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(54) **Title:** SPECIES SPECIFIC ANTIGEN SEQUENCES FOR TICK-BORNE RELAPSING FEVER (TBRF) AND METHODS OF USE

(57) **Abstract:** Compositions and methods for the detection and identification of Tick-Borne Relapsing Fever *Borrelia sp.* antibodies.

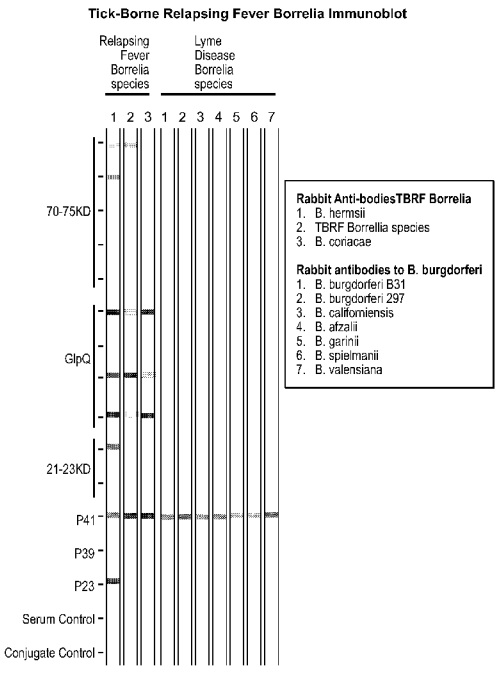

Figure 1

WO 2019/173569 A1

Species Specific Antigen Sequences for Tick-Borne Relapsing Fever (TBRF) and Methods of Use

Background

[0001] Borreliosis is caused by two groups of *Borrelia*, the *B. borgdorferi* group and the Tick-Borne Relapsing Fever (TBRF) *Borrelia* group. It was believed that the *B. borgdorferi* group was the only group that caused Lyme-like symptoms in infected subjects. However, it has been learned that TBRF *Borrelia* also causes Lyme-like symptoms. TBRF *Borrelia* is transmitted by hard (*Ixodes) and soft (Ornithodorus*) ticks.

[0002] *Borrelia* bacteria that cause TBRF are typically transmitted to humans through the bite of infected "soft ticks" of the genus *Ornithodorus*. Soft ticks differ in two important ways from the more familiar "hard ticks" (*e.g.*, the dog tick and the deer tick). First, the bite of soft ticks is brief, usually lasting less than half an hour. Second, soft ticks do not search for prey in tall grass or brush. Instead, they live within rodent burrows, feeding as needed on the rodent (for example, squirrels, chipmunks and prairie dogs) as it sleeps.

[0003] The main symptoms of TBRF are high fever (*e.g.*, 103° F), headache, muscle and joint aches. Symptoms can reoccur, producing a telltale pattern of fever lasting roughly 3 days, followed by 7 days without fever, followed by another 3 days of fever. Without antibiotic treatment, this process can repeat several times.

[0004] Humans typically come into contact with soft ticks when they sleep in rodent-infested cabins. The ticks emerge at night and feed briefly while the person is sleeping. The bites are painless, and most people are unaware that they have been bitten. Between meals, the ticks may return to the nesting materials in their host burrows.

[0005] There are several *Borrelia* species that cause TBRF, and these are usually associated with specific species of ticks. For instance, *B. hermsii* is transmitted by *O. hermsi* ticks, *B. parkerii* by *O. parkeri* ticks, and *B. turicatae* by *O. turicata* ticks. Each tick species has a preferred habitat and preferred set of hosts.

[0006] Soft ticks can live up to 10 years. Individual ticks will take many blood meals during each stage of their life cycle, and some species can pass the infection along through their eggs to their offspring. The long life span of soft ticks means that once a cabin or homestead is infested, it may remain infested unless steps are taken to find and remove the rodent nest.

[0007] Since TBRF can be caused by several species of *Borrelia*, tests need to be comprehensive by including all known species. Also, identification of the *Borrelia* species can aid in identifying the host rodent for eradication. Currently, the standard for identification is by identification of TBRF spirochetes in blood smears of a subject presenting symptoms consistent with TBRF. After obtaining the blood draw the sample must be cultured for at least 24 hours to facilitate identification. However, even early in the disease when spirochetes are highest, positive identification is only made about 70% of the time. (*See*, www.cdc.gov/relapsing-fever/clinicians/index.html). Thus, prior art materials and methods result in a delay in diagnosis and provide a relatively low level of sensitivity and specificity.

[0008] Therefore, what is needed are new materials and methods suitable for the identification of TBRF causative agents with decreased assay time and increased sensitivity and specificity.

Summary of the Invention

[0009] The present invention solves these problems in the prior art by providing antigen-specific amino acid sequences for TBRF *Borrelia* specific species. These novel amino acid sequences are used in assays to identify TBRF specific *Borrelia* in samples from subjects suspected of having TBRF. With the amino acid sequences of the present invention, identification of TBRF *Borrelia* in subject samples is performed with greater speed, sensitivity and specificity than the prior art methods. The amino acid sequences of the present invention can be used in diagnostic and scientific assays. Examples of suitable assays are Immunoblots, ELISA (enzyme-linked immunosorbent assay), etc. The amino acid sequences of the present invention can be used for the detection of TBRF *Borrelia* specific T-cells (*e.g.*, the IgXSPOT test; IGeneX, Palo Alto, CA). Further,

and importantly, antigens encoded by the amino acid sequences of the present invention can be used in vaccination protocols.

[0010] Thus, the present invention contemplates a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, said amino acid sequences consisting of 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, SEQ ID NO: 10, 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.

[0011] The present invention further contemplates that the sequences of the present invention, when bound, are bound to a substance selected from the group consisting of nitrocellulose, nylon, polyvinylidene difluoride (PVDF), magnetic beads and agarose.

[0012] The present invention further contemplates that the sequences of the present invention, when tagged, are tagged with an antibody with specificity for said amino acid sequence.

[0013] Further, the present invention contemplates a method of detecting *Borrelia* antisera in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), the method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled, tagged and/or bound amino acid sequences of the present invention and detecting a positive immunobinding reaction which indicates the presence of TBRF antisera in the sample.

[0014] The present invention further contemplates that two or more of the labeled and/or tagged and/or bound amino acid sequences of the present invention are mixed with the biological sample. A sample is considered positive for TBRF if at least two amino acid sequences are detected. In another embodiment, a sample is considered positive for TBRF if at least one amino acid sequences is detected.

[0015] The present invention further contemplates that the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG antibody linked to a

detectable moiety. The present invention contemplates that the detectable moiety may be selected from the group consisting of chromophores, radioactive moieties and enzymes. The present invention contemplates that the detectable moiety may comprise alkaline phosphatase. The present invention contemplates that the detectable moiety may comprise biotin.

[0016] The present invention further contemplates a method of detecting and distinguishing various species of *Borrelia* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences of the present invention and detecting a positive immunobinding reaction which indicates the presence of *Borrelia* in the subject, wherein detection of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 indicates the presence of *B. hermsii*, in the subject; detection of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 indicates the presence of *B. miyamotoi* in the subject; detection of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 indicates the presence of *B. turcica* in the subject; detection of SEQ ID NO: 6 and/or SEQ ID NO: 10 indicates the detection of *B. turicatae* in the sample; and, detection of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 indicates the detection of *B. coriaceae* in the sample. The labeled and/or tagged and/or bound amino acid sequences may be detected with anti-human IgG antibody conjugated to a detectable moiety. The present invention further contemplates that the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG antibody linked to a detectable moiety. The present invention contemplates that the detectable moiety may be selected from the group consisting of chromophores, radioactive moieties and enzymes. The present invention contemplates that the detectable moiety may comprise alkaline phosphatase. The present invention contemplates that the detectable moiety may comprise biotin. The present invention further contemplates that a sample is considered positive for *Borrelia* if at least one amino acid sequence is detected. The present invention further contemplates that a sample is considered positive for a specific species of *Borrelia* if at least one amino acid sequence identified with a specific species is detected.

