (KPL, Gaithersburg, MD, USA) at room temperature. After aspirating the final wash solution, strips for detecting IgG and IgM were incubated with alkaline phosphatase-conjugated goat anti-human IgG at 1:10,000 dilution and IgM at 1:6000 dilution respectively (KPL, Gaithersburg, MD, USA) for one hour. After three washes, bands were visualized by reaction with 5-bromo-4-chloro-3-indolylphosphatenitro-blue tetrazolium (BCIP/NBT, KPL, Gaithersburg, MD, USA). The reactions were terminated by washing with distilled water when a calibration control produced a visible band at 39 kDa. Alkaline phosphatase-conjugated rabbit antibody to the 39/93 kDa BBsl antigens (Strategic

Biosciences, Stow, MA, USA) diluted in human serum was used as the calibration control as previously described. Bands with lower intensity than the calibration control were reported as negative. The Lyme IB strips were also reacted with a mixture of human sera from patients with confirmed Lyme disease as a positive control and sera from uninfected persons as a negative control.

Scoring and Interpretation of Positive Serological Reactions

The following antigen bands in kDa were scored on the Lyme IB strips: for IgG—C6/Tier 1 antigen (SEQ ID NO: 45), 18 (group (xii) SEQ ID NO: 43), 23 (OspC; group (i) SEQ ID NOs: 1-10), 28 (group (xi) SEQ ID NOs: 41 and 42), 30 (group (x) SEQ ID NO: 40), 31 (OspA; group (v) SEQ ID NOs: 11-20), 34 (OspB; group (vi) SEQ ID NOs: 34-39), 39 (BmpA; group (ii) SEQ ID NOs: 21-26), 41 (FlaB; group (iii) SEQ ID NOs: 32 and 33), 45 (group (ix) SEQ ID NO: 31), 58 (group (viii) SEQ ID NO: 30), 66 (group (vii) SEQ ID NO: 29), and 93 (group (iv) SEQ ID NOs: 27 and 28); for IgM—C6/Tier 1 antigen (SEQ ID NO: 45), 23 (OspC; group (i) SEQ ID NOs: 1-10), 31 (Osp A; group (v) SEQ ID NOs: 11-20), 34 (OspB; group (vi) SEQ ID NOs: 34-39), 39 (BmpA; group (ii) SEQ ID NOs: 21-26), 41 (FlaB; group (iii) SEQ ID NOs: 32 and 33), and 93 (group (iv) SEQ ID NOs: 27 and 28). Tier 1 scoring: the sample was considered positive for Tier 1 if either IgM or IgG IB had a positive Tier-1 antigen band. Tier 2 scoring: IgG was considered positive if two from the following six antigens bands were present: 23, 31, 34, 39, 41 and 93 kDa. IgM IB was considered positive if two out of the five following bands were present: 23, 31, 34, 39 and 41 kDa. Results are summarized in Tables 7 and 8.

A sample was considered two-tier IgM-positive if positive by Tier 1 and IgM-positive by Tier 2. A sample was considered two-tier IgG positive if positive by Tier 1 and IgG-positive by Tier 2. A sample was considered two-tier IgM and IgG positive if positive by Tier 1 and IgM and IgG positive by Tier 2.

Results and Discussion

As shown in Tables 7 and 8, using the two-tier scoring criteria described above herein, the sensitivity of Lyme IgM immunoblot was 78.9%; the sensitivity of Lyme IgG immunoblot, 65.6%; and the overall sensitivity, 88.9%. The specificity was 97.9% for IgM; 100% for IgG; and 97.9% overall.

Table 7. Sensitivity of two-tier Lyme ImmunoBlot IgM and IgG (N=90)

Disease	N	IgM	IgG	IgM and IgG	Overall
Early Lyme	60	21	4	27	52
Cardiac Lyme	3	0	0	2	2
Lyme arthritis	20	0	5	15	20
Lyme arthritis	7	0	0	6	6
Total Lyme (+)	90	21	9	50	80
Total Lyme (+)	**90**	**71**	**59**		**80**
Sensitivity		**78.9%**	**65.6%**		**88.9%**

Table 8. Specificity of two-tier Lyme ImmunoBlot IgM and IgG (N=190)

Disease	N	IgM	IgG	IgM and IgG	Overall
Fibromyalgia	15	1	0	0	1
Mononucleosis	15	0	0	0	0
Multiple sclerosis	15	0	0	0	0
Periodontitis	15	0	0	0	0
Rheumatoid arthritis	15	1	0	0	1
Syphilis	15	1	0	0	1
Endemic Negative Controls	50	0	0	0	0
Non-Endemic Negative Controls	50	1	0	0	1
False Positive	0	4	0	0	4
True Negative	**190**	**186**	**190**	**190**	**186**
Specificity		**97.9%**	**100.0%**	**100%**	**97.9%**

Conclusion

Based on the data presented, two-tier Lyme ImmunoBlots have the sensitivity and specificity for clinical use, for detection of *B. burgdorferi* sensu lato antibodies in patients' sera.