[0017] The present invention contemplates a method of detecting *B. hermsii* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 of Clam 1 and detecting a positive immunobinding reaction of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 the detection of which indicates the presence of *B. hermsii* in the sample.

[0018] The present invention contemplates a method of detecting *B. miyamotoi* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or bound amino acid sequences selected from the group of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 of Clam 1 and detecting a positive immunobinding reaction of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 which indicates the presence of *B. miyamotoi* in the sample

[0019] The present invention contemplates a method of detecting *B. turcica* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 of Clam 1 and detecting a positive immunobinding reaction of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 which indicates the presence of *B. turcica* in the sample.

[0020] The present invention contemplates a method of detecting *B. turicatae* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 6 and/or SEQ ID NO: 10 of Clam 1 and detecting a positive immunobinding reaction of

SEQ ID NO: 6 and/or SEQ ID NO: 10 which indicates the presence of *B. turicatae* in the sample.

[0021] The present invention contemplates a method of detecting *B. coriaceae* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising: providing a biological sample obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 of Clam 1 and detecting a positive immunobinding reaction of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 of Clam 1 which indicates the presence of *B. coriaceae* in the sample.

[0022] The present invention further contemplates a composition comprising, consisting essentially of or consisting of one or more amino acid sequences selected from the group consisting of 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, SEQ ID NO: 10, 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, and an adjuvant.

[0023] The present invention further contemplates a nucleic acid sequence encoding any one or more of the amino acid sequences of the present invention. Said nucleic acid sequence may be labeled.

[0024] The present invention further contemplates a method of detecting T cells from a subject having or suspected of having tick-borne relapsing fever (TBRF), the method comprising: providing i) a sample comprising T cells obtained from a subject suspected of having TBRF, ii) a culture apparatus coated at least partially with anti-antibodies specific for one or more cytokines and iii) one or more sequences of Claim 1; mixing the blood sample with the one or more of the sequences of Clam 1 in the culture apparatus, and detecting any production of the one or more cytokines by the subject's T cells. In one embodiment, the anti-antibodies that are specific for a cytokine are anti-IFNγ antibodies and the cytokine is interferon gamma (IFNγ).

Brief Description of the Figures

[0025] Figure 1 shows an Immunoblot using the TBRF *Borrelia* specific antigenic peptide encoding amino acid sequences of the present invention.

Detailed Description of the Invention

[0026] The present invention provides novel compositions and methods for diagnosing, treating and vaccinating against infection by Tick-Borne Relapsing Fever causing *Borrelia sp.* The invention is based, in part, on the discovery of species-specific amino acid sequences encoding antigenic peptides (which may also be referred to as peptide antigens or antigens in the art), as described below. Further, the present invention provides nucleic acid sequences encoding the amino acid sequences of the present invention. The nucleic acid sequences may be labeled.

[0027] The present invention provides for antigenic amino acid sequences specific for various *Borrelia* species. The amino acid sequences of the present invention encode antigenic peptides that have high specificity and/or sensitivity for the indicated species.

[0028] The present invention, in one aspect, is a composition comprising one or more labeled and/or bound amino acid sequences, said amino acid sequences having 90%, 95%, 98%, 99%, 99.5% and/or 100% homology to 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, SEQ ID NO: 10, 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. Sequences less than 100% homologous may have deletions, additions and or substitutions of the 100% homologous sequence. One of ordinary skill in the art can easily determine if sequences less than 100% homologous can bind naturally or non-naturally occurring TBRF-related antibodies as well as the sensitivity and specificity of the antibody to the modified sequences. In other words, one of ordinary skill in the art will be able to identify sequences with significant homology to SEQ ID NOs: 1 – 20 of the present invention that give acceptable or equivalent

responses in the methods of the present invention without undue experimentation, in view of the teachings of this specification.

[0029] The present invention, in one aspect, is a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, said amino acid sequences consisting of 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, SEQ ID NO: 10, 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.

[0030] With regard to the present invention, the phrase "a composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, said amino acid sequences consisting of," 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 as an element of the "composition comprising."

[0031] In the context of the present invention, a "tagged" amino acid sequence is an amino acid sequence that is attached to a detectable moiety. 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 "labeled" and "tagged" may have overlap in that a tag may also, in some instances, function as a label. Further, tags useful with the present invention may be linked to a label.

[0032] The amino acid sequences of the present invention, or any tags attached to an amino acid sequence of the present invention, 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 invention.

[0033] Further, the amino acid sequences of the present invention 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 invention, 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 invention 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 invention 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 invention to a surface or substance.

[0034] The present invention, in one aspect, is a composition comprising, consisting essentially of or consisting of one or more labeled and/or tagged and/or bound amino acid sequences, said amino acid sequences consisting of 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, SEQ ID NO: 10, 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 and up to five and up to ten additional amino acids added to one or both of the 3'-prime and 5'-prime ends of the sequence, wherein said additional amino acids may or may not be the naturally contiguous amino acids.

[0035] The amino acid sequences of the present invention may be natural occurring and isolated from a natural source. Further, the amino acid sequences of the present invention may be non-natural, synthetic sequences, such as sequences produced by recombinant technology or sequences synthesized by protein synthesizing apparatuses. As such, the amino acid sequences of the present invention 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 embodiment, the amino acid sequences of the present invention are made recombinantly in *E. coli*.

[0036] The amino acid sequences of the present invention 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.

[0037] In another aspect, the present invention provides a method of quickly and accurately detecting *Borrelia* antisera in a sample from a subject suspected of having tick-borne relapsing fever (TBRF). A subject suspected of having TBRF can be identified as having symptoms such as a high fever (*e.g.*, 103° F), headache, muscle and joint aches. Symptoms typically reoccur, producing a telltale pattern of fever lasting roughly 3 days, followed by approximately 7 days without fever, followed by another 3 days of fever. Without proper antibiotic treatment, this process can repeat several times. Since the symptoms of TBRF can mimic, for example, viral flu-like symptoms, accurate diagnosis of TBRF is important for providing an effective treatment for the subject. The present invention provides a quick and easy diagnostic test for detecting the presence of antibodies specific for the causative *Borrelia* species, thereby satisfying the need for such a test.

[0038] The method of the present invention for detecting *Borrelia* antisera in a sample from a subject suspected of having TBRF, may comprise, for example, providing a biological sample (*e.g.*, blood, saliva) obtained from a subject suspected of having TBRF, mixing the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention and detecting a positive reaction which indicates the presence of TBRF antisera in the sample. The antisera may be detected by, for example, immunoblotting, Elispot, ELISA, Western blotting 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.

[0039] Briefly, 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.

[0040] 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.

[0041] 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.

[0042] The Enzyme-Linked ImmunoSpot (ELISPOT) method can detect human T cells that respond to TBRFspecific 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 invention 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.

[0043] 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 TBRF) T cells are isolated and cultured in each well and stimulated with recombinant antigens of one or more sequences of the present invention. TBRF-positive patient cells secrete cytokine in response to stimuli, which is captured by the antibody coated in the well and further detected by ELISA.

[0044] 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 invention, 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.

[0045] In one aspect of the method of the present invention, a positive result is indicated when two or more of the labeled and/or bound amino acid sequences of the present

invention are mixed with the biological sample and when at least two amino acid sequences are detected. In another aspect of the invention, a positive result is indicated when at least one of the labeled and/or bound amino acid sequences of the present invention are mixed with the biological sample and when at least one amino acid sequence is detected.

[0046] In the method of the present invention, any primary antibody bound to a peptide encoded by an amino acid sequence of the present invention may be detected with anti-human antibodies, such as IgG or IgM, used as the secondary antibody conjugated to a detectable moiety. As discussed above, 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 embodiment, the detectable moiety comprises alkaline phosphatase. In another embodiment the detectable moiety comprises biotin.

[0047] In another aspect of the invention, 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 TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. Amino acids may be labeled to confirm their presence if positive results are not obtained in the assay. The detection of a positive immunobinding reaction indicates the presence of *Borrelia* in the sample, wherein detection of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 indicates the presence of *B. hermsii*, in the sample; detection of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 indicates the presence of *B. miyamotoi* in the sample; detection of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 indicates the presence of *B. turcica* in the sample, detection of SEQ ID NO: 6 and/or SEQ ID NO: 10 indicates the detection of *B. turicatae* in the sample and, detection of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 indicates the detection of *B. coriaceae* in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to one of ordinary skill in the art.

[0048] In a preferred embodiment of the invention, a sample is considered positive for *Borrelia* if at least two amino acid sequences are detected. A sample is considered positive for a specific species of *Borrelia* if at least two amino acid sequences identified with a species are detected. In another embodiment of the invention, a sample is considered positive for *Borrelia* if at least one amino acid sequence is detected. A sample is considered positive for a specific species of *Borrelia* if at least one amino acid sequence identified with a species is detected.

[0049] In another aspect of the invention, a method is provided for detecting and distinguishing various species of *B. hermsii* in a sample. The sample may be from a subject suspected of having TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention specific for *B. hermsii*. The detection of a positive immunobinding reaction of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 indicates the presence of *B. hermsii*, in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to one of ordinary skill in the art. A sample is considered positive for *B. hermsii* if at least one amino acid sequence is detected.

[0050] In another aspect of the invention, a method is provided for detecting and distinguishing various species of *B. miyamotoi* in a sample. The sample may be from a subject suspected of having TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. The detection of, for example, a positive immunobinding reaction of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 indicates the presence of *B. miyamotoi* in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to one of ordinary skill in the art. A sample is considered positive for *B. miyamotoi* if at least one amino acid sequence is detected.

[0051] In another aspect of the invention, a method is provided for detecting and distinguishing various species of *B. turcica* in a sample. The sample may be from a subject suspected of having TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. The detection of, for example, a positive immunobinding reaction of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 indicates the presence of *B. turcica* in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to one of ordinary skill in the art. A sample is considered positive for *B. turcica* if at least one amino acid sequence is detected.

[0052] In another aspect of the invention, a method is provided for detecting and distinguishing various species of *B. turicatae* in a sample. The sample may be from a subject suspected of having TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. The detection of, for example, a positive immunobinding reaction of SEQ ID NO: 6 and/or SEQ ID NO: 10 indicates the detection of *B. turicatae* in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to one of ordinary skill in the art. A sample is considered positive for *B. turicatae* if at least one amino acid sequence is detected.

[0053] In another aspect of the invention, a method is provided for detecting and distinguishing various species of *B. coriaceae* in a sample. The sample may be from a subject suspected of having TBRF. The method may comprise, for example, providing a sample, for example, a biological sample obtained from a subject suspected of having TBRF and mixing or contacting the biological sample with one or more of the labeled and/or bound amino acid sequences of the present invention. The detection of, for example, a positive immunobinding reaction of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 indicates the detection of *B. coriaceae* in the sample. The assay used may be any of the assays described elsewhere in this specification, or as are known to

one of ordinary skill in the art. A sample is considered positive for *B. coriaceae* if at least one amino acid sequence is detected.

[0054] In another aspect of the present invention the amino acid sequences of the present invention may be used as vaccines in vaccination protocols. Vaccination protocols are well known to one of ordinary skill in the art. Briefly, vaccination is the administration of antigenic material (a vaccine) to stimulate an individual's immune system to develop adaptive immunity to a pathogen. Vaccines can prevent or ameliorate morbidity from infection.

[0055] Vaccines are typically administered with adjuvants. Adjuvants are compounds and mixtures of compounds designed or found to aid in stimulation of the immune system. For example an adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. Adjuvants may be added to a vaccine to modify the immune response by boosting it such as to produce a greater quantity of antibodies and provide longer-lasting protection, thus minimizing the amount of injected foreign material. Adjuvants may also be used to enhance the efficacy of a vaccine by helping to modify the immune response to particular types of immune system cells. There are different classes of adjuvants that can skew immune responses in different directions. For example, adjuvants may selectively skew the immune response by preferentially activating T cells or B cells depending on the purpose of the vaccine. Adjuvants may also be used in the production of antibodies from immunized animals. For example, rabbit anti-human IgG antibodies may be produced by inoculating a rabbit with conserved portions of a human IgG antibody in combination with an adjuvant. The most commonly used adjuvants include aluminum hydroxide, paraffin oil and Freud's complete and incomplete adjuvant.

[0056] Although not fully understood adjuvants are believed to apply their effects through different mechanisms. Some adjuvants, such as alum, function as delivery systems by generating depots that trap antigens at the injection site, providing a slow release that continues to stimulate the immune system. Other adjuvants augment or stimulation the host immune response by providing a general boost to the immune system.

[0057] In this regard, another aspect of the present invention is a composition comprising or consisting essentially of one or more amino acid sequences, said amino acid sequences having 90%, 95%, 98%, 99%, 99.5% and/or 100% homology selected from the group consisting of 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, SEQ ID NO: 10, 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 and an adjuvant. The adjuvant may be any adjuvant known to one of ordinary skill in the art suitable for use in humans. Combinations two or more adjuvants may be used. One of ordinary skill in the art will be able to identify sequences of the present invention that give acceptable or equivalent immunological responses without undue experimentation. By definition, with regard to the phrase "consisting essentially of," agents other than the recited sequences and adjuvant are not considered essential in the context of the present invention.

[0058] Isolated nucleic acid sequences, including polynucleotides and oligonucleotides, encoding the amino acid sequences of the present invention, 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 invention. Because of redundancy of the genetic code, multiple nucleic acid sequences may be suitable for the production of the peptide sequences of the present invention. One of ordinary skill in the art will be able to determine one or more nucleic acid sequences for the production of the amino acid sequences of the present invention. The nucleic acid sequences encoding the amino acid sequences of the present invention may be labeled by any suitable label known to one of ordinary skill in the art.

[0059] In this regard, nucleic acid sequences of suitable for the production of the amino acid sequences of the present invention may be substantially homologous to the naturally occurring sequences. Substantial homology of a nucleic acid sequence means either that (a) there is greater than about 65%, typically greater than about 75%, more typically greater than about 85%, preferably greater than about 95%, and more preferably greater than about 98% homology, most preferably 99% 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 ^{O}C, usually greater than about 30 ^{O}C and more usually greater than about 45 ^{O}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 invention.

[0060] Further, homologous nucleic acid sequences may be determined based on the nature of a nucleotide substitution in the nucleic acid sequence. For example, conservative nucleotide substitutions will be tolerated better and, therefore, can be more numerous in a particular nucleic acid sequence than non-conservative nucleotide substitutions. One or 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 invention without adversely affecting the species specificity of the encoded antigenic peptide, without undue experimentation.

[0061] The present invention will now be described in view of the following, non-limiting, exemplification.