Equivalents

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

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

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

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

What is claimed is:

CLAIMS

1. A panel for detecting IgM- or IgG-class antibodies, the panel comprising SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups:

(i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10,

(ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26,

(iii) SEQ ID NO: 32, and SEQ ID NO: 33,

(iv) SEQ ID NO: 27 and SEQ ID NO: 28;

(v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20,

(vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39,

(vii) SEQ ID NO: 29,

(viii) SEQ ID NO: 30,

(ix) SEQ ID NO: 31,

(x) SEQ ID NO: 40,

(xi) SEQ ID NOs: 41 and 42, and

(xii) SEQ ID NO: 43.

2. The panel of claim 1, wherein the labelled and/or tagged and/or bound amino acid sequences are bound to a substance selected from the group consisting of nitrocellulose, nylon, polyvinylidene difluoride (PVDF), magnetic beads, and agarose.

3. The panel of claim 1 further comprising one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

4. The panel of claim 1, wherein each of the one or more amino acid sequences are tagged with an antibody with specificity for the amino acid sequence.

5. A method for detecting IgM- or IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease, the method comprising:

(a) providing a screening panel comprising SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups:

(i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10,

(ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26,

(iii) SEQ ID NO: 32, and SEQ ID NO: 33,

(iv) SEQ ID NO: 27 and SEQ ID NO: 28;

(v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20,

(vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39,

(vii) SEQ ID NO: 29,

(viii) SEQ ID NO: 30,

(ix) SEQ ID NO: 31,

(x) SEQ ID NO: 40,

(xi) SEQ ID NOs: 41 and 42, and

(xii) SEQ ID NO: 43;

(b) providing the biological sample obtained from the subject suspected of having Lyme disease;

(c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and

(d) detecting specific binding of IgM- or IgG-class antibodies, if present in the

biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when:

(1) a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i)–(iii),

(2) a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii),

(3) a positive immunobinding reaction with IgM-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (iii) and (v), or

(4) a positive immunobinding reaction with IgG-class antibodies is detected for SEQ ID NO: 45 and for at least one amino acid sequence from at least two of groups (i) – (vi),

and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject.

6. The method of claim 5 wherein the binding of IgM-class antibodies is detected through the use of an anti-human IgM antibody linked to a detectable moiety.

7. The method of claim 5 wherein the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety.

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

9. The method of Claim 8, wherein the detectable moiety comprises alkaline phosphatase.

10. The method of Claim 8, wherein the detectable moiety comprises biotin.

11. The method of claim 5, wherein the one or more Bbsl species comprise *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297.

12. The method of claim 5, wherein the screening panel of step (a) further comprises at least one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

13. A method for detecting IgM-class and IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease, the method comprising:

(a) providing a screening panel comprising SEQ ID NO: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups:

(i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10,

(ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26,

(iii) SEQ ID NO: 32, and SEQ ID NO: 33,

(iv) SEQ ID NO: 27 and SEQ ID NO: 28;

(v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20,

(vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39,

(vii) SEQ ID NO: 29,

(viii) SEQ ID NO: 30,

(ix) SEQ ID NO: 31,

(x) SEQ ID NO: 40,

(xi) SEQ ID NOs: 41 and 42, and

(xii) SEQ ID NO: 43;

(b) providing the biological sample obtained from the subject suspected of having Lyme disease;

(c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and

(d) detecting specific binding of IgM-class and IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when a positive immunobinding reaction with IgM-class or IgG-class antibodies is detected for SEQ ID NO: 45 and:

(1)(A) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i)-(iii), and

(1)(B) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii); or

(2)(A) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (v), and

(2)(B) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi),

and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject.

14. The method of claim 13 wherein the binding of IgM-class antibodies is detected through the use of an anti-human IgM antibody linked to a detectable moiety.

15. The method of claim 13 wherein the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety.

16. The method of claim 14 or 15, wherein the detectable moiety is selected from the group consisting of chromophores, radioactive moieties, and enzymes.

17. The method of Claim 16, wherein the detectable moiety comprises alkaline phosphatase.

18. The method of Claim 16, wherein the detectable moiety comprises biotin.

19. The method of claim 13, wherein the one or more Bbsl species comprise *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297.

20. The method of claim 13, wherein the screening panel of step (a) further comprises at least one or more of SEQ ID NO: 44 and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

21. A method for detecting IgM- or IgG-class antibodies resulting from infection by one or more *Borrelia burgdorferi* sensu lato (Bbsl) species, if present in a biological sample obtained from a subject suspected of having Lyme disease, the method comprising:

(a) providing a screening panel comprising a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups:

(i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10,

(ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26,

(iii) SEQ ID NO: 32, and SEQ ID NO: 33,

(iv) SEQ ID NO: 27 and SEQ ID NO: 28,

(v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20,

(vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39,

(vii) SEQ ID NO: 29,

(viii) SEQ ID NO: 30,

(ix) SEQ ID NO: 31,

(x) SEQ ID NO: 40,

(xi) SEQ ID NOs: 41 and 42, and

(xii) SEQ ID NO: 43;

(b) providing the biological sample obtained from the subject suspected of having Lyme disease;

(c) contacting the biological sample with the screening panel of step (a) under conditions appropriate for specific antibody binding to an epitope; and

(d) detecting specific binding of IgM- or IgG-class antibodies, if present in the biological sample, with amino acid sequences included in the selected groups of the screening panel of step (a), wherein the sample is scored as positive for infection by one or more Bbsl species when:

(1) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii),

(2) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least five of groups (i) – (iv) and (vii) – (xii),

(3) a positive immunobinding reaction with IgM-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (iii) and (v), or

(4) a positive immunobinding reaction with IgG-class antibodies is detected for at least one amino acid sequence from at least two of groups (i) – (vi),

and wherein a positive score for infection indicates the presence of antibodies to one or more Bbsl species in the subject.

22. The method of claim 21 wherein the binding of IgM-class antibodies is detected through the use of an anti-human IgM antibody linked to a detectable moiety.

23. The method of claim 21 wherein the binding of IgG-class antibodies is detected through the use of an anti-human IgG antibody linked to a detectable moiety.

24. The method of claim 22 or 23, wherein the detectable moiety is selected from the group consisting of chromophores, radioactive moieties, and enzymes.

25. The method of Claim 24, wherein the detectable moiety comprises alkaline phosphatase.

26. The method of Claim 24, wherein the detectable moiety comprises biotin.

27. The method of claim 21, wherein the one or more Bbsl species comprise *B. afzelii*, *B. garinii*, *B. californiensis*, *B. spielmanii*, *B. mayonii*, *B. valaisiana*, *B. bavariensis*, *B. burgdorferi* B31, and *B. burgdorferi* 297.

28. The method of claim 21, wherein the screening panel of step (a) further comprises at least one or more of SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46, and variants thereof which retain the immunological binding profile of the corresponding non-variant.