Exemplification

Example 1

[0062] Borreliosis is caused by two groups of *Borrelia, B. burgdorferi* group and the Tick-Borne Relapsing Fever (TBRF) *Borrelia* group. It was believed that *B. burgdorferi* group is the only group that causes Lyme-like symptoms. However it is now known that

TBRF *Borrelia* can also cause Lyme-like symptoms. TBRF *Borrelia* can be transmitted by hard (*Ixodes*) and soft (*Ornithodorus)* ticks.

[0063] TBRF ImmunoBlot

[0064] The TBRF *Borrelia* immunoblot of the present invention is designed to detect antibodies to TBRF *Borrelia* species [including, but not limited to *Borrelia miyamotoi, B. hermsii, B. turicatae and B. coriaceae*] specific antigens in human serum. For diagnostic purposes, immunoblot test results may be used in conjunction with clinical symptoms and other evidence available to the diagnosing physician.

[0065] The TBRF *Borrelia* ImmunoBlot Test is a qualitative immunoblot assay that detects antibodies directed against TBRF associated *Borrelia species* in sera of patients suspected of having TBRF *Borrelia* infection. Recombinant TBRF *Borrelia* antigens (protein sequences listed below) were applied as straight lines onto nitrocellulose strips, where they bind. The strips were then be used in the TBRF *Borrelia* ImmunoBlot Test.

[0066] Species specific *Borrelia* amino acid sequences of the present invention:

B. hermsii - BipA-1H

MSESNWEIDEPGSVQDIRNSVASELQKPENIGQRGKSVGKEVGKDAAASGEGAVVAVGSKQN
TLQNSENSSQEGAGSALQKPGDSPQKGVASQEGTNGALQGVVAAGGVSVGGSGVGAAASD
GNSSSSQEAESVDLKNVLADSHGVGASNLNIKAEGDISTGHGTEGVIASGDLTNTIITSGVTAAA
SPAIVSGDERGVAAIKDSIAVVLETQKEAQKETEKETENVKFAVLGTVKKIVDGVADGIANVIEYG
LENGID [SEQ ID NO: 1]

B. hermsii - BipA-2H

MNKVKDGIKTASKGAETFVTAAMQGAGAVLGSLQDVGNFVMDATLSMGDMFAGIKSDSDVSS
ANSGMTVNSGMTVSLSSNETQVIGHLEEYLKSAIKVNGNESSRQSKLENGRQKFFAWLREKDT
DFSKRKELVQAMQRVYNFIKEKSSNSRELQTWVLGVVGDDDTVVDVDRDDELNSDVEIDFLIK
KTLSSRDYSGFAVSLLFQALADTLYDAENDRDKPEEQIFKDLKAVFSDNAGEDKGFGEFKSIIED
KSQA [SEQ ID NO: 2]

B. miyamotoi - BipA

MAKAGNFDEVLDLDGDDEEAEIDEIEQNLSEGVDGNIDQNPGNVDGHIVLVDAQGVSVPTVVE
HSAVSSSVERRVVTVGQNVESRRLTKVERLEKYLESAIKSGGKLNEQQRRLKNGKQMLFTWL
NEDANASKRAELEQDMQKLYGLIKESITDSSSQFSSRDGEYSDEAIDGLLNSLFSSSFDNTFAL
DLFFQALLNTLCDFKKDNCKDEEVIFADIRKVFSDESDTKGHFGYLKSKLKDELVISDEGDYGED
EDFEED [SEQ ID NO: 3]

B. turcica - BipA1

MEEEASIESEAMPLDEAKISSLDKETVPAVQADGTPEQIVASVGLEGEESKKFEHLKGSLGDAIK
VNGKGEEKRKENEERQKEFFDWLDKNDPDLSKRKELAELMKKVYGLLKEHAQNSEQIKSFVE
GTPKDENVKKIGVTSARDIKTDEQVEALIKAVLGHSEESGTNLSLFFQKLGDAFGTEDGESQKS
NEKILEELKRVCESSDEIKKLKEDLKIEEKVQS [SEQ ID NO: 4]

B. turcica - BipA2

MNIDAVADLLADQQEAASTGLKDASTGLKDTSTGLSKLDKKEKKVSSLKETLENSSNVLYESSS
PTKTRQEEFFKWLEENDSDYSKRKQLEESMDKVFSLIKDSASSSTEIKEIIAKGQSDAGIIKAGIK
TADDIKTDEQVDALVKFVTGTGDDLDLESGSSSIKAFFGTLAEVFDDDLNDVMTDKKGQKRGH
DKVFEDLKKVFSEDSDGPFDILKDALKQALKNN [SEQ ID NO: 5]

B. turicatae - BipA-1H

MSTSYWSVDNDGFVQGTKSFVDSPLRKPDRFDQEVSAGGKKIEKAVSRNLRVAGGQRQGIAD
DGIGVAGVREAGGVLKDAGNAVQRDINGSGEGIKNDVIQNPEGVGVQVAVGSADTGADSGQE
AGKVFQNLGDTGTQSIQRVVSSSDLNSDLGVGSKDGISTNGMSTNHVTENENSINSITSTSSGL
NTALQMAGTSTRTSGYEGEITTNTQDRTFVETGTQDSKAQYSDFSDQDIRDKVLGSVVGGVV
[SEQ ID NO: 6]

B. hermsii - GlpQ-s

MASMTGGQQMGRGSEKMSQNQKSPLVIAHRGASGYLPEHTLEAKAYAYALGADYLEQDIVLT
KDNIPVIMHDPELDTTTNVAKLFPERARENGRYYSVDFTLDELKSLSLSERFDPASRNPIYPNRF
PLNEHDFKIPTLEEEIQFIQGLNKSTGRNVGIYPEIKKPLWHKQQGKDISKIVIEILNKYGYKSKED
KIYLQTFDFDELKRIREELGYQGKLIMLVGENDWDEAPTDYEYIKSEEGMAEVAKYSDGIGPWIP
QIIIEGKITDLTSLAHKHNMEVHPYTFRIDALPSYVKDADELLDLLFNKAKVDGIFTDFTDTVVNFIT
KIKPKGE [SEQ ID NO: 7]

B. miyamotoi - GlpQ-s
MASMTGGQQMGRGSEMGENKKSPLIIAHRGASGYLPEHTLEAKAYAYALGADYLEQDIVLTKD

NIPVIMHDPEIDTTTNVAQLFPNRARENGRYYATDFTLTELKSLNLSERFDPENKKPIYPNRFPL
NEYNFKIPTLEEEIQFIQGLNKSTGKNVGIYPEIKKPFWHKQQGKDISKIVIEILNKYGYKSKEDKI
YLQTFDFDELKRIRKELGYQGKLIMLVGENDWNEAPTDYEYIKSEEGIAEVAKYSDGIG [SEQ
ID NO: 8]

B. turcica - GlpQ

MASMTGGQQMGRGSKVSMNKALPLVIAHRGASGYLPEHTLEAKAFAYALGAHYLEQDIVLTKD
DIPIIMHDPEIDTTTNVAEIFPERARKDGRYYSVDFTLRELKSLKLSERFDPKTGKPIYPNRFPLN
EYNFKIPTLEEEIQFIQGLNKSTGRNVGIYPEIKKPFWHKQQGKDISKIVIEMLNKYGYKSKEDKIY
LQIFDFDELKRIREELGYKGKLVMLIGENDWNEAPTDYEYIKSEEGIAEVAKYSDGIGPWIPQVII
DGKVTGLTSLAHKHKMEVHPYTMRIDALPSYVKDANELLNLLFNKAKVDGVFTDFPDVVLGFIR
K [SEQ ID NO: 9]