FIG. 1A

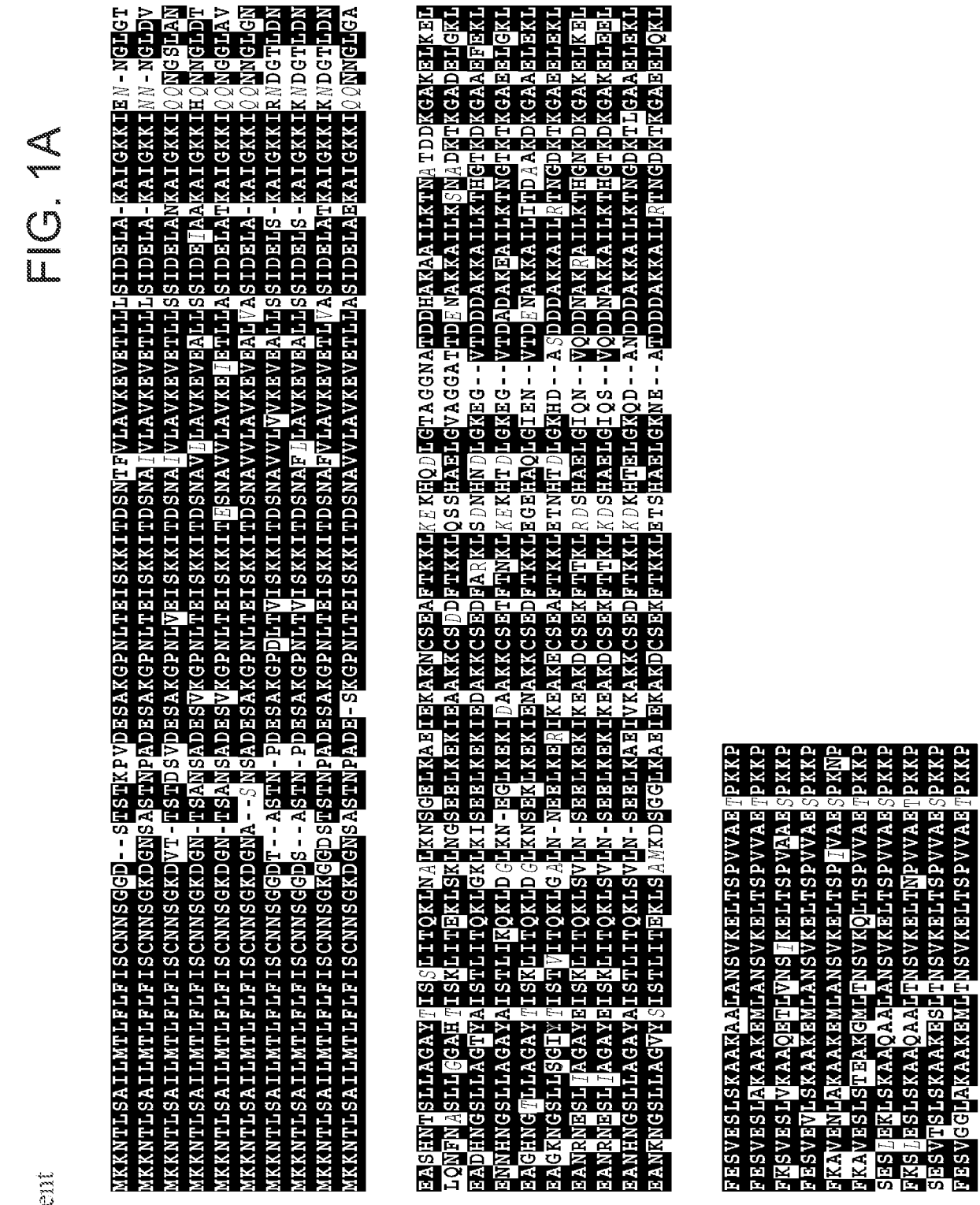
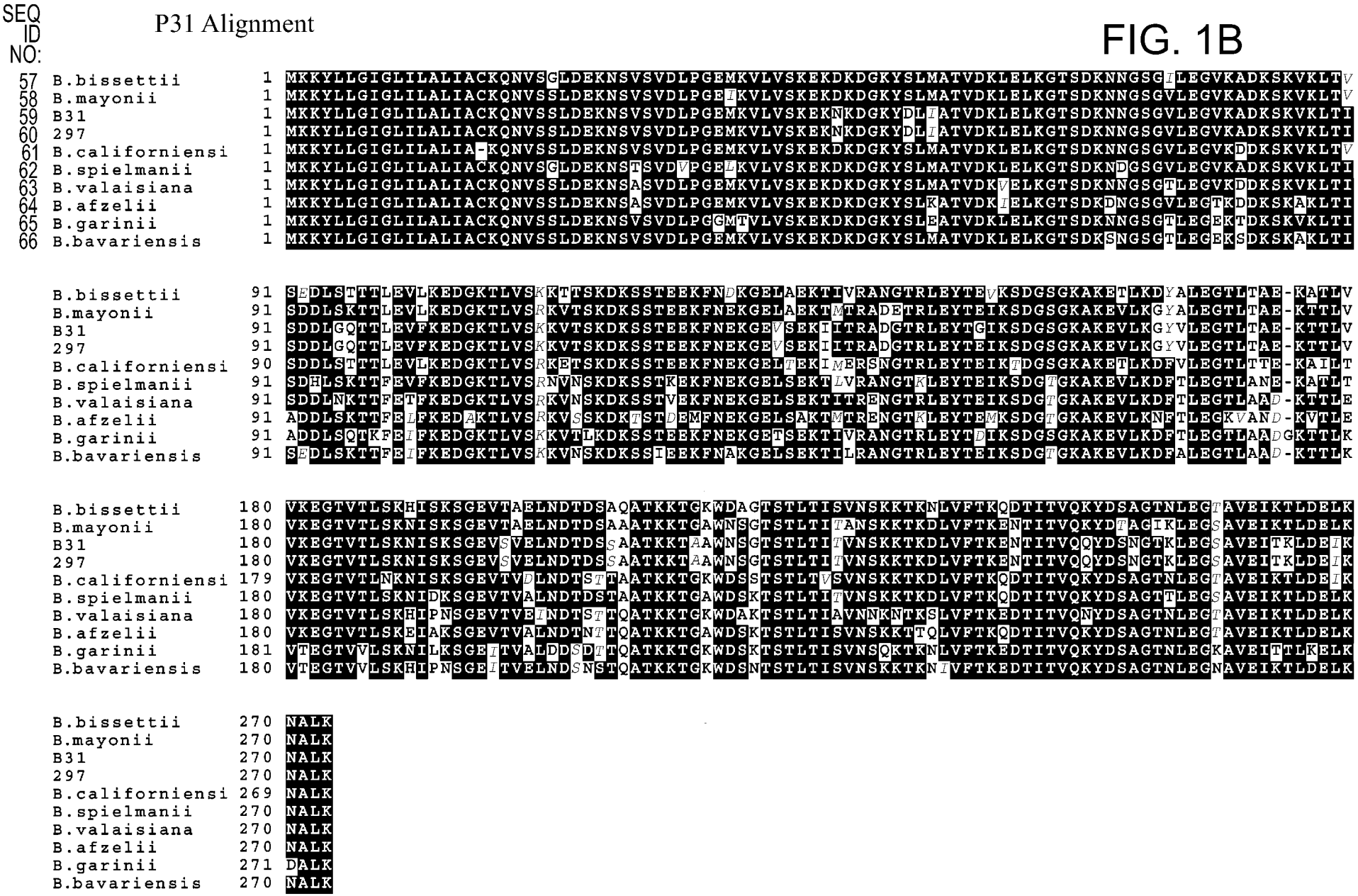

P23 Alignment
SEQ ID NO:
47 B.spielmanii
48 B.bissettii
49 B.valaisiana
50 B31
51 297
52 B.mayonii
53 B.garinii
54 B.bavariensis
55 B.afzelii
56 B.californiensi

FIG. 1B

P31 Alignment



SEQ ID NO:

P39 Alignment

FIG. 1C

```
67 B31            1 MNKILLLLILLESIVFLSCSGKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFKIELVLKESSS
68 297            1 MNKILLLLILLESIVFLSCSGKGSLGSEIPKVSLIIDGTFDDKSFNESALNGVKKVKEEFKIELVLKESSS
69 afzelii        1 MNKLLLLLILFEGVIFLSCSGKSGLESGIPKVSLVIDGTFDDKSFNESALNGVKKLKEEFEIELVLKESST
70 spielmanii     1 MNKLLLFILFEGIIFLSCSDKGGLENKIPKVSLIIDGTFDDKSFNESALNGVKKLKEEFEIDLVLKESST
71 valaisiana     1 MSKLLLLLILFESIIFLSCSGKGSLEGGIPKVSVIIDGTFDDKSFNESALNGIKKVKEEFKVEFVLKESSS
72 garinii        1 MNKSLLLILFECIIFLSCGGKGSLESEIPKVSLIIDGTFDDKSFNESALNGIKKVKEEFKIEPVLKESSI
73 Consensus      1 MNKLLLLLILFE.!!FLSCSGKGSLES.IPKVSL!IDGTFDDKSFNESALNG!KKVKEEFK!#LVLKESS.

   B31           71 NSYLSDLEGLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYSNDPIPANLVGMTFRAQEGAFL
   297           71 NSYLSDLEGLKDAGSDLIWLIGYRFSDVAKVAALQNPDMKYAIIDPIYSNDPIPANLVGMTFRAQEGAFL
   afzelii       71 NSYLSDLEGLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPVYSNEPIPANLVGMTFRAQEGAFL
   spielmanii    71 NSYVSDLEGLKDAGSNLIWLIGYKFSDVAKAVSLQNSEMKYAIIDPVYSSEPIPANLVGMTFRAQEGAFL
   valaisiana    71 NSYLSDLEGLKDTGSNLIWLIGYRFSDVAKVVSLQNSEVKYAIIDPVYSNEPIPANLVGMTFRAQEGAFL
   garinii       71 NSYLSDLEGLKDTGSNLIWLIGYKFSDVAKAVSLQNPEIKYAIIDPIYSDEPIPANLVGMTFRSQEGAFL
   Consensus     62 NSYLSDLEGLKDAGS#LIWLIGYKFSDVAKAVSLQNS#MKYAIIDP!YSN#PIPANLVGMTFRAQEGAFL

   B31          141 TGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGAKYANKDIKISTQYIGSFADLEAGRSVATRMYSDE
   297          141 TGYIAAKLSKTGKIGFLGGIEGEIVDAFRYGYEAGAKYANKDIKISTQYIGSFADLEAGRSVATRMYSDE
   afzelii      141 TGYIAAKVSKTGKIGFLGGIEGDIVDAFRYGYEAGAKYANKDIKIFSQYIGSFSDLEAGRSVATKMYSDG
   spielmanii   141 TGYIASKVSKTGKIGFLGGIEGDIVDAFRYGYEAGAKYANKDIKIFSQYIGSFADIEAGRSVATKMYSDG
   valaisiana   141 TGYIASKVSKTGKIGFLGGIKSEIVDAFRYGYEAGAKYANKDIKIFTHYIGSFADLEASRSIAIKMYSDG
   garinii      141 TGYIAAKVSKTGKIGFLGGIEGEIVDSFRYGYEAGAKYANKDIKISAYYIGSFADLEAGRSVATKMYSDG
   Consensus    128 TGYIAAKVSKTGKIGFLGGIEG#IVDAFRYGYEAGAKYANKDIKIF.QYIGSFADLEAGRS!ATKMYSDG

   B31          211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQAYLAPDNVITSTTKDVGRALNIFTSNHLKTNTFEG
   297          211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQAYLAPDNVITSTTKDVGRALNIFTSNHLKTNTSEG
   afzelii      211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPNNVITSTTKDVGRSLNLFTSNYLKTNTFEG
   spielmanii   211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPNNVITSSTKDVGRSLNLFTSNYLKTNNFEG
   valaisiana   211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPDNVITSSTKDVGRALNIFTSNYLKTNTFEG
   garinii      211 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAPNNIITSATKDVGRSLNIFTSNYLKTNTFEG
   Consensus    195 IDIIHHAAGLGGIGAIEVAKELGSGHYIIGVDEDQSYLAP#N!ITS.TKDVGRSLNIFTSNYLKTNTFEG

   B31          281 GKLINYGLKEGVVGFVRNPKMISFELEKEIDNLSSKIINKEIIVPSNKESYEKFLKEFI
   297          281 GKLINYGLKEGVVGFVRNPKMISFELEKEIDNLSSKIINKEIIVPSNKESYEKFLKEFI