B. turicatae - GlpQ

MASMTGGQQMGRGSEKMSMTNKKPPLIIAHRGASGYLPEHTLEAKAFAYALGADYLEQDIVLT
KDNVPIIMHDPELDTTTNVAKLFPERARENGRYYSVDFTLDELKSLSLSERFDLETRKPIYPNRF
PLNEYNVKIPTLEEEIQFIQGLNKSTGRNVGIYPEIKKPLWHKQQGKDISKIVIEILNKYGYKSKED
KIYLQTFDFDELKRIREELGYQGKLIMLVGENDWDEAPTDYEYIKSQEGMTEVAKYADGIGPWI
PQIIIDGKITDLTSLAHKYNMEVHAYTFRIDSLPSYVKDANELLDLLFNKAKIDGLFTDFTDTVVKF
VKQ [SEQ ID NO: 10]

B. hermsii - BpcA

MASMTGGQQMGRGSSDANLLKTLDNNQKQALIYFKDTLQDKKYLNDLTASQKNFLDDLEKNK
KDPGLQDKLKKTLSSEYDGSQFNKLLNELGNAKVKQFLQQLHIMLQSIKDGTLTSFSYANFKDL
QTLEQKKERALQYINGRLYVEYYFYINGISNADNFFESVMQLLET [SEQ ID NO: 11]

B. miyamotoi - BpcA

MLDHNLQPNKINNIISSLDSNQKQALIFFKNLVKNKQYSKDLEQASKSYLENLKEKNNQNLNLQN
KLNQGLNCDYDDSKIEKLFDQLGNDKMKKFLQQLHLMLKSINDGTLISFSSSNFRDTTTLSQKK
EKALEYIKSQLYIEFYFHSNDISDTEFFFQRTIALLETQN [SEQ ID NO: 12]

B. hermsii - P41

MRNNSINATNLSKTQEKLSSGHRINRASDDAAGMGVAGKINAQIRGLSQASRNTSKAINFIQTT
EGNLNEVERVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQAQYNQMHMLSNKS

AAQNVKTAEELGMQPAKINTPASLAGSQASWTLRVHVGANQDEAIAVNIYASNVANLFAGEGA
QAAPVQEIGQQEEGQAAPAPAAAPAQGGVNSPINVTTAVDANMSLAKIEGAIRMVSDQRANLG
AFQNRLESIKDSTEYAIENLKASYAQIKDATMTDEVVASTTHSILTQSAMAMIAQANQVPQYVLS
LLR [SEQ ID NO: 13]

B. miyamotoi - P41

MRNNGINAANLSKTQEKLSSGYRINRASDDAAGMGVAGKLNSQIRGLSQASRNTSKAINFIQTT
EGNLNEVEKVLVRMKELAVQSGNGTYSDSDRGSIQIEIEQLTDEINRIADQAQYNQMHMLSNKS
AAQNVKTAEELGMQPAKINTPASLAGSQASWTLRVHVGANQDEAIAVNIYAANVANLFNGEGA
QAAPAQEGAQQEGVQAVPAPAAAPVQGGVNSPINVTTAIDANMSLSKIEDAIRMVTDQRANLG
AFQNRLESVKASTDYAIENLKASYAQVKDAIMTDEIVASTTNSILTQSAMAMIAQANQVPQYVLS
LLR [SEQ ID NO: 14]

B. turcica - P41

MRNNGINASNLSKTQEKLSSGYRINRASDDAAGMGVAGKINAQIRGLSQASRNTSKAINFIQTT
EGNLNEVEKVLVRMKELAVQSGNGTYSDADRGSIQIEIEQLTDEINRIADQSQYNQMHMLSNKS
AAQNVKTAEELGMQPAKINTPASLSGAQASWTLRVHVGANQDEAIAVNIYAANVPNLFAGEGA
QTAAAAPAQAGTQQEGAQEPAAAAAPAQGGVNSPVNVTTTVDANMSLAKIENAIRMISDQRAN
LGAFQNRLESIKNSTEYSIENLKASYAQIKDATMTDEIVSSTTNSILTQSAMAMIAQANQVPQYVL
SLLR [SEQ ID NO: 15]

B. hermsii - BmpA10

MSKAGSGLTKIAILVDGTFDDESFNGSAWKGAKKVEKEFGLEIMMKESNANSYLADLESLKNN
GSNFLWLIGYKFSDFAIIAALENPESKYVIIDPVYESDLVIPENLSAITFRTEEGAFLVGYIAAKMSK
TGKIGFLGGFDDVVVNTFRYGYEAGAIYANKHINIDNKYIGNFVNTETGKNMANAMYAEGVDIIY
HVAGLAGLGVIESARDLGDGHYVIGVDQDQSHLAPDNVITSSIKDIGRVLNIMISNYLKTNAFEG
GQVLSYGLKEGFLDFVKNPKMISFELEKELDDLSEGIINGKIIVPNNERTYNQFMRKIL [SEQ ID
NO: 16]

B. hermsii - OspC10

MFLFISCNNGGPELKGNEVAKSDGTVLDLSKISTKIKNAGAFAASVQEVATLVKSVDELASAIGK
KIKEDGTLDTLNNKNGSLLAGAFQVILTVEAKLKELEKQDGLSVELRAKVTSAKSASSGLVNKLK
GGHAELGIEGATDENAQKAIKKDNGDQSKGAEELGKLNTAIGALLSAANDAVEAAIKELTAAPA
KPATPAKP [SEQ ID NO: 17]

***B. coriaceae* - GlpQ**

```
1   MKSMKPKLLM LIINIFLIIS CQNEKVSMNE KSPLIIAHRG ASGYLPEHTL EAKAYAYALG
61  ADYLEQDIVL TKDNVPIIMH DSELDTTTNV AKLFPERARE NGKYYAVDFT LDEIKSLSIS
121 ERFDPETREP IYPNRFPLNE YNFKIPTLEE EIQFIQGLNK STGKNVGIYP EIKKPFWHKQ
181 QGKDISKIVI EILNQYGYKS KEDKIYLQTF DFDELKRIRE ELGYQGKLIM LVGENDWNEA
241 PTDYEYIKSQ EGMTEVAKYA DGIGPWISQI IIDGQVTDLI SLAHKHNMEV HPYTFRIDAL
301 PSYVKDANEL LDLLFNKAQV DGIFTDFVDK AMEFVKK [SEQ ID NO: 18]
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***B. coriaceae* - BIPA**

```
1   MKSFSVFILF LSTFTLSCKF YDTANASDLQ ADGDKFSQGF SSFNDILPFS DLKINKDVST
61  GSLKAQGTSS IKGDEKKGKG TSKDPIKDQE ASGLKGVGVA GAGAKSFGDD GKKEEVVSKD
121 SLKNEGTSGT AEVLKVSKEV EVAGVDTAKP AGGNGEEVAS ISENYLQNQE TLVAQGAGVG
181 SVGDAIGDRS LFFKNTDSNN AEQVVATEDL LVGASEGVNT SDLGLKVAIP TDHVRGDVVA
241 TETQNAEKKG DKTQNTELAS LDIKDNITVN VVDGTKININ KNSSNTNESI NVTKDGVNTV
301 IKGVETSIKT ADGKVVVKKR TLKKGLKKKN SKKQASKSKT PEAAVVGNKK NVDTNMSSVI
361 GLDSEALGKD KNIDLDSKSD ETYVIERVEK LAKYLQSAIK INGKKVEEQD KLEAGRQKFF
421 EWLSKNDTDL LKRKALVQDL QKIYDLMKDK IADSTELQDW FQIVSDDIGD EETNIIDVES
481 YYELSSDTEI DFLLERTLED ENYSGFSISL FMQALADTLY DIQNDSHKSG EEILQELKRV
541 FDDTFYKIRG FEEFKSQIAA ED [SEQ ID NO: 19]
```

***B. coriaceae* - BPCA**

```
1   MKLTKKYLLA VLLLSLINCD LLSKNKILTS HLLNTLDNNK KEALVTFKNL LQDKSHLEYL
61  KSEQAKMLTN FTEDDGIEQP HLQEKLKGTL SSEYNENQLN QLFSELGYEK TKQFLDNLHK
121 MLQAIKDGTL RAFHDSSSFK DYNTTLEAKK AEALSSVKKE LYVQYYFYIN DLQTADDFFV
181 LTRNHLMIFK NNL [SEQ ID NO: 20]
```

[0067] ImmunoBlotting: Immunoblotting is well known to one of ordinary skill in the art. With regard to the present invention, to perform the TBRF *Borrelia* ImmunoBlot test, patient serum was incubated with TBRF *Borrelia* ImmunoBlot strips, produced as described above, in each trough of an incubation tray. If specific antibodies to TBRF *Borrelia* antigens were present in a sample, they were bound to the corresponding antigen bands. After washing away unbound antibodies, the bound TBRF *Borrelia* specific antibodies were detected with alkaline phosphatase (AP) conjugated goat or rabbit anti-human IgG or IgM antibody. After removing the unbound conjugated antibody, the strips were incubated with BCIP/NBT, an AP chromogenic substrate. A dark purple colored precipitate developed on the antigen-antibody complexes. Bands were visualized and scored for intensity relative to the positive, negative and calibrator (calibration) controls.

Example 2

[0068] Specificity of TBRF Borrelia ImmunoBlot

[0069] Method: TBRF ImmunoBlot strips were tested with rabbit anti-TBRF *Borrelia* serum samples and *Borrelia burgdorferi* serum samples. Rabbit antibodies to the following *Borrelia* species were tested: *B. hermsii*, *TBRF Borrelia species*, *B. coriaceae*, *B. burgdorferi B31*, *B. burgdorferi 297*, *B. californiensis*, *B. afzalii*, *B. garinii*, *B. spielmanii* and *B. valensiana.*

[0070] Result Summary: As shown in Figure 1, in the columns numbered 1 – 3 under the heading "Relapsing Fever Borrelia species," antibodies to *B. hermsii,* TBRF *Borrelia sp.,* and *B. coriaceae* were detected. In the columns numbered 1 – 7 under the heading "Lyme Disease Borrelia species," only antibodies to 41 kDa were detected with the rabbit anti-*B. burgdorferi* specific serum samples. The numbers refer to the antibodies denoted in the Figure key.

[0071] Conclusion: Based on the data presented above, the TBRF ImmunoBlot is very specific for the detection of TBRF *Borrelia* specific antibodies.

Example 3

[0072] TBRF Borrelia ImmunoBlots Validation Study – Clinical Sensitivity and Specificity

[0073] A total of 171 patient samples were tested as per TBRF *Borrelia* ImmunoBlot IgM and IgG protocols to determine clinical sensitivity and specificity. The following patient samples (Table 1) were tested as per TBRF *Borrelia* Immunoblot IgM and IgG test protocols. The ImmunoBlots were read by in-house criteria. The ImmunoBlot (IgM or IgG) was considered positive if 2 of the following bands were present: 21-23, 41, 70-75kDa and GlpQ. If only one of the following bands is present, the ImmunoBlot was considered boarder-line positive: 21-23, 70-75kDa and GlpQ. Results are summarized below in Tables 2a, 2b and 2c.

Table 1: List of Patient Samples Tested			
Study Set #	**Source**	**Samples**	**n**
Set 1	In-house	34 samples tested by TBRF Western blots (Lyme-like symptoms, most negative by Lyme Western Blot and Lyme ImmunoBlots)	34
Set 2	In-house	10 samples - 2 samples/ patient collected at different time points (Negative by Lyme Western and ImmunoBlots)	20
Set 3	CDC	CDC – Specificity Samples (Provided Blinded by CDC)	50
Set 4	NY biologics	Specificity Samples (purchased from NY Biologics)	25
Set 5	In-house	Autoimmune and Allergy patient samples (Previously left over PT samples)	42
Total Samples			**171**

Table 2a

Results: Overall Summary.

Table 2: Clinical Sensitivity - Overall Summary (see Table 2a and 2b)								
Sample Size		Result	Lyme ImmunoBlot			TBRF ImmunoBlot		
			IgM	IgG	IgM &/or IgG	IgM	IgG	IgM &/or IgG
Set 1	34	Positive	2	1	2	15	8	18
		Negative	32	33	32	19	26	16
Set 2	10 (old)	Positive	0	0	0	6	3	7
		Negative	10	10	10	4	7	3
	10 (new)	Positive	0	0	0	8	5	10
		Negative	10	10	10	2	5	0
Total	54	Positive	2	1	2	29	16	35
		Negative	52	53	52	25	38	19

Table 2b: Performance of TBRF Immunoblot on Clinical Samples

Set 1: Patients with Lyme-like symptoms				
Patients Samples with antibodies to:	**n**	**IgM (+)**	**IgG (+)**	**IgM and/or IgG (+)**
Patients negative for Lyme	30	15	9	18
Bartonella	2	0	0	0
Ehrlichia	2	0	0	0
Total Positive		**15**	**9**	**18**
Total Negative				16

[0074] To see how a patient's response immunologically to infection two samples were collected from each of 10 patients (including patients with a history of a tick bite and TBRF PCR positives). Results are presented below in Table 2c

Table 2c: Performance of TBRF Immunoblot on 10 Positive Clinical Samples

Set 2: Patients with Lyme-like Symptoms, negative by Lyme ImmunoBlots and Lyme Western blots (10 patient samples collected at 2 time points)				
Patient Samples	**n**	**IgM (+)**	**IgG (+)**	**IgM and/or IgG (+)**
1st collection	10	6	3	7
		4	7	3
2nd collection (6 weeks to 2 years latter)	10	8	5	10
		2	5	0

[0075] **Sensitivity:** Based on the data presented above for patients with Lyme-like symptoms, of the 44 patients (total 54 samples) with Lyme-like symptoms, 2 patients had antibodies to *B. burgdorferi* and TBRF *Borrelia*; 29 patients had antibodies to TBRF *Borrelia*. Four patients with other tick-borne diseases were negative by TBRF ImmunoBlot. Two sets of serum samples were collected from 10 patients, to see patient's immune response to infection. We collected two samples at different time points from 10 patients (including patients with a history of a tick bite and/or TBRF PCR positive samples). The second sample was collected 6 weeks to 2 years after the first sample (See Table 2b and Table 2c for detailed results). As shown above, 7 patients (4 IgM (+), 1 IgG (+) and 2 with IgM and IgG) were positive initially. When the second sample was tested all patients were positive, 5 IgM (+), 2 IgG(+) and 3 IgM and IgG (+).

[0076] **Specificity:** Based on the data presented below in Table 3, the specificity of the TBRF *Borrelia* ImmunoBlot was 95.0% for IgM and 97.5% for IgG.

[0077] **Conclusion:** The specificity of TBRF ImmunoBlot was 95.0% for IgM and 97.5% for IgG. With 10 patients (Set 2), we demonstrated that the immune response varied between individuals.

[0078] The results show that the TBRF ImmunoBlots can be used in to detect TBRF *Borrelia*-specific antibodies in patients suspected of TBRF Borreliosis. The results can be used by the physician in conjunction with patient history and symptoms. Detailed results are summarized below in Table 3.

Table 3: Clinical Specificity - Overall Summary

Source	Sample Types	n	IgM (+)	IgG (+)	IgM and/or IgG (+)
50 CDC Samples (n=50, Set 3)	Endemic Controls	10	0	0	0
	Fibromyalgia	5	0	0	0
	Mononucleosis	5	0	0	0
	Multiple Sclerosis	5	1	0	1
	Non-endemic Controls	10	0	0	0
	Periodontitis	5	0	0	0
	Rheumatoid Arthritis	5	1	0	1
	Syphilis	5	0	1	1
	Antibodies to:	n	IgM (+)	IgG (+)	IgM and/or IgG (+)
New York Biologics (n=25, Set 4)	Rapid Plasma Reagin (RPR)	8	1	1	2
	Epstein-Barr virus (EBV)	4	0	0	0
	Human immunodeficiency virus 1 (HIV-1)	4	0	0	0
	Cytomegalovirus (CMV)	5	0	1	1
Autoimmune and Allergy (n=42, Set 5)	Antinuclear antibody (ANA+)	5	0	0	0
	Antinuclear antibody (ANA-)	4	0	0	0
	DNA (+)	1	0	0	0
	Rheumatoid factor (+)	9	2	0	2
	Rheumatoid Factor (-)	8	0	0	0
	IgG (+)	13	1	0	1
	Spec. IgE (+)	4	0	0	0
	Spec. IgE (-)	2	0	0	0
In-house (n=4, Set 1)	Bartonella henselae	2	0	0	0
	Human Granuloytic Ehrlichia	2	0	0	0
False Positive			6	3	9
True Negative		121	115	118	112
Specificity			95%	97.5%	93%

We Claim

1. A composition comprising one or more labeled and/or tagged and/or bound amino acid sequences, said amino acid sequences consisting of 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, SEQ ID NO: 10, 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.

2. The composition of Claim 1, wherein said 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 composition of Claim 1, wherein each of said one or more amino acid sequences are tagged with an antibody with specificity for said amino acid sequence.

4. A method of detecting *Borrelia* antisera in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences of Clam 1; and

detecting a positive immunobinding reaction which indicates the presence of TBRF antisera in the sample.

5. The method of Claim 4, wherein two or more of the labeled and/or tagged and/or bound amino acid sequences of Clam 1 are mixed to the biological sample and a sample is considered positive for TBRF if at least two amino acid sequences are detected.

6. The method of Claim 4, wherein the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG antibody linked to a detectable moiety.

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

8. The method of Claim 6, wherein said detectable moiety comprises alkaline phosphatase.

9. The method of Claim 6, wherein said detectable moiety comprises biotin.

10. A method of detecting and distinguishing various species of *Borrelia* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences of Clam 1; and

detecting a positive immunobinding reaction which indicates the presence of *Borrelia* in the subject, wherein detection of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 indicates the presence of *B. hermsii*, in the subject; detection of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 indicates the presence of *B. miyamotoi* in the subject; detection of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 indicates the presence of *B. turcica* in the subject; detection of SEQ ID NO: 6 and/or SEQ ID NO: 10 indicates the detection of *B. turicatae* in the sample; and, detection of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 indicates the detection of *B. coriaceae* in the sample.

11. The method of Claim 10, wherein said the labeled and/or tagged and/or bound amino acid sequences are detected with anti-human IgG antibody conjugated to a detectable moiety.

12. The method of Claim 11, wherein said detectable moiety is selected from the group consisting of chromophores, radioactive moieties and enzymes.

13. The method of Claim 12, wherein said detectable moiety comprises alkaline phosphatase.

14. The method of 12, wherein said detectable moiety comprises biotin.

15. The method of Claim 10, wherein a sample is considered positive for *Borrelia* if at least one amino acid sequence is detected.

16. The method of Claim 10, wherein a sample is considered positive for a specific species of *Borrelia* if at least one amino acid sequence identified with a species is detected.

17. A method of detecting *B. hermsii* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 of Clam 1; and

detecting a positive immunobinding reaction of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 16 and/or SEQ ID NO: 17 the detection of which indicates the presence of *B. hermsii* in the sample.

18. A method of detecting *B. miyamotoi* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 of Clam 1; and

detecting a positive immunobinding reaction of SEQ ID NO: 3, SEQ ID NO: 8, SEQ D NO: 12 and/or SEQ ID NO: 14 which indicates the presence of *B. miyamotoi* in the sample

19. A method of detecting *B. turcica* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 of Clam 1; and

detecting a positive immunobinding reaction of SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 9 and/or SEQ ID NO: 15 which indicates the presence of *B. turcica* in the sample.

20. A method of detecting *B. turicatae* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 6 and/or SEQ ID NO: 10 of Clam 1; and

detecting a positive immunobinding reaction of SEQ ID NO: 6 and/or SEQ ID NO: 10 which indicates the presence of *B. turicatae* in the sample.

21. A method of detecting *B. coriaceae* in a sample from a subject suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing a biological sample obtained from a subject suspected of having TBRF;

mixing the biological sample with one or more of the labeled and/or tagged and/or bound amino acid sequences selected from the group of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 of Clam 1; and

detecting a positive immunobinding reaction of SEQ ID NO: 18, SEQ ID NO: 19 and/or SEQ ID NO: 20 of Clam 1 which indicates the presence of *B. coriaceae* in the sample.

22. A composition consisting essentially of one or more amino acid sequences selected from the group consisting of 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, SEQ ID NO: 10, 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, and an adjuvant.

23. A nucleic acid sequence encoding any one or more of the amino acid sequences of Claim 1.

24. A method of detecting T cells from a subject having or suspected of having tick-borne relapsing fever (TBRF), said method comprising:

providing i) a sample comprising T cells obtained from a subject suspected of having TBRF, ii) a culture apparatus coated at least partially with anti-antibodies specific for one or more cytokines and iii) one or more sequences of Claim 1;

mixing the blood sample with the one or more of the sequences of Clam 1 in the culture apparatus, and;

detecting any production of said one or more cytokines by the subject's T cells.

25. The method of Claim 25, wherein said anti-antibodies specific for a cytokine are anti-IFNγ antibodies and said cytokine is interferon gamma (IFNγ).

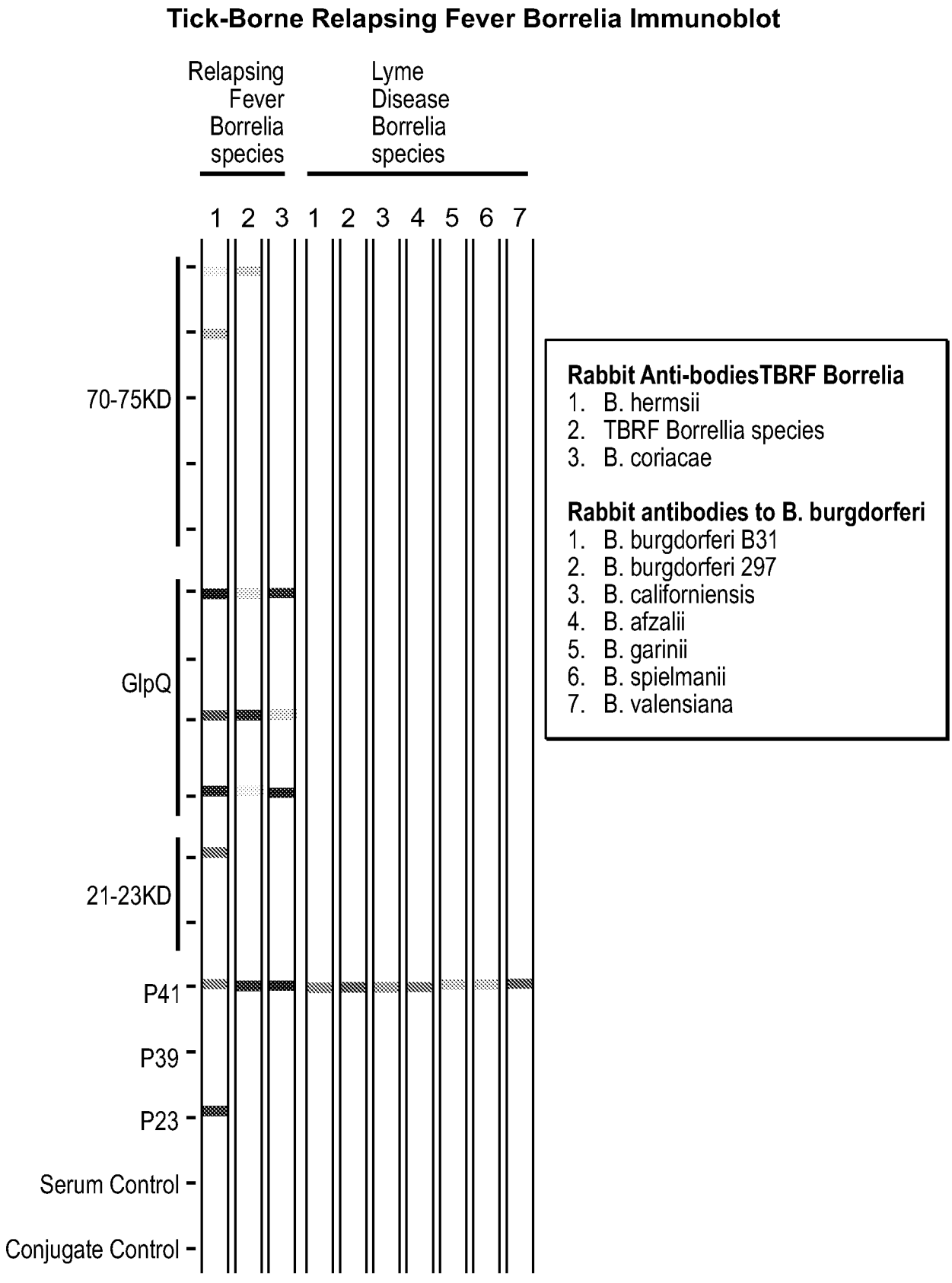
Tick-Borne Relapsing Fever Borrelia Immunoblot
Relapsing Fever Borrelia species
Lyme Disease Borrelia species
1 2 3 1 2 3 4 5 6 7
70-75KD
GlpQ
21-23KD
P41
P39
P23
Serum Control
Conjugate Control
Rabbit Anti-bodiesTBRF Borrelia
1. B. hermsii
2. TBRF Borrellia species
3. B. coriacae
Rabbit antibodies to B. burgdorferi
1. B. burgdorferi B31
2. B. burgdorferi 297
3. B. californiensis
4. B. afzalii
5. B. garinii
6. B. spielmanii
7. B. valensiana

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/21113

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.: 5
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, 6-25, directed to a composition comprising a labeled and/or tagged and/or bound peptide (claims 1-3, 22), a nucleic acid encoding said peptide (claim 23), a method of using it for detecting Borrelia in a sample (claims 4, 6-21, 24, 25). The peptide will be searched to the extent that the amino acid sequence encompasses SEQ ID NO: 1. It is believed that claims 1-4, 6-25 encompass this first named invention, and thus these claims will be searched without fee to the extent that amino acid sequence encompasses SEQ ID NO: 1. Additional amino acid sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected amino acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a peptide of the amino acid sequence SEQ ID NO: 20, i.e., claims 1-4, 6-25.

********** See Supplemental Sheet to continue **********

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, 6-25, restricted to SEQ ID NO: 1

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 19/21113

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/569, G01N 33/571, C07K 16/12, C07K 14/20, C07K 14/195, A61K 39/00 (2019.01)
CPC - C07K 14/20, C07K 16/1207, A61K 39/00, A61K 2039/53, G01N 33/56911, G01N 2333/20, Y02A 50/401, Y02A 50/57, C07K 2319/00, C07K 2319/02

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	UniProtKB/TrEMBL D5I3L2_BORHE. Immunogenic protein A (24 June 2015) [Retrieved from the Internet 24 April 2019: <https://www.uniprot.org/uniprot/D5I3L2.txt?version=4>] in entirety; amino acids 25-283, 99.7% identity to SEQ ID NO: 1	1-4, 6-25

☐ 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

"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
02 May 2019	10 JUL 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/21113

In Continuation of Box III. Observations where unity of invention is lacking:

The inventions listed as Group I+ 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:

The special technical feature of inventions of Group I+ is a specific amino acid sequence recited therein.

The inventions of Group I+ share the technical features of a composition comprising a labeled and/or tagged and/or bound peptide. Some inventions of Group I+ share the technical features of a method of using it for detecting Borrelia in a sample. However, these shared technical feature do not represent an improvement over prior art as being anticipated by a paper titled "Serological detection of Tick-Borne Relapsing Fever in Texan domestic dogs" by Esteve-Gasent, et al. (PLoS One. 2017, 12(12):e0189786) (hereinafter "Esteve -Gasent").

Esteve-Gasent discloses a composition comprising a labeled peptide (pg 4, Fig 1 and its legend, "Purification and clean-up of rGlpQ for ELISA test use... (C) Purified rGlpQ [recombinant Borrelial antigen glycerophosphodiester phosphodiesterase] after fusion protein removal and ready to use in ELISA and Immunoblot assays").

Esteve-Gasent discloses a method of using a composition comprising a bound peptide for detecting Borrelia in a sample (Abstract, "immunoblot assays showed that 17 out of 853 samples tested were considered to be seropositive, which constitutes 1.99% of all Texas samples tested in this study"; pg 5, "Immunoblot assays. Borrelia turicatae in-house assay. To confirm the ELISA seropositive samples, rGlpQ and Borrelia turicatae whole cell lysate were separated in 12% SDS-PAGE gels and wet transferred onto nitrocellulose membrane ? the membranes were incubated with 1:1000 dilutions of each canine serum sample for 1 hour at room temperature. The membranes were then washed again 3 times with TBS-T for 5 minutes each. Next, the membranes were incubated for 1 hour at room temperature with 1:3000 dilutions of anti-dog HRP conjugated antibody... After washing 6 times for 5 minutes each with TBS-T, the membranes were incubated with Chemiluminescent ECL detection reagents ... Borrelia burgdorferi commercially available assay. To confirm potential cross- reaction with B. burgdorferi, twenty-six dog serum samples were evaluated by Immunoblot using a commercially available human B. burgdorferi strip system... For each sample or control strip (positive and negative), a channel in a 12-strip plate was filled with 2 ml of 1X sample diluent/wash solution ... 20 mkL of each of the samples (1:100) was added to the appropriately marked channel and incubated at room temperature for 30 minutes. Strips were washed three times by adding 2 mL of sample diluent/wash solution to each channel of the strip incubation tray and incubated for 5 minutes with vigorous agitation. Two ml of anti-dog alkaline phosphatase conjugated IgG antibody ... diluted 1:2,000 was added to each strip containing well, and incubated for 30 minutes at room temperature. Strips were then washed 3 times and 2 mL of color developing solution was added to each channel. All strips were incubated for 6 minutes to allow color development. Strips were then ... evaluated. The presence or absence of the following 13 bands was then recorded: a93, a66, a60, a58, a45, a41 (Flagella), a39 (BmpA), a34, a31 (OpsA), a30, a28, a23 (OpsC) and a18... The presence of reactivity of two or more of the following antigens a93, a30, a 23 and/or a18 together with any of the other bands (excluding a66, a58 and a45) will be considered positive for LD"). 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 inventions.

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