   afzelii      281 GKLINYGLKEGVVGFVRNPKMIPFEVEKEIDSLSSKIINKEVIVPYNKESYEKFLKEFI
   spielmanii   281 GKLINYGLKEGVVGFVRNPKMIPFEVEKEIDSLSGKIINKEVIVPYNKESYEKFLKEFL
   valaisiana   281 GKLINYGLKEGVVGFVRNPKMIPFELEKEIDSISSKIINKEVIVPYNKESYEKFLKEFI
   garinii      281 GRLINYGLKEGVVGFVKNPKMIPFELEKEIDNLSSKIINKEIIVPYNKESYEKFLKE..
   Consensus    262 GKLINYGLKEGVVGFVRNPKMIPFELEKEIDSLSSKIINKE!IVPYNKESYEKFLKEFI
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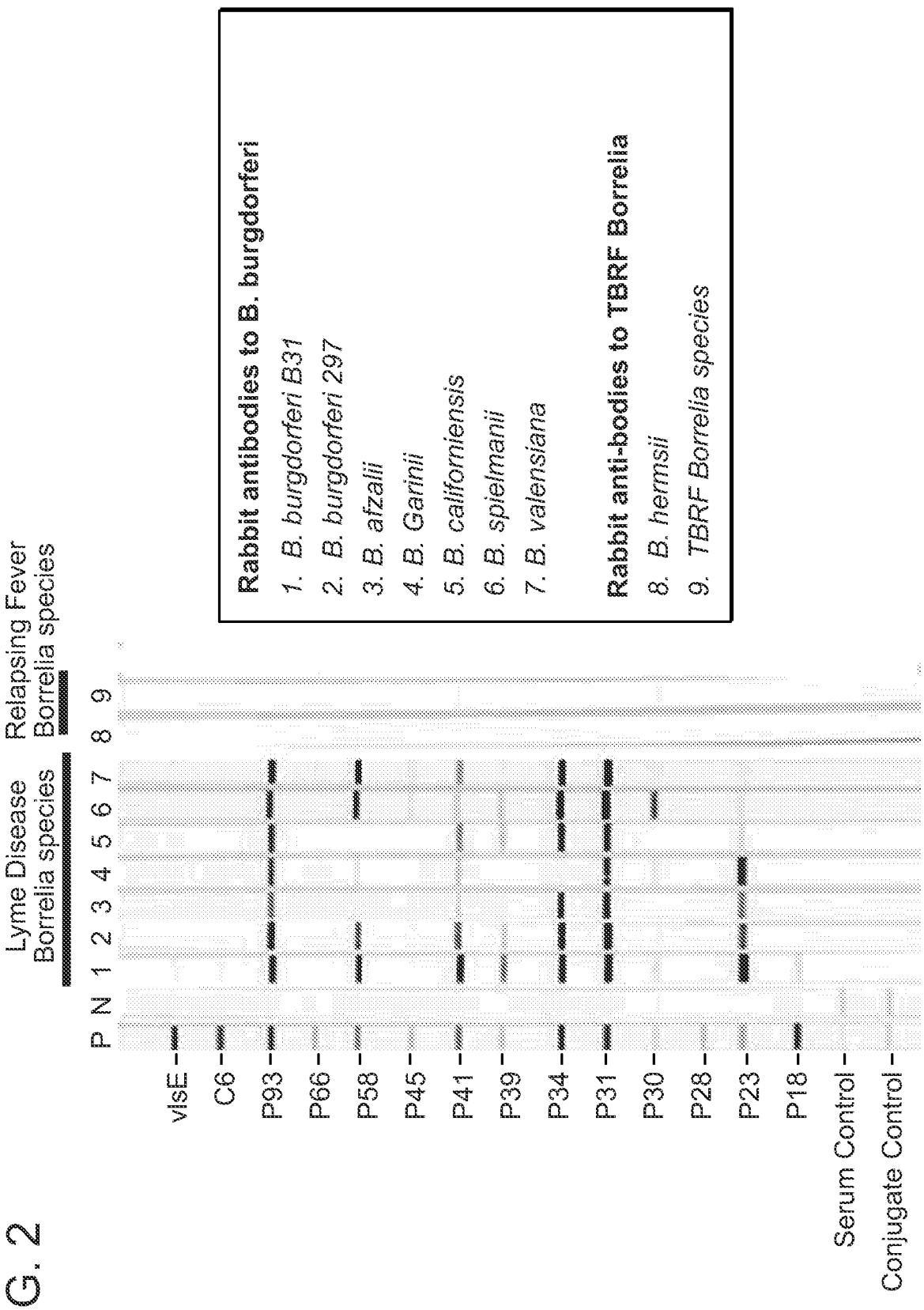
FIG. 2


Lyme Disease
Borrelia species
Relapsing Fever
Borrelia species
P N 1 2 3 4 5 6 7 8 9
vlsE
C6
P93
P66
P58
P45
P41
P39
P34
P31
P30
P28
P23
P18
Serum Control
Conjugate Control
Rabbit antibodies to B. burgdorferi
1. B. burgdorferi B31
2. B. burgdorferi 297
3. B. afzalii
4. B. Garinii
5. B. californiensis
6. B. spielmanii
7. B. valensiana
Rabbit anti-bodies to TBRF Borrelia
8. B. hermsii
9. TBRF Borrelia species

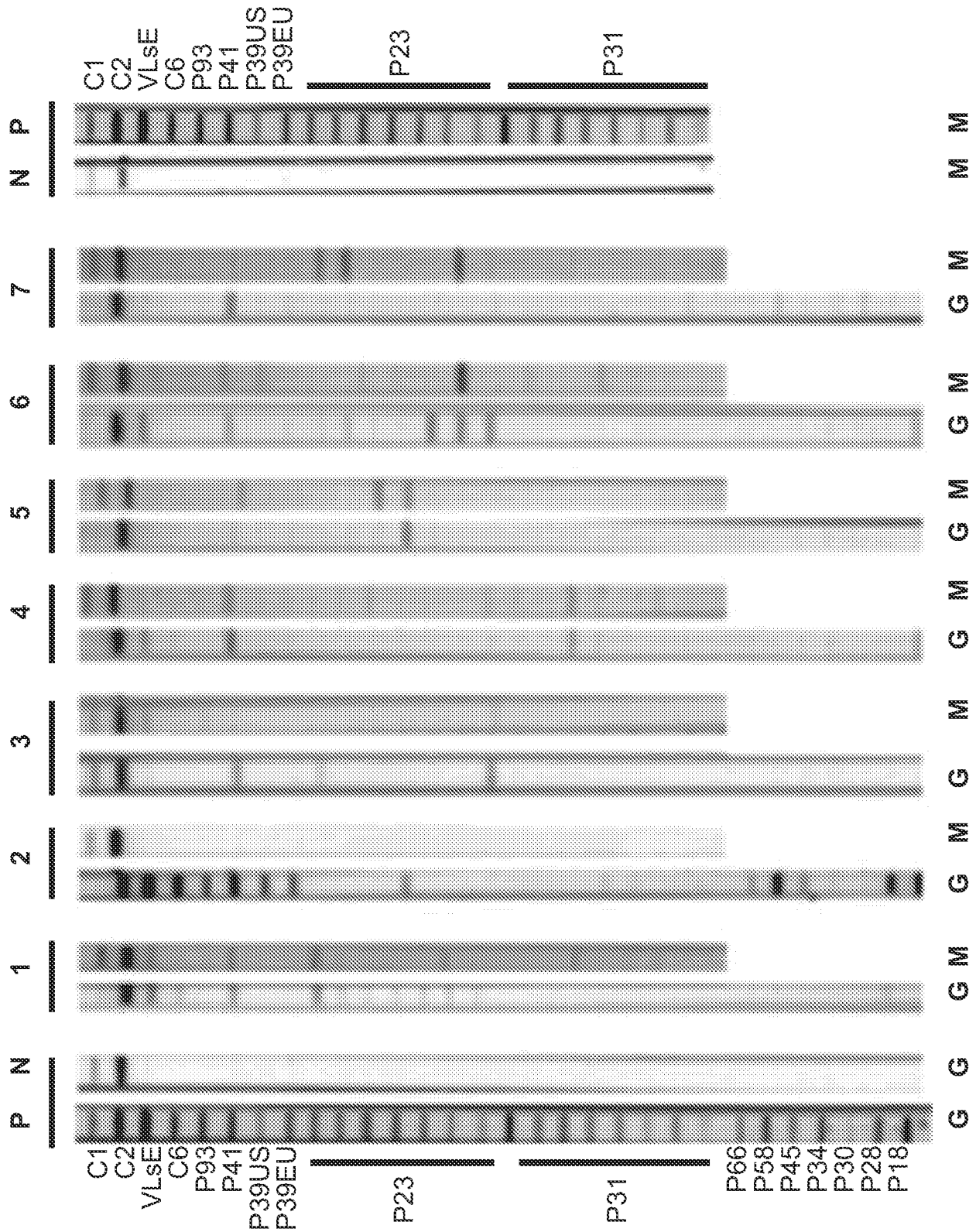

C1
C2
VLsE
C6
P93
P41
P39US
P39EU
P23
P31
P66
P58
P45
P34
P30
P28
P18
P
N
1
2
3
4
5
6
7
G
M

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/27163

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/04, G01N 33/569, G01N 33/68 (2021.01)

CPC - C12Q 1/04, G01N 2333/20, G01N 2458/00, G01N 2469/20

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)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0162875 A1 (DATTWYLER et al.) 25 June 2009 (25.06.2009) para [0028], [0060], [0063], [0065]; Claim 20	1-4
A	WO 2008/031133 A2 (INTERCELL AG) 20 March 2008 (20.03.2008) Abstract, Claim 14; SEQ ID NO: 470	1-4
A	WO 2011/112805 A2 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 15 September 2011 (15.09.2011) Claim 5; SEQ ID NO 4	1-4

☐ Further documents are listed in the continuation of Box C. ☐ 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
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"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)
"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
7 September 2021	SEP 23 2021
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. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/27163

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-4, directed to a panel for detecting IgM- or IgG-class antibodies, the panel comprising SEQ: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences.

Group II, claims 5-28, directed to a method for detecting IgM- or IgG-class antibodies resulting from infection by one or more Borreha burgdotferi sensu lato (Bbsl) species, if present in a biological sample obtained from a subject.

----------Please see Supplemental Sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/27163

Continuation of Box No. III Observations where unity of invention is lacking:

The inventions listed as Groups I-II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of a composition comprising or consisting of a panel, that is not required by Group II.

Group II has the special technical feature of contacting a biological sample with a screening panel under conditions appropriate for specific antibody binding to an epitope; and detecting specific binding of IgG and/or IgM class antibodies, it present in the biological sample, that is not required by Group I.

Common technical features:

Groups I-II share the common technical features of detecting IgM- or IgG-class antibodies, and a panel comprising SEQ: 45 and a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant, and wherein the plurality of groups of labelled and/or tagged and/or bound amino acid sequences is selected from the following groups: (i) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10,
(ii) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26,
(iii) SEQ ID NO: 32, and SEQ ID NO: 33,
(iv) SEQ ID NO: 27 and SEQ ID NO: 28;
(v) SEQ ID NO: 11, SEQ ID NO: 12, SEQ fD NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16. SEQ ID NO: 17, SEQ ID NO: 18, SEQ ED NO: 19, and SEQ ID NO: 20,
(vi) SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39,
(vii.) SEQ ID NO: 29,
viii) SEQ ID NO: 30,
(ix) SEQ ID NO: 31,
(x) SEQ ID NO: 40,
(xi) SEQ ID NOs: 41 and 42, and.
(xii) SEQ ID NO: 43.

However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is previously made obvious by US 2009/0162875 A1 to Dattwyler et al. (hereinafter 'Dattwyler') and US 2012/0142023 A1 to Ascoli et al., (hereinafter 'Ascoli').

Dattwyler teaches a panel for detecting 1gM- or IgG-class antibodies, the panel comprising a plurality of groups of labelled and/or tagged and/or bound amino acid sequences, wherein the labelled and/or tagged and/or bound amino acid sequences comprise amino acid sequences and variants thereof which retain the immunological binding profile of the corresponding non-variant (para [0063] - "The diagnostic method comprises taking a sample of body fluid or tissue likely to contain antibodies. 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 the detection of early 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)."; para [0065] - "In embodiments of the invention, the assay may comprise (1) immobilizing the antibody(s) in the sample, adding a peptide of the invention, and then detecting the degree of antibody bound to the peptide, e.g. by the peptide being labeled or by adding a labeled substance (conjugate, binding partner), such as a labeled antibody, which specifically recognizes the peptide; (2) immobilizing a peptide of the invention, adding the sample containing an antibody(s), and then detecting the amount of antibody bound to the peptide, e.g. by adding a labeled substance (conjugate, binding partner), such as a labeled antibody, which specifically recognizes the antibody; or (3) reacting the peptide and the sample containing antibody(s) 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 (conjugate, binding partner), such as a labeled antibody, which specifically recognizes the peptide."; para [0028] - "The invention includes a peptide represented by SEQ ID NO:1, as well as active variants of this peptide. An "active variant" of this peptide, or of other peptides described herein, refers to a peptide which retains the ability to specifically recognize (bind to) an antibody against a causative agent of Lyme disease."; para [0060] - "Thus, "a" peptide of the present invention, as used above, can be two or more peptides, which can be the same or different.").

Ascoli teaches a test strip for detection of IgG or IgM antibodies specific for Borrelia burgdorferi, comprising a plurality of bound amino acid sequences including a sequence with 99.9% identity t SEQ ID NO: 29 (para [0026] "test strips to detect the presence of B. burgdorferi or to detect and diagnose LD. The test strip contains at least two regions, each of which containing a protein selected from...P66 (SEQ ID NO: 15)"; [0033] "a four (4) window cassette for B. burgdorferi-specific IgG or IgM antibody detection in accordance with the preferred embodiment of the present invention"; note, SEQ ID NO: 15 residues 25-618 have 99.9% identity to SEQ ID NO: 29, and 100% identity except for a single mismatch L to M corresponding to the residue at position 1 of the claimed sequence). The addition of a Met at the start of the sequence was a practice well known in the art of molecular biology at the time, in order to design peptides that can be expressed since Met it is a start codon recognized by translation machinery, as evidenced by Dattwyler (para [0101] "We unexpectedly found that shortening the C terminal portion of the peptide by 7 and 8 amino acids showed a trend toward increased its sensitivity...the critical immunodiagnostic epitope of the 26-residue IR6 sequence is confined within a 17-residue segment of the IR6 sequence and the N-terminal sequence (Met) is essential for maintenance of antigenicity") and thus the use of a fragment of the antibody detection sequence taught by Ascoli comprising a Met at the N terminus of a fragment thereof that retains binding activity would have been obvious to one of ordinary skill in the art during the ordinary course of experimentation. Further it would have been obvious to one of ordinary skill in the art to have applied the antibody detection sequences to the panel taught by Dattwyler, in order to detect antibodies indicative of Borrelia burgdorferi in a sample.

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As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/27163

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. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ 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: