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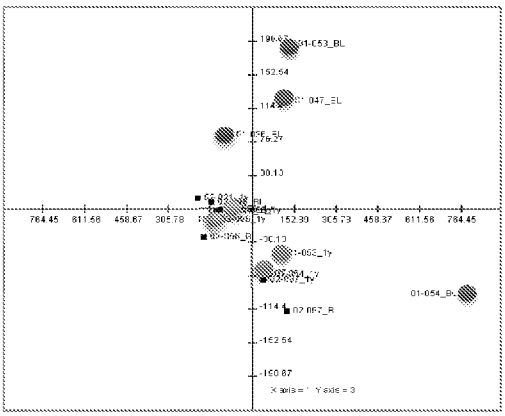


FIGURE 1

Figure 1. PCA analysis of 562 proteins quantified in all 16 samples showing that all 4 Lyme patients were clearly distinguished from the controls at time of diagnosis (i.e., baseline). RED big dots: 4 Lyme disease patients at baseline, BLUE big dots: 4 Lyme disease patients at 1 year post initial diagnosis, **Black** small squares: 4 controls at baseline and 1 year later.

(57) **Abstract:** Reagents and methods to use said reagents for detection of markers of various aspects of Lyme disease have been identified in biological fluids thus permitting diagnosis or classification of Lyme disease subjects as well as indicating suitable methods of treatment.

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CIRCULATING BIOMARKER SIGNATURES FOR LYME DISEASE DIAGNOSIS AND TREATMENT

Cross Reference to Related Applications

[0001] This application claims priority from U.S. provisional applications 62/613,009 filed 2 January 2018 and 62/665,382 filed 1 May 2018. The contents of these applications are incorporated by reference herein in their entirety.

Technical Field

[0002] The invention is directed to reagents and methods for detecting Lyme disease in subjects. In particular it includes reagents that detect miRNA, mRNA and peptides or proteins whose levels are altered in Lyme disease or in various stages thereof.

Background Art

[0003] Lyme disease (also known as Lyme borreliosis) is a zoonotic infection that is transmitted by certain *Ixodes* tick species and caused by a group of related spirochetes referred to formally as *Borrelia burgdorferi* sensu lato, or more simply as Lyme borrelia. With over 25,000 cases reported annually, it is the most common vector-borne infection in the United States. Lyme disease is also an infection of public health importance in parts of Europe and Asia.

[0004] The most common clinical manifestation is a characteristic skin lesion called erythema migrans. The spirochete may spread hematogenously to other skin locations resulting in secondary erythema migrans skin lesions or to non-skin sites such as the joints, nervous system, or heart leading to a variety of extracutaneous clinical manifestations. About 20-30% of patients don't respond to antibiotic treatment well and have higher risk to progress to post-treatment Lyme disease syndrome (PTLDS).

[0005] In clinical practice, the mainstay of laboratory diagnosis is detection of antibody to *Borrelia burgdorferi*. However, this test method has a number of limitations including poor sensitivity in early infection (<40%). In addition, antibodies will often persist for a long time measured in years; therefore, they do not provide information on the presence of active versus a past and resolved infection. Antibodies to *Borrelia burgdorferi* do not indicate who has a disseminated infection or who has coinfection with another tick-transmitted pathogen

such as *Babesia microti.* Thus, research to find a biomarker or biomarkers other than antibody production is warranted to better characterize patients with this infection.

Disclosure of the Invention

[0006] In one aspect, the invention is directed to a single panel of reagents for determining in a test subject,

[0007] (a) the presence or absence of Lyme disease;

[0008] (b) the probability that the subject will develop chronic Lyme disease symptoms;

[0009] (c) the probability that the subject will respond to a Lyme disease treatment; or

[0010] (d) the probability that the subject has PTLDS.

[0011] The single panel comprises, in an organized array on a single solid support, one or more detection reagents selected from the group consisting of antibodies, aptamers, oligonucleotide probes and combinations thereof that detect one or more miRNA markers of Lyme disease and/or one or more protein markers of Lyme disease, and wherein the detection of one or more miRNA markers and/or one or more protein markers indicates (a), (b), (c) or (d).

[0012] The one or more miRNA markers may comprise one or more miRNA markers selected from the group consisting of hsa-miR-423, hsa-miR-21, hsa-miR-130b, hsa-miR-615, hsa-miR-19b, hsa-miR-485, and hsa-miR-193a.

[0013] The one or more protein markers are proteins or peptides encoded by the genes having the symbols ACO1, ACY1, AFM, AGXT, ALDH1A1, ALDOB, AMBP, ANKRD65, APCS, APOA4, APOB, APOC2, APOC4, ApoE, APOF, APOM, ASH1L, ASTN1, BHMT, C1QB, C1QC, C1QL1, C1QL4, C1R, C1S, C2, C3, C4A, C4B, C4BPA, C4BPB, C5, C6, C7, C8A, C8B, C8G, C9, CA1, CD59, CD5L, CD93, CES1, CFB, CFD, CFH, CFHR1, CFHR5, CFI, CLSTN3, CNDP1, CNTFR, CPB2, CPN2, CRP, CSPG5, CST6, CTSB, CTSS, CTTNBP2, DDT, DEFA1, DEFA1B, DLG5, DMGDH, EEF1G, EPHA4, F10, F12, F9, FBP1, FOXN2, FSCN1, FTL, GABRA5, GC, GOLGB1, GOT1, GP6, GPR180, GPT, GSG2, GSTM2, GSTO1, HABP2, HBB, HGFAC, HMGXB4, HP, HPCAL4, HSPA9, IFNA2, IGFALS, IL1RAP, IQGAP1, ITGA2B, ITIH2, ITIH4, KIAA1462, KNG1, KRT9, LAP3, LCAT, LPA, LTBP3, LTN1, MAP2, MYL4, MYL6, NQO1, OIT3, OLFM1, PARVB, PF4, PGLYRP2, PHLDB3, PKLR, PLG, PLXDC1, POLR3H, PON1, PPP1R13L, PSMA1, PSMA4, PSMA5, PSMA7, PSMB1, PSMB4, PSME1, PYGL, RCN1, RRBP1, RUNDC3A,

RYR2, S100A9, SAA1, SEC23A, SELL, SERPINA12, SERPINA7, SERPINF2, SKAP2, SLC5A1, SMC1A, SOD2, SRCIN1, SSC5D, STK40, TRANK1, TRAP1, UNC45A, VTN, WDR48, ZBED6, ZNF238, ASL, HPD, MECOM, and MYH6. The peptides or proteins may be detected as such or in the form of their encoding mRNA.

[0014] In addition, the invention is directed to methods for detecting the presence or absence of Lyme disease, the probability that the subject will develop chronic Lyme disease symptoms or that the subject will respond to a particular Lyme disease treatment or that the subject has PTLDS by detecting the alteration in the level of one or more of the markers described above in a biological fluid of the subject. Where appropriate, the invention further includes treating the subject in accordance with the results of these methods.

Brief Description of the Drawings

[0015] Figure 1 is a depiction of principal component analysis (PCA) of 562 proteins that differentiate Lyme disease patients from controls.

[0016] Figures 2A– D depict volcano plotting of the 562 proteins described in Figure 1.

[0017] Figure 3 shows the level of the brain-specific protein MAP1A at various times relative to diagnosis.

[0018] Figures 4A-E show the relative abundance of various specific proteins measured by SRM at various time points in Lyme patients.

[0019] Figure 5 is a graph showing the concentration of hsa-miR-130b-5p concentration in Lyme patients with PTLDS as compared to patients without PTLDS.

[0020] Figure 6 is a graph showing the concentration of hsa-let-7a-5p in Lyme patients with clinical symptoms at baseline compared to Lyme patients with no symptoms at baseline.

[0021] Figures 7A-7C are a series of graphs showing the level of accuracy for diagnosis of Lyme disease onset using the array of markers miR-130b-5p, miR-19b-3p, miR-485-5p and miR-193a-5p. Figure 7A shows all Lyme patients vs. controls; Figure 7B shows PTLDS patients vs. controls and Figure 7C shows non-PTLDS patients vs. controls.

[0022] Figure 8 shows a similar assessment of the accuracy using a different set of miRNAs with respect to PTLDS vs. non-PTLDS patients.

Modes of Carrying Out The Invention

[0023] The subject matter in this specification includes all possible combinations of any individual features. For example, where a particular feature is disclosed in the context of a particular aspect or embodiment of the subject matter, or a particular claim, that feature can also be used, to the extent possible, in combination with other features disclosed and/or in the context of other particular aspects and embodiments of the subject matter, and in the subject matter generally.

[0024] The invention employs biological fluids of a subject. The fluids are typically blood, serum or plasma, and may also include, for example, urine or cerebrospinal fluid or saliva or lung lavage. Typical subjects are human, but other susceptible species, in particular, mammals in general may be tested. Controls or other bases for comparison with a test subject are based on determination of the levels of the various markers in subjects not exhibiting the classification tested for, or may be literature values for the markers in the relevant fluids or may, in particular in the case of evaluation of treatment regimens, be derived from the same subject at various time points.

[0025] In one embodiment, a single panel is provided for determining the probability of one or more classifications described herein in a test subject. In some embodiments, the single panel comprises two or more detection reagents which may be antibodies, aptamers, oligonucleotide probes or combinations thereof that detect one or more miRNA markers of Lyme disease and/or one or more protein markers of Lyme disease. The single panel comprises the one or more detection reagents in an organized array on a single solid support. The number of reagents in the organized array is typically a multiplicity – for example, 3, 5, 10, 50, 100 including intermediate integers. Also, when detection of protein or peptide is described, such detection may be either detection of the protein or peptide directly or detection of the mRNA encoding the protein or peptide or detection of both.

[0026] In the invention methods, the one or more miRNA markers and/or protein markers can be detected in a biological fluid of a test subject using any method available to a person of ordinary skill in the art including, but not limited to, the use of the single panel assay described above. Other detection methods can also be used, such as chromatographic methods, flow cytometry, mass spectrometry and other instrumental methods designed for multiplex detections. The difference in the level of the interaction between the detection reagents such as those on the single panel and the biological sample obtained from the test

subject, as compared to a corresponding biological sample from a control subject, indicates a probability of one or more classifications in a test subject.

[0027] In some embodiments, the biological sample is compared to one or more corresponding biological samples from one or more control subjects, one or more previous biological samples from the same test subject, or one or more biological sample from one or more classifications of subjects.

[0028] Classifications include the presence or absence of Lyme disease in the test subject, the nature of or success of a Lyme disease treatment regimen for the test subject, the probability of developing chronic Lyme disease symptoms, such as the post-treatment Lyme disease syndrome (PTLDS), and the probability of Lyme disease treatment efficacy for the test subject.

[0029] The one or more miRNA markers and/or protein markers are detected at one or more time points and used to perform one or more classifications including but not limited to diagnosing Lyme disease, diagnosing Lyme disease subtype and/or stage, determining the most effective treatment strategy, selecting among various treatment options including treatment type, dosage, and duration, and/or determining patient prognosis. Time points for detection include, but are not limited to, prior to diagnosis, at or about the time of diagnosis, and/or at one or more subsequent time points that may include time points prior to, during, and/or following one or more treatment regimens. In some embodiments, the detection of one or more miRNA markers and/or protein markers can be used to differentiate PTLDS patients from patients who returned to normal after treatment (i.e., non-PTLDS).

[0030] In some embodiments, the one or more miRNA markers comprise one or more miRNA markers from the group consisting of miR-423-5p, miR-21-5p, miR-130b-5p, miR-615-3p, miR-19b-3p, miR-485-5p, and miR-193a-5p.

[0031] In some embodiments, the miRNA markers comprise one to four of the miRNA markers miR-423-5p, miR-21-5p, miR-130b-5p, and miR-615-3p for diagnosing Lyme disease.

[0032] In some embodiments, the miRNA markers comprise one to four of the miRNA markers miR-130b-5p, miR-19b-3p, miR-485-5p, and miR-193a-5p for diagnosing Lyme disease.

[0033] In some embodiments, the one or more miRNA markers comprise one to four of the miRNA markers miR-130b-5p, miR-485-5p, miR-615-3p, and miR-423-5p to differentiate PTLDS patients from non-PTLDS patients.

[0034] The one or more miRNA markers comprise any one or more miRNA markers listed in Table 3, Table 7, Table 8, and/or Table 9. The average diagnostic accuracy achieved in differentiating PTLDS from non-PTLDS patients in differentiating Lyme disease patients from controls or the probability that the subject will develop chronic Lyme disease symptoms or the probability that the subject will respond to a Lyme disease treatment at one or more time points is at least or is at least about or is or is about 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 89%, 90%, 91%, 92%, 94%, 96%, and 98%.

[0035] In some embodiments, the one or more protein markers comprises one or more protein markers from the group consisting of proteins with the gene symbols of ACO1, ACY1, AFM, AGXT, ALDH1A1, ALDOB, AMBP, ANKRD65, APCS, APOA4, APOB, APOC2, APOC4, ApoE, APOF, APOM, ASH1L, ASTN1, BHMT, C1QB, C1QC, C1QL1, C1QL4, C1R, C1S, C2, C3, C4A, C4B, C4BPA, C4BPB, C5, C6, C7, C8A, C8B, C8G, C9, CA1, CD59, CD5L, CD93, CES1, CFB, CFD, CFH, CFHR1, CFHR5, CFI, CLSTN3, CNDP1, CNTFR, CPB2, CPN2, CRP, CSPG5, CST6, CTSB, CTSS, CTTNBP2, DDT, DEFA1, DEFA1B, DLG5, DMGDH, EEF1G, EPHA4, F10, F12, F9, FBP1, FOXN2, FSCN1, FTL, GABRA5, GC, GOLGB1, GOT1, GP6, GPR180, GPT, GSG2, GSTM2, GSTO1, HABP2, HBB, HGFAC, HMGXB4, HP, HPCAL4, HSPA9, IFNA2, IGFALS, IL1RAP, IQGAP1, ITGA2B, ITIH2, ITIH4, KIAA1462, KNG1, KRT9, LAP3, LCAT, LPA, LTBP3, LTN1, MAP2, MYL4, MYL6, NQO1, OIT3, OLFM1, PARVB, PF4, PGLYRP2, PHLDB3, PKLR, PLG, PLXDC1, POLR3H, PON1, PPP1R13L, PSMA1, PSMA4, PSMA5, PSMA7, PSMB1, PSMB4, PSME1, PYGL, RCN1, RRBP1, RUNDC3A, RYR2, S100A9, SAA1, SEC23A, SELL, SERPINA12, SERPINA7, SERPINF2, SKAP2, SLC5A1, SMC1A, SOD2, SRCIN1, SSC5D, STK40, TRANK1, TRAP1, UNC45A, VTN, WDR48, ZBED6, ZNF238, ASL, HPD, MECOM, and MYH6.

[0036] In some embodiments, the one or more protein markers comprises one or more of the proteins with the gene symbols of AFM, AGXT, ALDOB, APCS, APOA4, APOB, APOC4, C5, C6, C9, CES1, CFH, CFHR1, CRP, CST6, FBP1, F9, GC, HPCAL4, ITIH2, ITIH4, LCAT, OLFM1, PGLYRP2, SERPINA7, S100A9, and SLC5A1, or one or more protein markers listed in Table 1 or Table 2, and may further evaluate a protein marker for the

protein with the gene symbol of PF4. In some embodiments, the one or more detection reagents detect combinations of one or more peptides of the one or more protein markers and one or more mRNA that encode the one or more protein markers.

[0037] For evaluating the probability that a test subject is afflicted with Lyme disease, the invention method comprises contacting a biological sample obtained from the test subject with the single panel of the invention or one or more markers assessing the level of interaction between the one or more detection reagents on the single panel or marker. A difference in the level of the interaction in the test subject as compared to a corresponding biological sample from a control subject indicates a probability that the test subject is afflicted with Lyme disease, wherein the control subject is a normal subject that is not afflicted with Lyme disease.

[0038] Similarly, a difference in the level of the interaction in the test subject as compared to a corresponding biological sample from a control subject indicates a probability that the test subject is or is not afflicted with Lyme disease of a particular subset, wherein the particular subset is associated with one or more treatment regimens. In some embodiments, the particular subset is the subject with or without treatment according to a regimen so that a treatment regimen can be evaluated.

[0039] A difference in the level of said interaction in the subject undergoing treatment as a function of time indicates the treatment efficacy of the current and/or previous treatment regimen(s) of the test subject where the subject has been subjected to the treatment.

[0040] A difference in the level of the interaction in the test subject as compared to a corresponding biological sample from a control subject indicates a probability that the test subject has, or is likely to develop, chronic symptoms of Lyme disease, such as post-treatment Lyme disease syndrome (PTLDS).

[0041] The one or more protein markers may be acute phase and/or innate immune system response proteins, that may be highly enriched in *Borrelia burgdorferi* affected organs. These protein markers are used as biomarkers for early diagnosis of Lyme disease, or to distinguish subjects with Lyme disease who later develop PTLDS from those with Lyme disease who would later return to health after treatment over the same period of time.

[0042] In some embodiments, protein markers comprise one or more protein markers from the group consisting of proteins with the gene symbols set forth above, and comprise at least three, ten, sixteen, twenty-four, or thirty protein markers from this set.

[0043] In some embodiments, a subset is contemplated wherein protein markers comprises one, or at least three, six, nine, twelve, or fifteen of the proteins with the gene symbols of AFM, AGXT, ALDOB, APCS, APOA4, APOB, APOC4, C5, C6, C9, CES1, CFH, CFHR1, CRP, CST6, F9, FBP1, GC, HPCAL4, ITIH2, ITIH4, LCAT, OLFM1, PF4, PGLYRP2, SERPINA7, S100A9, and SLC5A1, or

[0044] one or more protein markers from the group consisting of proteins with the gene symbols of AFM, ALDOB, APOA4, APOB, C9, CFHR1, CRP, CST6, F9, FBP1, GC, ITIH2, ITIH4, PF4, PGLYRP2, and S100A9, or

[0045] one or more protein markers from the group consisting of proteins with the gene symbols of AFM, ALDOB, APOA4, C9, CRP, CST6, FBP1, ITIH2, PGLYRP2, and S100A9, or

[0046] one or more protein markers from the group consisting of proteins with the gene symbols of AFM, ALDOB, APOA4, CST6, FBP1, ITIH2, and PGLYRP2, or

[0047] one or more protein markers selected from the group consisting of proteins with the gene symbols of ALDOB, APOB, C9, CFHR1, CRP, CST6, F9, GC, ITIH4, and PF4, or

[0048] one or more markers selected from the group consisting of proteins with the gene symbols of ALDOB, C9, CRP, and CST6, or one or more markers selected from the group consisting of proteins with the gene symbols of AFM, ALDOB, CST6, and PGLYRP2, or

[0049] one or more markers from the group consisting of proteins with the gene symbols of C9, CST6, FBP1, and ALDOB.

[0050] In some embodiments, one or more ratios of the level of detection for a first protein marker to the level of detection for a second protein marker are determined. The one or more ratios are used to diagnose subjects afflicted with Lyme disease, to distinguish Lyme disease subjects in the early phase after tick bite, and/or to predict the likelihood that the subject would later develop PTLDS. The first protein marker may be any protein marker disclosed herein and the second protein marker may be any protein marker disclosed herein. In exemplary embodiments, the gene symbol for first protein marker is C9, FBP1, and ALDOB, and in some embodiments, the second protein marker is a protein with the gene symbol of CST6. Thus, suitable ratios are those of C9/CST6, FBP1/CST6, or ALDOB/CST6. Various numbers of ratios may be determined *e.g.* at least five, at least ten, at least twenty, at least thirty, or at least forty ratios of the levels of detection for first protein

markers to the level of detection for second protein markers. In some embodiments, a combination of C9/CST6 and FBP1/CST6 and ALDOB/CST6 is determined.

[0051] The levels of one or more of the protein markers for proteins with the gene symbols of AFM, ALDOB, APOA4, C9, CRP, CST6, FBP1, ITIH2, PGLYRP2, and S100A9 are altered in the serum of a test subject who is afflicted with Lyme disease as compared to a corresponding biological sample from a control subject who is not afflicted with Lyme disease, or the levels of one or more of the protein markers for proteins with the gene symbols of AFM, ALDOB, APOA4, CST6, FBP1, ITIH2, and PGLYRP2 are altered in the serum of a test subject who would later develop PTLDS as compared to a corresponding biological sample from a test subject who would not develop PTLDS over the same period of time.

[0052] In some embodiments, the levels of one or more of the protein markers for proteins with the gene symbols of ALDOB, APOB, C9, CFHR1, CRP, CST6, F9, GC, ITIH4, and PF4 are cooperative in classifying Lyme disease subjects.

[0053] In some embodiments, the levels of one or more of the protein markers for proteins with the gene symbols of ALDOB, C9, CRP, and CST6 are cooperative in classifying Lyme disease subjects.

[0054] In some embodiments, the levels of one or more of the protein markers for proteins with the gene symbols of AFM, ALDOB, CST6, and PGLYRP2 provide predictive value to distinguish test subjects who are afflicted with Lyme disease and later develop PTLDS from test subjects who are afflicted with Lyme disease and returned to health after treatment over the same period of time.

[0055] In some embodiments, the ratios of one or more protein pairs can be used to distinguish and classify test subjects with Lyme disease in the early phase of the disease after tick bite, or the ratios of one or more protein pairs can be used to distinguish test subjects with Lyme disease who are likely to develop PTLDS from those who are not likely to develop PTLDS, or the ratio comprises one or more of the protein pairs of C9/CST6, FBP1/CST6, or ALDOB/CST6.

[0056] In some embodiments, the biological sample is obtained from the test subject prior to any diagnosis of Lyme disease, at or around the time of diagnosis of Lyme disease, or at any time point following diagnosis of Lyme disease.

Protein Markers

[0057] Table 1 lists peptide sequences determined by mass spectrometry generated by proteins with altered serum levels in Lyme or PTLDS.

TABLE 1.

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
P21399	ACO1	DFNDPSQDPDFTQVVELDLK		Yes	Yes
P21399	ACO1	FVEFFGPGVAQLSIADR		Yes	Yes
P21399	ACO1	GPFLLGIK		Yes	Yes
Q03154	ACY1	GPEEEHPSVTLFR		Yes	Yes
Q03154	ACY1	LHEAVFLR		Yes	Yes
Q03154	ACY1	TPVLLHDHDER		Yes	Yes
Q03154	ACY1	TVQPKPDYGAAVAFFEETAR		Yes	Yes
P43652	AFM	LPNNVLQEK	liver	Yes	Yes
P21549	AGXT	ALNAPPGTSLISFSDK	liver	Yes	Yes
P21549	AGXT	LQALGLQLFVK	liver	Yes	Yes
P00352	ALDH1A1	IFINNEWHDSVSGK		Yes	Yes
P00352	ALDH1A1	TIPIDGNFFTYTR		Yes	Yes
P00352	ALDH1A1	YILGNPLTPGVTQGPQIDK		Yes	Yes
P05062	ALDOB	ALQASALAAWGGK	liver	Yes	Yes
P05062	ALDOB	ELSEIAQSIVANGK	liver	Yes	Yes
P02760	AMBP	GECVPGEQEPEPILIPR	liver	Yes	Yes
E5RJM6	ANKRD65	GPAGLVTQLLR	liver	Yes	Yes
E5RJM6	ANKRD65	LLEAPGPGPAAAEAEDAR	liver	Yes	Yes
P02743	APCS	IVLGQEQDSYGGK	liver	Yes	Yes
P06727	APOA4	LGPHAGDVEGHLSFLEK	liver/small intestine	Yes	Yes
P06727	APOA4	SELTQQLNALFQDK	liver/small intestine	Yes	Yes
P04114	APOB	GFEPTLEALFGK	liver	Yes	Yes
P02655	APOC2	TYLPAVDEK	liver	Yes	Yes
P55056	APOC4	AWFLESK	liver	Yes	Yes
P55056	APOC4	ELLETVVNR	liver	Yes	Yes
P02649	ApoE	CLAVYQAGAR		Yes	Yes
P02649	ApoE	LAVYQAGAR		Yes	Yes
P02649	ApoE	LGADMEDVCGR		Yes	Yes
P02649	ApoE	LGADMEDVR		Yes	Yes
Q13790	APOF	SGVQQLIQYYQDQK	liver	Yes	Yes
O95445	APOM	AFLLTPR		Yes	Yes
O95445	APOM	DGLCVPR		Yes	Yes
O95445	APOM	WIYHLTEGSTDLR		Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
Q9NR48	ASH1L	ALLNGGTSVTEK		Yes	Yes
Q9NR48	ASH1L	LGLGTVVGLVNK		Yes	Yes
Q9NR48	ASH1L	LGTGTTAVFINK		Yes	Yes
Q9NR48	ASH1L	LHNTVSSLAATFGSK		Yes	Yes
O14525	ASTN1	SITVSALPFLR	brain	Yes	Yes
O14525	ASTN1	GFDLVLGEQPSDK		Yes	Yes
O14525	ASTN1	ITLHVPEHLIADGSR		Yes	Yes
O14525	ASTN1	IYNLFNGYTSGK		Yes	Yes
Q93088	BHMT	AGASIIGVNCHFDPTISLK		Yes	Yes
Q93088	BHMT	AIAEELAPER		Yes	Yes
P02746	C1QB	LEQGENVFLQATDK		Yes	Yes
P02747	C1QC	FNAVLTNPQGDYDTSTGK		Yes	Yes
P02747	C1QC	GEPGIPAIPGIR		Yes	Yes
P02747	C1QC	TNQVNSGGVLLR		Yes	Yes
O75973	C1QL1	AHGGNSNK		Yes	Yes
O75973	C1QL1	FDDVVTNLGNNYDAASGK		Yes	Yes
O75973	C1QL1	VAFYAGLK		Yes	Yes
Q86Z23	C1QL4	FDDVVTNVGNAYEAASGK		Yes	Yes
Q86Z23	C1QL4	VHGGNTNK		Yes	Yes
P00736	C1R	CLPVCGKPVNPVEQR		Yes	Yes
P00736	C1R	FCGQLGSPLGNPPGK		Yes	Yes
P00736	C1R	LPVANPQACENWLR		Yes	Yes
P09871	C1S	GDSGGAFAVQDPNDK		Yes	Yes
P09871	C1S	IIGGSDADIK		Yes	Yes
P09871	C1S	TNFDNDIALVR		Yes	Yes
P06681	C2	ECQGNGVWSGTEPICR		Yes	Yes
P06681	C2	GALISDQWVLTAAHCFR	liver	Yes	Yes
P01024	C3	IHWESASLLR	liver	Yes	Yes
P01024	C3	SGIPIVTSPYQIHFTK	liver	Yes	Yes
P01024	C3	VLLDGVQNPR	liver	Yes	Yes
P0C0L4	C4A	VLSLAQEQVGGSPEK	liver	Yes	Yes
P0C0L5	C4B	HLVPGAPFLLQALVR	liver	Yes	Yes
P0C0L5	C4B	SCGLHQLLR	liver	Yes	Yes
P04003	C4BPA	LSLEIEQLELQR	liver	Yes	Yes
P04003	C4BPA	YTCLPGYVR	liver	Yes	Yes
P20851	C4BPB	LIQEAPKPECEK	liver	Yes	Yes
P01031	C5	TDAPDLPEENQAR	liver	Yes	Yes
P13671	C6	DLHLSDVFLK	liver	Yes	Yes
P10643	C7	LSGNVLSYTFQVK		Yes	Yes
P10643	C7	LTPLYELVK		Yes	Yes
P10643	C7	YSAWAESVTNLPQVIK		Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
P07357	C8A	AIDEDCSQYEPIPGSQK	liver	Yes	Yes
P07358	C8B	IPGIFELGISSQSDR	liver	Yes	Yes
P07358	C8B	SGFSFGFK	liver	Yes	Yes
P07360	C8G	SLPVSDSVLSGFEQR	liver	Yes	Yes
P07360	C8G	VQEAHLTEDQIFYFPK	liver	Yes	Yes
P02748	C9	LSPIYNLVPVK	liver	Yes	Yes
P00915	CA1	LYPIANGNNQSPVDIK		Yes	Yes
P00915	CA1	VLDALQAIK		Yes	Yes
P13987	CD59	AGLQVYNK		Yes	Yes
P13987	CD59	FEHCNFNDVTTR		Yes	Yes
O43866	CD5L	LVGGDNLCSGR		Yes	Yes
Q9NPY3	CD93	LLDDLVTCASR		Yes	Yes
P23141	CES1	EGYLQIGANTQAAQK	liver	Yes	Yes
P23141	CES1	GNWGHLDQVAALR	liver	Yes	Yes
P00751	CFB	LEDSVTYHCSR	liver	Yes	Yes
P00746	CFD	ATLGPAVRPLPWQR		Yes	Yes
P00746	CFD	LAVLVLLGAAACAAPPR		Yes	Yes
P00746	CFD	THHDGAITER		Yes	Yes
P08603	CFH	LEDSVTYHCSR		Yes	Yes
Q03591	CFHR1	ITCTEEGWSPTPK	liver	Yes	Yes
Q03591	CFHR1	INHGILYDEEK	liver	Yes	Yes
Q03591	CFHR1	ISSVGGEATFCDFPK		Yes	Yes
Q9BXR6	CFHR5	ENYLLPEAK	liver	Yes	Yes
Q9BXR6	CFHR5	GWSTPPICSFTK	liver	Yes	Yes
Q9BXR6	CFHR5	LQGSVTVTCR	liver	Yes	Yes
P05156	CFI	AQLGDLPWQVAIK	liver	Yes	Yes
P05156	CFI	HGNTDSEGIVEVK	liver	Yes	Yes
P05156	CFI	VFSLQWGEVK	liver	Yes	Yes
Q9BQT9	CLSTN3	VNDVNEFAPVFVER	brain	Yes	Yes
Q9BQT9	CLSTN3	IEYAPGAGSLALFPGIR	brain	Yes	Yes
Q9BQT9	CLSTN3	LHGSGVPFEAVILDK		Yes	Yes
Q96KN2	CNDP1	ALEQDLPVNIK		Yes	Yes
Q96KN2	CNDP1	EWVAIESDSVQPVPR		Yes	Yes
P26992	CNTFR	EYIIQVAAK		Yes	Yes
Q96IY4	CPB2	SFYANNHCIGTDLNR	liver	Yes	Yes
P22792	CPN2	LLNIQTYCAGPAYLK	liver	Yes	Yes
P02741	CRP	ESDTSYVSLK	liver	Yes	Yes
P02741	CRP	GYSIFSYATK	liver	Yes	Yes
O95196	CSPG5	ALAHYDNVICQDDPSAPHK		Yes	Yes
O95196	CSPG5	EAGSAVEAEELVK		Yes	Yes
Q15828	CST6	AQSQLVAGIK	skin	Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
Q15828	CST6	DLSPDDPQVQK	skin	Yes	Yes
P07858	CTSB	HYGYNSYSVSNSEK		Yes	Yes
P25774	CTSS	GIDSDASYPYK		Yes	Yes
Q8WZ74	CTTNBP2	APEDAAGAAAEAAK		Yes	Yes
Q8WZ74	CTTNBP2	LGTPEALLGPK		Yes	Yes
P30046	DDT	ELALGQDR		Yes	Yes
P30046	DDT	FFPLESWQIGK		Yes	Yes
P30046	DDT	LCAAAASILGKPADR		Yes	Yes
P30046	DDT	SHSAHFFEFLTK		Yes	Yes
P59665	DEFA1	YGTCIYQGR		Yes	Yes
P59665	DEFA1	IPACIAGER	blood	Yes	Yes
P59665	DEFA1B	YGTCIYQGR	blood	Yes	Yes
Q8TDM6	DLG5	GSEPLGISIVSGEK		Yes	Yes
Q8TDM6	DLG5	HCLLDIAPHAIER		Yes	Yes
Q8TDM6	DLG5	IASSYYPEGDGDSSHLPAK		Yes	Yes
Q8TDM6	DLG5	LAPVVIPAQFLEEQK		Yes	Yes
Q9UI17	DMGDH	EGEEKPPLSAETQWK		Yes	Yes
Q9UI17	DMGDH	LEEETGQVVGFHQPGSIR		Yes	Yes
Q9UI17	DMGDH	NYPAVIIQEPLVLTEPTR		Yes	Yes
P26641	EEF1G	ILGLLDAYLK		Yes	Yes
P26641	EEF1G	LDPGSEETQTLVR		Yes	Yes
P54764	EPHA4	GLNPLTSYVFHVR		Yes	Yes
P54764	EPHA4	VYPANEVTLLDSR		Yes	Yes
P00742	F10	ETYDFDIAVLR	liver	Yes	Yes
P00742	F10	NCELFTR	liver	Yes	Yes
P00748	F12	VVGGLVALR	liver	Yes	Yes
P00740	F9	SALVLQYLR	liver	Yes	Yes
P00740	F9	SCEPAVPFPCGR	liver	Yes	Yes
P09467	FBP1	APVILGSPDDVLEFLK	liver	Yes	Yes
P09467	FBP1	EAVLDVIPTDIHQR	liver	Yes	Yes
P32314	FOXN2	LQESDSLATSIDPK		Yes	Yes
P32314	FOXN2	SSVSSLSSVDEVYEFIPK		Yes	Yes
Q16658	FSCN1	LSCFAQTVSPAEK		Yes	Yes
Q16658	FSCN1	YLTAEAFGFK		Yes	Yes
P02792	FTL	DDVALEGVSHFFR		Yes	Yes
P02792	FTL	LNQALLDLHALGSAR		Yes	Yes
P31644	GABRA5	LPLNNLLASK	brain	Yes	Yes
P31644	GABRA5	STNAFTTGK	brain	Yes	Yes
P02774	GC	ELSSFIDK	liver	Yes	Yes
P02774	GC	HLSLLTTLSNR	liver	Yes	Yes
Q14789	GOLGB1	AQVVDLLQQELTAAEQR		Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
Q14789	GOLGB1	VLLDDTQSEAAR		Yes	Yes
P17174	GOT1	IGADFLAR		Yes	Yes
P17174	GOT1	ITWSNPPAQGAR		Yes	Yes
P17174	GOT1	IVASTLSNPELFEEWTGNVK		Yes	Yes
P17174	GOT1	VGGVQSLGGTGALR		Yes	Yes
Q9HCN6	GP6	ASFPIITVTAAHSGTYR		Yes	Yes
Q9HCN6	GP6	YGFDQFALYK		Yes	Yes
Q86V85	GPR180	IGHFEFHGDHALLCVR		Yes	Yes
Q86V85	GPR180	LQQSSHGYSCSEK		Yes	Yes
P24298	GPT	ALCVINPGNPTGQVQTR		Yes	Yes
P24298	GPT	ALELEQELR		Yes	Yes
P24298	GPT	AWALDVAELHR		Yes	Yes
P24298	GPT	LLVAGEGHTR		Yes	Yes
Q8TF76	GSG2	SILHQLTASLAVAEASLR		Yes	Yes
P28161	GSTM2	LGLDFPNLPYLIDGTHK		Yes	Yes
P28161	GSTM2	NQVFEPSCLDAFPNLK		Yes	Yes
P78417	GSTO1	GSAPPGPVPEGSIR		Yes	Yes
Q14520	HABP2	LIANTLCNSR		Yes	Yes
P68871	HBB	EFTPPVQAAYQK		Yes	Yes
P68871	HBB	VNVDEVGGEALGR		Yes	Yes
Q04756	HGFAC	VANYVDWINDR	liver	Yes	Yes
Q04756	HGFAC	YEYLEGGDR	liver	Yes	Yes
Q9UGU5	HMGXB4	DEQGALLLGHELQSFLK		Yes	Yes
Q9UGU5	HMGXB4	VTIVADHPGIDFGELSK		Yes	Yes
P00738	HP	VTSIQDWVQK	liver	Yes	Yes
Q9UM19	HPCAL4	DCPSGILNLEEFQQLYIK	brain	Yes	Yes
P38646	HSPA9	DAGQISGLNVLR		Yes	Yes
P01563	IFNA2	HDFGFPQEEFGNQFQK	brain	Yes	Yes
P01563	IFNA2	SFSLSTNLQESLR		Yes	Yes
P35858	IGFALS	DFALQNPSAVPR	liver	Yes	Yes
Q9NPH3	IL1RAP	VAFPLEVVQK		Yes	Yes
P46940	IQGAP1	LGLAPQIQDLYGK		Yes	Yes
P46940	IQGAP1	LGNFFSPK		Yes	Yes
P08514	ITGA2B	LSLNAELQLDR		Yes	Yes
P08514	ITGA2B	VAIVVGAPR		Yes	Yes
P19823	ITIH2	VQFELHYQEVK	liver	Yes	Yes
Q14624	ITIH4	ILDDLSPR	liver	Yes	Yes
Q14624	ITIH4	LGVYELLLK	liver	Yes	Yes
Q9P266	KIAA1462	TSLSVDQAPTPK		Yes	Yes
P01042	KNG1	DIPTNSPELEETLTHTITK	liver	Yes	Yes
P01042	KNG1	YFIDFVAR	liver	Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
P35527	KRT9	TLLDIDNTR		Yes	Yes
P28838	LAP3	GSDEPPVFLEIHYK		Yes	Yes
P28838	LAP3	GVLFASGQNLAR		Yes	Yes
P04180	LCAT	SSGLVSNAPGVQIR	liver	Yes	Yes
P08519	LPA	NPDAVAAPYCYTR	liver	Yes	Yes
P08519	LPA	TPAYYPNAGLIK	liver	Yes	Yes
Q9NS15	LTBP3	CPQLQYTGVQKPGPVR		Yes	Yes
Q9NS15	LTBP3	GYTQDNNIVNYGIPAHR		Yes	Yes
Q9NS15	LTBP3	SAVEIAPTQVTETDECR		Yes	Yes
Q9NS15	LTBP3	VVFAPVICK		Yes	Yes
O94822	LTN1	FLSTLLDSFSSSR		Yes	Yes
P11137	MAP2	EEFVETCPSEHK	brain	Yes	Yes
P11137	MAP2	LINQPLPDLK	brain	Yes	Yes
P11137	MAP2	VGSLDNAHHVPGGGNVK	brain	Yes	Yes
P11137	MAP2	YSAWAESVTNLPQVIK	brain	Yes	Yes
P12829	MYL4	ITYGQCGDVLR	heart	Yes	Yes
P60660	MYL6	EAFQLFDR		Yes	Yes
P15559	NQO1	ALIVLAHSER		Yes	Yes
P15559	NQO1	FGLSVGHHLGK		Yes	Yes
Q8WWZ8	OIT3	LYTISEGYVPNLR	liver	Yes	Yes
Q8WWZ8	OIT3	NHGIFPFTLEIFK	liver	Yes	Yes
Q99784	OLFM1	LTGISDPVTVK	brain	Yes	Yes
Q9HBI1	PARVB	ARPEDVVNLDLK		Yes	Yes
Q96PD5	PGLYRP2	EFTEAFLGCPAIHPR	liver	Yes	Yes
Q96PD5	PGLYRP2	GCPDVQASLPDAK	liver	Yes	Yes
Q6NSJ2	PHLDB3	ELLEQQAASEQR		Yes	Yes
Q6NSJ2	PHLDB3	LQGQQLEALTR		Yes	Yes
P30613	PKLR	GDLGIEIPAEK	liver	Yes	Yes
P00747	PLG	CTTPPPSSGPTYQCLK	liver	Yes	Yes
P00747	PLG	EAQLPVIENK		Yes	Yes
Q8IUK5	PLXDC1	GSFTFQAALHHDGR		Yes	Yes
Q9Y535	POLR3H	IPPWQFER	brain	Yes	Yes
Q9Y535	POLR3H	LEDAYVFPGDGASHTK	brain	Yes	Yes
P27169	PON1	IFFYDSENPPASEVLR	liver	Yes	Yes
P27169	PON1	IQNILTEEPK	liver	Yes	Yes
Q8WUF5	PPP1R13L	GSPLAEGPQAFFPER		Yes	Yes
Q8WUF5	PPP1R13L	TPLYLQPDAYGSLDR		Yes	Yes
P25786	PSMA1	AQPAQPADEPAEK		Yes	Yes
P25786	PSMA1	LVSLIGSK		Yes	Yes
P25789	PSMA4	LLDEVFFSEK		Yes	Yes
P25789	PSMA4	RPFGVSLLYIGWDK		Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
P25789	PSMA4	SALALAIK		Yes	Yes
P25789	PSMA4	TTIFSPEGR		Yes	Yes
P28066	PSMA5	AIGSASEGAQSSLQEVYHK		Yes	Yes
P28066	PSMA5	GVNTFSPEGR		Yes	Yes
P28066	PSMA5	LFQVEYAIEAIK		Yes	Yes
P28066	PSMA5	LGSTAIGIQTSEGVCLAVEK		Yes	Yes
O14818	PSMA7	DIVVLGVEK		Yes	Yes
O14818	PSMA7	LTVEDPVTVEYITR		Yes	Yes
P20618	PSMB1	DVFISAAER		Yes	Yes
P20618	PSMB1	GAVYSFDPVGSYQR		Yes	Yes
P20618	PSMB1	LSEGFSIHTR		Yes	Yes
P28070	PSMB4	AIHSWLTR		Yes	Yes
P28070	PSMB4	FQIATVTEK		Yes	Yes
Q06323	PSME1	TENLLGSYFPK		Yes	Yes
P06737	PYGL	GIVGVENVAELK		Yes	Yes
P06737	PYGL	YEYGIFNQK		Yes	Yes
Q15293	RCN1	HWILPQDYDHAQAEAR		Yes	Yes
Q15293	RCN1	IDNDGDGFVTTEELK		Yes	Yes
Q15293	RCN1	ISWEEYK		Yes	Yes
Q15293	RCN1	TFDQLTPDESK		Yes	Yes
Q9P2E9	RRBP1	TAGPLESSETEEASQLK		Yes	Yes
Q59EK9	RUNDC3A	TPVVIDYTPYLK		Yes	Yes
Q59EK9	RUNDC3A	GYLEELVR		Yes	Yes
Q59EK9	RUNDC3A	LQLQLEEAAAQNQR		Yes	Yes
Q92736	RYR2	DSSLSAVLNSIDVK		Yes	Yes
P06702	S100A9	LGHPDTLNQGEFK		Yes	Yes
P0DJI8	SAA1	EANYIGSDK		Yes	Yes
P0DJI8	SAA1	GPGGVWAAEAISDAR		Yes	Yes
Q15436	SEC23A	AVLNPLCQVDYR		Yes	Yes
Q15436	SEC23A	HLLQAPVDDAQEILHSR		Yes	Yes
P14151	SELL	SLTEEAENWGDGEPNNK		Yes	Yes
Q8IW75	SERPINA12	IFEEHGDLTK	skin	Yes	Yes
Q8IW75	SERPINA12	GLQVDTFSR	skin	Yes	Yes
Q8IW75	SERPINA12	SGIYQVGYDDK	skin	Yes	Yes
P05543	SERPINA7	GWVDLFVPK	liver	Yes	Yes
P08697	SERPINF2	WFLLEQPEIQVAHFPFK	liver	Yes	Yes
O75563	SKAP2	DAEEWVQQLK		Yes	Yes
O75563	SKAP2	DHSFLGFEWQK		Yes	Yes
O75563	SKAP2	SIYLQEFQDK		Yes	Yes
P13866	SLC5A1	TTAVTRPVETHELIR	heart	Yes	Yes
Q14683	SMC1A	SGVISGGASDLK		Yes	Yes

Protein Markers with Peptide Sequences Identified by Mass Spectrometry in Patient Serum					
UniProt ID	**Gene Symbol**	**Peptide Sequence**	**Organ origin**	**Detected in serum**	**Can distinguish Lyme patients vs controls or PTLDS vs non-PTLDS**
P04179	SOD2	DFGSFDK		Yes	Yes
P04179	SOD2	GDVTAQIALQPALK		Yes	Yes
P04179	SOD2	LTAASVGVQGSGWGWLGFNK		Yes	Yes
Q9C0H9	SRCIN1	TSIPVLTSFGAR		Yes	Yes
A1L4H1	SSC5D	LPWTWDTPSGR		Yes	Yes
A1L4H1	SSC5D	WTSHTTATLTPQAPR		Yes	Yes
Q8N2I9	STK40	AGPFILGPR		Yes	Yes
Q8N2I9	STK40	HLVSEGDLLK		Yes	Yes
O15050	TRANK1	SVAEALQDLLFER		Yes	Yes
Q12931	TRAP1	YSNFVSFPLYLNGR		Yes	Yes
Q12931	TRAP1	AFLDALQNQAEASSK		Yes	Yes
Q9H3U1	UNC45A	LDQAVLDLQR		Yes	Yes
P04004	VTN	VDTVDPPYPR	liver	Yes	Yes
Q8TAF3	WDR48	NGVNALQLDPALNR		Yes	Yes
Q8TAF3	WDR48	SADPPPAIWVATTK		Yes	Yes
Q8TAF3	WDR48	SGGDLTLHYR		Yes	Yes
Q8TAF3	WDR48	TLISASSDTTVK		Yes	Yes
P86452	ZBED6	GKPGSHLGTSTLQR		Yes	Yes
P86452	ZBED6	LPSETYFFTK		Yes	Yes
P86452	ZBED6	NSLEDFFPQGADLETYK		Yes	Yes
P86452	ZBED6	SDLLSDTLHGEK		Yes	Yes
Q99592	ZNF238	DSADVDCVLDLSVK		Yes	Yes
Q99592	ZNF238	FHCELVNSLSVK		Yes	Yes
P04424	ASL			Yes	Yes
P32754	HPD			Yes	Yes
Q03112	MECOM			Yes	Yes
P13533	MYH6			Yes	Yes

[0058] Table 2 lists the proteins of Table 1 for which in most cases SRM assay has also been developed.

TABLE 2.

List of Protein Markers Identified						
UniProt ID	Gene symbol	Organ origin	detected in serum	MS platform	Can distinguish Lyme patients vs controls or PTLDS vs nonPTLDS	SRM assay developed
P21399	ACO1		YES	ITRAQ	YES	YES
Q03154	ACY1		YES	ITRAQ	YES	YES
P43652	AFM	liver	YES	iTRAQ/SRM	YES	YES
P21549	AGXT	liver	YES	SRM	YES	YES
P00352	ALDH1A1		YES	ITRAQ	YES	YES
P05062	ALDOB	liver	YES	iTRAQ/SRM	YES	YES
P02760	AMBP	liver	YES	SRM	YES	YES
E5RJM6	ANKRD65		YES	ITRAQ	YES	YES
P02743	APCS	liver	YES	SRM	YES	YES
P06727	APOA4	liver/small intestine	YES	iTRAQ/SRM	YES	YES
P04114	APOB	liver	YES	iTRAQ/SRM	YES	YES
P02655	APOC2	liver	YES	SRM	YES	YES
P55056	APOC4	liver	YES	iTRAQ/SRM	YES	YES
P02649	ApoE	Brain	YES	iTRAQ/SRM	YES	YES
Q13790	APOF	liver	YES	iTRAQ/SRM	YES	YES
O95445	APOM	0	YES	iTRAQ/SRM	YES	YES
Q9NR48	ASH1L		YES	ITRAQ	YES	YES
O14525	ASTN1	brain	YES	SRM	YES	YES
Q93088	BHMT		YES	ITRAQ	YES	YES
P02746	C1QB	CC	YES	SRM	YES	YES
P02747	C1QC	CC	YES	SRM	YES	YES
O75973	C1QL1	CC	YES	SRM	YES	YES
Q86Z23	C1QL4	CC	YES	SRM	YES	YES
P00736	C1R	CC	YES	SRM	YES	YES
P09871	C1S	CC	YES	SRM	YES	YES
P06681	C2	liver	YES	SRM	YES	YES
P01024	C3	liver	YES	SRM	YES	YES
P0C0L4	C4A	liver	YES	SRM	YES	YES
P0C0L5	C4B	liver	YES	SRM	YES	YES
P04003	C4BPA	liver	YES	iTRAQ/SRM	YES	YES
P20851	C4BPB	liver	YES	SRM	YES	YES
P01031	C5	liver	YES	SRM	YES	YES

List of Protein Markers Identified

UniProt ID	Gene symbol	Organ origin	detected in serum	MS platform	Can distinguish Lyme patients vs controls or PTLDS vs nonPTLDS	SRM assay developed
P13671	C6	liver	YES	SRM	YES	YES
P10643	C7	CC	YES	SRM	YES	YES
P07357	C8A	liver	YES	SRM	YES	YES
P07358	C8B	liver	YES	SRM	YES	YES
P07360	C8G	liver	YES	SRM	YES	YES
P02748	C9	liver	YES	iTRAQ/SRM	YES	YES
P00915	CA1	0	YES	iTRAQ/SRM	YES	YES
P13987	CD59	0	YES	SRM	YES	YES
O43866	CD5L		YES	ITRAQ	YES	YES
Q9NPY3	CD93	0	YES	iTRAQ/SRM	YES	YES
P23141	CES1	liver	YES	iTRAQ/SRM	YES	YES
P00751	CFB	liver	YES	SRM	YES	YES
P00746	CFD	CC	YES	SRM	YES	YES
P08603	CFH	CC	YES	SRM	YES	YES
Q03591	CFHR1	liver	YES	SRM	YES	YES
Q9BXR6	CFHR5	liver	YES	SRM	YES	YES
P05156	CFI	liver	YES	SRM	YES	YES
Q9BQT9	CLSTN3	brain	YES	SRM	YES	YES
Q96KN2	CNDP1		YES	ITRAQ	YES	YES
P26992	CNTFR	0	YES	iTRAQ/SRM	YES	YES
Q96IY4	CPB2	liver	YES	SRM	YES	YES
P22792	CPN2	liver	YES	SRM	YES	YES
P02741	CRP	liver	YES	iTRAQ/SRM	YES	YES
O95196	CSPG5		YES	ITRAQ	YES	YES
Q15828	CST6	skin	YES	iTRAQ/SRM	YES	YES
P07858	CTSB	0	YES	iTRAQ/SRM	YES	YES
P25774	CTSS		YES	ITRAQ	YES	YES
Q8WZ74	CTTNBP2		YES	ITRAQ	YES	YES
P30046	DDT		YES	ITRAQ	YES	YES
P59665	DEFA1	blood	YES	iTRAQ/SRM	YES	YES
P59665	DEFA1B	blood	YES	iTRAQ/SRM	YES	YES
Q8TDM6	DLG5		YES	ITRAQ	YES	YES
Q9UI17	DMGDH		YES	ITRAQ	YES	YES
P26641	EEF1G	0	YES	iTRAQ/SRM	YES	YES
P54764	EPHA4	0	YES	iTRAQ/SRM	YES	YES
P00742	F10	liver	YES	SRM	YES	YES
P00748	F12	liver	YES	SRM	YES	YES
P00740	F9	liver	YES	SRM	YES	YES

List of Protein Markers Identified

UniProt ID	Gene symbol	Organ origin	detected in serum	MS platform	Can distinguish Lyme patients vs controls or PTLDS vs nonPTLDS	SRM assay developed
P09467	FBP1	liver	YES	iTRAQ/SRM	YES	YES
P32314	FOXN2		YES	ITRAQ	YES	YES
Q16658	FSCN1	0	YES	iTRAQ/SRM	YES	YES
P02792	FTL	0	YES	iTRAQ/SRM	YES	YES
P31644	GABRA5	brain	YES	SRM	YES	YES
P02774	GC	liver	YES	SRM	YES	YES
Q14789	GOLGB1		YES	ITRAQ	YES	YES
P17174	GOT1		YES	ITRAQ	YES	YES
Q9HCN6	GP6	0	YES	iTRAQ/SRM	YES	YES
Q86V85	GPR180		YES	ITRAQ	YES	YES
P24298	GPT		YES	ITRAQ	YES	YES
Q8TF76	GSG2		YES	ITRAQ	YES	YES
P28161	GSTM2		YES	ITRAQ	YES	YES
P78417	GSTO1		YES	ITRAQ	YES	YES
Q14520	HABP2	Liver	YES	SRM	YES	YES
P68871	HBB	0	YES	iTRAQ/SRM	YES	YES
Q04756	HGFAC	liver	YES	iTRAQ/SRM	YES	YES
Q9UGU5	HMGXB4	0	YES	iTRAQ/SRM	YES	YES
P00738	HP	liver	YES	SRM	YES	YES
Q9UM19	HPCAL4	brain	YES	SRM	YES	YES
P38646	HSPA9	0	YES	iTRAQ/SRM	YES	YES
P01563	IFNA2	brain	YES	SRM	YES	YES
P35858	IGFALS	liver	YES	SRM	YES	YES
Q9NPH3	IL1RAP	0	YES	iTRAQ/SRM	YES	YES
P46940	IQGAP1	0	YES	iTRAQ/SRM	YES	YES
P08514	ITGA2B	0	YES	iTRAQ/SRM	YES	YES
P19823	ITIH2	liver	YES	SRM	YES	YES
Q14624	ITIH4	liver	YES	SRM	YES	YES
Q9P266	KIAA1462	0	YES	iTRAQ/SRM	YES	YES
P01042	KNG1	liver	YES	SRM	YES	YES
P35527	KRT9	Skin	YES	SRM	YES	YES
P28838	LAP3	0	YES	iTRAQ/SRM	YES	YES
P04180	LCAT	liver	YES	SRM	YES	YES
P08519	LPA	liver	YES	iTRAQ/SRM	YES	YES
Q9NS15	LTBP3		YES	ITRAQ	YES	YES
O94822	LTN1		YES	ITRAQ	YES	YES
P11137	MAP2	brain	YES	SRM	YES	YES
P12829	MYL4	heart	YES	SRM	YES	YES

List of Protein Markers Identified

UniProt ID	Gene symbol	Organ origin	detected in serum	MS platform	Can distinguish Lyme patients vs controls or PTLDS vs nonPTLDS	SRM assay developed
P60660	MYL6	0	YES	iTRAQ/SRM	YES	YES
P15559	NQO1	0	YES	iTRAQ/SRM	YES	YES
Q8WWZ8	OIT3	liver	YES	iTRAQ/SRM	YES	YES
Q99784	OLFM1	brain	YES	SRM	YES	YES
Q9HBI1	PARVB	0	YES	iTRAQ/SRM	YES	YES
Q96PD5	PGLYRP2	liver	YES	SRM	YES	YES
Q6NSJ2	PHLDB3		YES	ITRAQ	YES	YES
P30613	PKLR	liver	YES	SRM	YES	YES
P00747	PLG	liver	YES	SRM	YES	YES
Q8IUK5	PLXDC1	0	YES	iTRAQ/SRM	YES	YES
Q9Y535	POLR3H	brain	YES	iTRAQ/SRM	YES	YES
P27169	PON1	liver	YES	iTRAQ/SRM	YES	YES
Q8WUF5	PPP1R13L	0	YES	iTRAQ/SRM	YES	YES
P25786	PSMA1	0	YES	iTRAQ/SRM	YES	YES
P25789	PSMA4		YES	ITRAQ	YES	YES
P28066	PSMA5		YES	ITRAQ	YES	YES
O14818	PSMA7	0	YES	iTRAQ/SRM	YES	YES
P20618	PSMB1		YES	ITRAQ	YES	YES
P28070	PSMB4		YES	ITRAQ	YES	YES
Q06323	PSME1	0	YES	iTRAQ/SRM	YES	YES
P06737	PYGL		YES	ITRAQ	YES	YES
Q15293	RCN1		YES	ITRAQ	YES	YES
Q9P2E9	RRBP1		YES	ITRAQ	YES	YES
Q59EK9	RUNDC3A		YES	ITRAQ	YES	YES
Q92736	RYR2		YES	ITRAQ	YES	YES
P06702	S100A9	0	YES	SRM	YES	YES
P0DJI8	SAA1	Liver	YES	SRM	YES	YES
Q15436	SEC23A		YES	ITRAQ	YES	YES
P14151	SELL	Bone Marrow	YES	SRM	YES	YES
Q8IW75	SERPINA12	skin	YES	SRM	YES	YES
P05543	SERPINA7	liver	YES	SRM	YES	YES
P08697	SERPINF2	liver	YES	SRM	YES	YES
O75563	SKAP2		YES	ITRAQ	YES	YES
P13866	SLC5A1	heart	YES	SRM	YES	YES
Q14683	SMC1A	0	YES	iTRAQ/SRM	YES	YES
P04179	SOD2		YES	ITRAQ	YES	YES
Q9C0H9	SRCIN1		YES	ITRAQ	YES	YES
A1L4H1	SSC5D	0	YES	iTRAQ/SRM	YES	YES

List of Protein Markers Identified

UniProt ID	Gene symbol	Organ origin	detected in serum	MS platform	Can distinguish Lyme patients vs controls or PTLDS vs nonPTLDS	SRM assay developed
Q8N2I9	STK40		YES	ITRAQ	YES	YES
O15050	TRANK1		YES	ITRAQ	YES	YES
Q12931	TRAP1		YES	ITRAQ	YES	YES
Q9H3U1	UNC45A		YES	ITRAQ	YES	YES
P04004	VTN	liver	YES	SRM	YES	YES
Q8TAF3	WDR48		YES	ITRAQ	YES	YES
P86452	ZBED6		YES	ITRAQ	YES	YES
Q99592	ZNF238		YES	ITRAQ	YES	YES
P04424	ASL		YES	ITRAQ	YES	NO
P32754	HPD		YES	ITRAQ	YES	NO
Q03112	MECOM		YES	ITRAQ	YES	NO
P13533	MYH6		YES	ITRAQ	YES	NO

TABLE 3.

Select miRNA Markers
miR-423-5p
miR-21-5p
miR-130b-5p
miR-615-3p
miR-19b-3p
miR-485-5p
miR-193a-5p

[0059] The following examples illustrate, but do not limit the invention.

EXAMPLES

Example 1: Global proteome profiling unveils protein expression differences in Lyme disease patient sera.

[0060] In furtherance of the goal of identifying protein biomarkers to diagnose acute Lyme disease and to predict the development of chronic symptoms, changes in the blood proteome were investigated in a small set of longitudinal serum samples from patients with acute Lyme disease. 16 banked serum samples were obtained. This longitudinal cohort included four Lyme disease patients who provided blood samples at the time of initial diagnosis (*Baseline)*, and at *1 year* after diagnosis. Sera from four matched individuals without Lyme disease were also included as controls. Note that all four Lyme patients in this study (Patient ID: 01-036, 01-047, 01-053 and 01-054) presented with the characteristic "bulls-eye rash" (*erythema migrans*, or *EM*). A two-pronged, mass spectrometry-based approach to protein biomarker discovery was applied: 1) global, quantitative profiling by iTRAQ offers an unbiased assessment of changes in protein levels as a result of disease; and 2) SRM provides a highly sensitive and highly specific way to measure specific peptides that correspond to proteins of interest. SRM was used to validate candidates identified by iTRAQ, as well as to measure levels of organ-specific proteins in blood.

[0061] *Serum sample preparation:* An immune-affinity depletion LC column from Agilent (Mars14 column) was used to selectively remove the top 14 abundant plasma proteins from each of the 16 individual serum samples. This procedure generally results in a 20-fold enrichment of low-abundant proteins. The flow-through fractions were digested to generate tryptic peptides. 60 µg of each peptide sample were labeled with isobaric isotopic labeling reagent for global quantitation analysis (iTRAQ; Q-Exactive Plus LC/MS, Thermo/Fisher), while the remainder of the peptide samples were spiked-in with heavy isotope-labeled synthetic peptides and analyzed by SRM in a triple quadruple mass spectrometer (Agilent 6490).

[0062] Compared to other methods, isobaric stable isotope labeling of peptides using iTRAQ or TMT tags is a highly efficient and reliable method for in-depth quantification of complex proteomes with a broad labeling capability of up to 8-10 samples. Recent technical advances in fragmentation techniques using the Q-Exactive mass spectrometer enable rapid extraction of all ions from the Higher-energy Collision Dissociation (HCD) cell and dramatically increase speed and sensitivity of the instrument in HCD mode. As a result, ions for peptide quantification and peptide identification can now be collected in a single MS/MS scan resulting in more peptide ions to be analyzed in the same machine time. To assess the changes in the blood proteome associated with the development of Lyme disease, an approach was used that combines abundant blood protein depletion, iTRAQ isobaric-labeling, extensive fractionation with peptide-level high pH C18 fractionation (pH 10, 12 fractions) and a high-sensitivity Q-Exactive Plus mass spectrometer for serum protein detection and quantification.

[0063] Results (one single mass spectrometer run per fraction) from the study identified about 1,429 proteins from two iTRAQ sets (FDR < 0.01), of which 1,302 proteins (~91%) were quantified with isobaric tags from iTRAQ (m/z 113, 114, 115, 116, 117, 118, 119 and 121) and 1,140 proteins were quantified with more than one unique peptide. Sixty-eight proteins showed altered (>1-fold change) abundances in Lyme disease at either the baseline time point or 1-year after initial diagnosis.

[0064] Principal Components Analysis (PCA) using 562 proteins that were quantifiable in all 16 samples (FIG. 1) clearly distinguish the four Lyme disease patients at the baseline time point (red dots), from both the Lyme patients after 1 year and the four control subjects (black squares). Furthermore, the Lyme patients after 1 year are indistinguishable from the

controls. These results suggest that there are indeed signatures of early infection in patient blood.

[0065] Volcano plot analysis confirmed the PCA findings (FIG. 2). A significant number of proteins were differentially expressed in the four Lyme sera at baseline compared to 1 year after initial infection (FIG. 2A). In this study, we observed 22 differentially expressed proteins (DEPs) that were up-regulated at baseline compared to 1 year after diagnosis, and 6 DEPs that were down-regulated.

Table 4 shows differentially expressed proteins (gene symbols) in Volcano analysis with levels >0.5-fold difference and p-value <0.05 between 2 conditions; BL = baseline; 1 y = 1 year after diagnosis; Cntl = Control.

TABLE 4

	Up	Down
Lyme BL *vs.* 1 y	CRP FBP1 UGP2 RCN1 LRG1 LBP DOT1L SAA2-SAA4 C9 SERPINA3 B4GALT1 DEFA1 ITIH3 CDHR2 ITGAL HDLBP DCI ICAM1 SERPINB1 KRT10 CECR1 CORO1A	HGFAC TBC1D2 APOH RBP4 CCNB3 APOA4
Cntl BL *vs.* 1 y	COL1A2 DNAJA4 RSBN1L MID2 MYL12B	MDN1 DCD ARID1B
BL Lyme *vs.* Cntl	CRP PSMA4 RCN1 C9 SAA2-SAA4 LBP ITIH3 MARCO SERPINA3 ITGAL	CDH1 NID2 MAPRE2 HRG IL1RAP MSLN NOTCH2 SERPINA4 IGJ EXT2 OGN CA2 POSTN B3GNT2 EXT1 APOA4 LUM GPNMB CLIC1 FERMT3 MRC2 CA1 HRNR MED30 HGFAC PRDX2 MYL12B AFM EXTL2 NRCAM RBP4 CNDP1 UBP4
1y Lyme *vs.* Cntl	TUBB	MDN1 KRT1 FBP1

[0066] As shown in Table 4, the proteins that were up-regulated at baseline include CRP, FBP1, UGP2, RCN1, LRG1, LBP, DOT1L, SAA2-SAA4, C9, SERPINA3, B4GALT1, DEFA1, ITIH3, CDHR2, ITGAL, HDLBP, DCI, ICAM1, SERPINB1, KRT10, CECR1, and CORO1A. The proteins that were down-regulated at baseline compared to 1 year after diagnosis include HGFAC, TBC1D2, APOH, RBP4, CCNB3, and APOA4. Furthermore, When Lyme patients were compared to controls, both at the baseline time point, 43 DEPs were observed, whereas only 4 DEPs were identified when comparing Lyme patients to controls at the 1-year time point. As shown in Table 4, the proteins that were up-regulated in Lyme patients compared to controls at baseline include CRP, PSMA4, RCN1, C9, SAA2-SAA4, LBP, ITIH3, MARCO, SERPINA3, and ITGAL. The proteins that were down-regulated in Lyme patients compared to controls at baseline include CDH1, NID2, MAPRE2, HRG, IL1RAP, MSLN, NOTCH2, SERPINA4, IGJ, EXT2, OGN, CA2, POSTN, B3GNT2, EXT1, APOA4, LUM, GPNMB, CLIC1, FERMT3, MRC2, CA1, HRNR, MED30, HGFAC,

PRDX2, MYL12B, AFM, EXTL2, NRCAM, RBP4, CNDP1, and UBP4. As also shown in Table 4, the protein that was up-regulated in Lyme patients compared to controls at the 1-year time point after diagnosis includes TUBB. The proteins that were down-regulated in Lyme patients compared to controls at the 1-year time point after diagnosis include MDN1, KRT1, and FBP1.

[0067] Gene ontology analysis indicated that sets of proteins elevated more than 1-fold in Lyme patients were highly enriched for being associated with acute phase response, proteasome function, and carbohydrate biosynthesis (Enrichment score cutoff: p-value < 0.01).

[0068] Organ-specific proteins (OSP) are proteins that are expressed predominantly in one or two major organs in the body. It is believed that their presence in the blood can reflect the health of the corresponding organ(s). Through exhaustive analysis of many different types of expression datasets, both in the public domain and generated in-house, lists of proteins that are highly enriched in more than 19 different human tissues and organs were assembled. In this iTRAQ experiment, among the 156 OSPs quantified, 17 OSPs were elevated >0.5-fold in Lyme patients at either the baseline or 1-year time points.

Table 5 shows 17 organ-specific proteins that change > 0.5-fold in Lyme disease sera, as compared to the average of 4 controls (linear ratios). Missing values for some samples indicates that the relevant peptides were not detected in the single iTRAQ run; a more comprehensive analysis, employing multiple MS runs, would be expected to generate data across all samples.

TABLE 5

Gene symbol	Description	Organ	01-036BL	01-036-1y	01-047BL	01-047-1y	01-053BL	01-053-1y	01-054BL	01-054-1y	Avg. Lyme-BL	Avg. Lyme-1y	Avg Lyme BL/1y
CAMP	Cathelicidin antimicrobial peptide	Bone marrow	1.913	3.164	1.332	1.387	1.913	1.464	1.611	1.365	1.692	1.845	0.917
CTSG	Cathepsin G	Bone marrow	1.034	0.703	1.296	0.763	2.530	1.162	2.799	1.408	1.930	1.009	1.913
MAP1A	Isoform 2 of Microtubule-associated protein 1A	Brain					1.650	1.114	1.539	1.038	1.595	1.076	1.482
MYO16	Unconventional myosin-XVI	Brain					1.695	1.265	1.557	1.011	1.626	1.138	1.429
PDE4DIP	Isoform 4 of Myomegalin	Brain					2.060	1.152	1.892	0.879	1.976	1.015	1.948
MYH6	Myosin-6	Heart	0.848	5.785	0.911	2.006					0.879	3.896	0.226
ALDOB	Fructose-bisphosphate aldolase B	Liver	1.249	1.063	1.271	0.874	1.609	1.462	5.728	1.363	2.464	1.190	2.070
ASL	Isoform 2 of Argininosuccinate lyase	Liver					1.733	1.152	4.477	0.879	3.105	1.015	3.059
BHMT	Betaine--homocysteine S-methyltransferase 1	Liver					1.218	1.056	5.945	0.985	3.582	1.020	3.511
C9	Complement component C9	Liver	1.607	1.137	2.143	1.020	2.170	1.676	2.180	1.250	2.024	1.271	1.593
CES1	Isoform 2 of Liver carboxylesterase 1	Liver	0.927	0.557	3.486	1.235	1.511	1.782	4.100	1.385	2.506	1.240	2.021
CRP	C-reactive protein	Liver	2.426	1.388	4.640	0.685	8.265	1.946	3.847	1.088	4.795	1.277	3.755
DMGDH	Dimethylglycine dehydrogenase	Liver					3.109	0.759	1.606	0.786	2.357	0.772	3.052
GOT1	Aspartate aminotransferase	Liver	0.982	2.413	2.092	2.646	1.472	1.610	3.936	1.501	2.135	2.043	1.045
GPT	Alanine aminotransferase 1	Liver					1.060	1.170	3.381	0.893	2.220	1.032	2.153
HP	Haptoglobin	Liver	0.894	0.844	3.334	0.999	2.610	1.419	10.772	1.761	5.752	1.241	4.636
HPD	Isoform 2 of 4-hydroxyphenylpyruvate dioxygenase	Liver					1.767	1.336	5.071	1.370	3.419	1.353	2.527

[0069] As shown in Table 5, the OSPs that were elevated greater than 0.5-fold in Lyme patients include the proteins with the gene symbols of CAMP, CTSG, MAP1A, MYO16, PDE4DIP, MYH6, ALDOB, ASL, BHMT, C9, CES1, CRP, DMGDH, GOT1, GPT, HP, and HPD.

[0070] As shown in Table 6, there are a number of proteins that are highly enriched in a variety of human tissues and organs of patients with Lyme disease both at the time of diagnosis and one year later.

Table 6 shows organs most likely affected by Borrelia include liver, heart, brain, kidney, skin and skeletal muscle. In Phase I **nine** liver (TOP), **two** heart (MIDDLE), and **nine** immune- and defense response-related (BOTTOM) proteins were identified that were significantly elevated in Lyme patients compared to matched controls.

TABLE 6

↑ elevated protein concentration in serum.
_ no significant concentration changes in serum

Patient group		Patient at diagnosis				Patient 1 year later				control at enrollment				Control 1 year later			
Patient name		01_036_1	01_047_1	01_053_1	01_054_1	01_036_6	01_047_6	01_053_6	01_054_6	02_021_1	02_056_1	02_096_1	02_097_1	02_021_3	02_056_3	02_096_3	02_097_3
	AGXT	4.2X ↑	–	–	52X ↑	–	–	2.4X ↑	2.1X ↑	2.6X ↑	–	–	–	–	–	–	–
	FBP1	5.7X ↑	–	2.9X ↑	43X ↑	–	–	–	–	–	–	–	–	–	–	–	–
	ALT1	5.0X ↑	2.6X ↑	3.6X ↑	37X ↑	3.0X ↑	–	–	–	–	–	–	–	–	–	–	–
liver proteins	CES1	3.4X ↑	–	2.6X ↑	35X ↑	–	2.1 ↑	–	–	2.4X ↑	–	–	–	–	–	–	–
	ALDOB	4.9X ↑	–	3.7X ↑	28X ↑	4.0X ↑	–	–	–	2.6X ↑	–	–	–	–	–	–	–
	ADH4	4.5X ↑	–	–	23X ↑	2.2X ↑	–	2.9X ↑	–	–	–	–	–	–	–	–	–
	APOC1	–	–	2.3X ↑	4.6X ↑	8.9X ↑	3.5X ↑	–	–	–	–	–	–	–	–	–	–
	LCAT	–	–	–	3.2X ↑	2.3X ↑	–	–	–	–	–	–	–	–	–	–	–
	APOC2	–	–	2.4X ↑	3.1X ↑	6.6X ↑	–	–	–	–	–	–	–	–	–	–	–

Patient group		Patient at diagnosis				Patient 1 year later				control at enrollment				Control 1 year later			
Patient name		01_036_1	01_047_1	01_053_1	01_054_1	01_036_6	01_047_6	01_053_6	01_054_6	02_021_1	02_056_1	02_096_1	02_097_1	02_021_3	02_056_3	02_096_3	02_097_3
heart proteins	ACTA1	–	–	6.5X ↑	3.7 ↑	5.5X ↑	3.4X ↑	–	–	–	–	–	–	–	–	–	–
	ADPRHL1	–	–	2.1X ↑	–	–	2.3X ↑	3.1X ↑	–	–	–	–	–	–	–	–	–

Patient group		Patient at diagnosis				Patient 1 year later				control at enrollment				Control 1 year later			
Patient name		01_036_1	01_047_1	01_053_1	01_054_1	01_036_6	01_047_6	01_053_6	01_054_6	02_021_1	02_056_1	02_096_1	02_097_1	02_021_3	02_056_3	02_096_3	02_097_3
	CRP	12X ↑	22X ↑	46X ↑	27X ↑	–	–	–	–	2.7X ↑	–	–	–	–	–	–	–
	APOA2	4.1X ↑	–	14X ↑	8.4X ↑	4.3X ↑	–	–	–	–	–	–	–	–	–	–	–
	C9	3.2X ↑	3.5X ↑	3.0X ↑	3.9X ↑	–	–	2.0X ↑	–	–	–	–	–	–	–	–	–
immune &	PON1	2.1X ↑	–	3.0X ↑	3.0X ↑	4.0X ↑	–	–	–	–	–	–	–	–	–	–	–
defense response	CFB	–	–	2.1X ↑	3.1X ↑	–	–	–	–	–	–	–	–	–	–	–	–
	APOF	–	–	–	2.5X ↑	4.0X ↑	2.1X ↑	–	–	–	–	–	–	–	–	–	–
	C6	–	–	–	2.4X ↑	–	–	–	–	–	–	–	–	–	–	–	–
	C8A	–	–	–	2.4X ↑	–	–	–	–	–	–	–	–	–	–	–	–
	MMP9	2.7X ↑	2.9X ↑	–	–	2.1X ↑	–	–	–	–	–	–	–	–	–	–	–

[0071] As disclosed in FIG. 3, MAP1A was increased more than one-fold one year after infection in two of the four Lyme patients.

[0072] Elevated levels of two *bone marrow* proteins—cathelicidin antimicrobial peptide (CAMP) and cathepsin G (CTSG)—both of which are involved in antibacterial activities, in a subset of the four Lyme patients presents the intriguing possibility of an effect of *B. burgdorferi* infection on the bone marrow, which has not been previously reported in the literature. Interestingly, two cases with chronic Lyme disease were observed in 1997 with positive *B. burgdorferi* DNA (OspA gene) in their bone marrow biopsy (Fein, L. et al., Bone Marrow as a Source for Borrelia burgdorferi DNA (1997) *J Spiro Tick Diseases.* 4(3):58-60).

Another chronic Lyme case was described in 2003 with epithelioid granulomas in a bone marrow trephine biopsy (Hans M. et al., Bone marrow manifestation of Lyme disease (Lyme Borreliosis). (2003) *British Journal of Haematology.* 120(5): 723). Three *brain* proteins (MAP1A, MYO16, and PDE4DIP) and 11 *liver* proteins (ALDOB, ASL, BHMT, C9, CES1, CRP, DMGDH, GOT1, GPT, HP, and HPD) were also higher at the baseline time point, indicating effects on these target organs in the early phase of *B. burgdorferi* infection. Interestingly, Myosin-6 (MYH6), a *heart*-enriched protein, showed no change at baseline but increased more than 2-fold after 1 year of initial infection, suggesting a potential long-term effect in the heart. This data supports the feasibility of developing a blood-based diagnostic for early detection of Lyme disease.

[0073] In another set of experiments, 130 liver-enriched proteins (represented by 204 peptides) with heavy isotope labeled counterparts were mainly focused on. 78 of these proteins (119 peptides) were detected in serum by Selected Reaction Monitoring (SRM). A few heart-enriched proteins were also tested.

[0074] Distinct patterns of serum protein level changes were observed in Lyme patient samples, either compared with controls, or compared in Lyme patients at different time points (baseline vs. one year later). Examples of representative patterns are illustrated in Figure 4.

[0075] Some proteins (e.g., the proteins with gene symbols C5, C6, C8A, C9, CFB, and CRP) were found to have levels highly or moderately elevated in all four patients at the time of diagnosis compared to control samples at enrollment and one year later and Lyme patient samples collected one year later. Examples are shown in FIG. 4A with C9 and FIG. 4B with C5.

[0076] Some proteins (e.g., the proteins with gene symbols APOB and CFH) exhibited levels significantly higher in all four Lyme patients one year after diagnosis as compared to controls, but not as compared to Lyme patients at the baseline time point An example is shown in FIG. 4C with APOB.

[0077] Some proteins (e.g., the protein with gene symbol HPX) exhibited levels significantly higher in all four Lyme patients at both baseline and one year time points as compared to controls at enrollment and at the one year time point. An example is shown in FIG. 4D with HPX.

[0078] Some proteins (e.g., the protein with gene symbol NDUF4) exhibited levels significantly higher at the baseline time point but lower at the one year later time point in all four Lyme patients as compared to controls. An example is shown in FIG. 4E with NDUF4.

[0079] These data suggest that one or more of these organ-specific proteins in Lyme patients may help to predict which patients will develop chronic symptoms and possibly even which kind of symptom(s). They may also provide insights into how patients who respond to antibiotic therapy rapidly differ from those who progress to chronic conditions.

[0080] In an extensive follow-up study, proteins and peptides were analyzed to determine their relevance to Lyme disease detection/diagnosis and their association with the development of chronic symptoms. Table 1 provides a list of protein markers (identified by Uniprot Protein ID and Gene symbol), including 302 corresponding peptide sequences, which were detected by mass spectrometry (e.g., SRM and/or iTRAQ) in patient serum. These protein markers demonstrated some measurable difference in abundance which can distinguish all Lyme disease patients from healthy controls and/or PTLDS patients from non-PTLDS patients. As shown by Table 1, some of the detected protein markers are organ-specific proteins. Also shown by Table 1 is that some proteins were detected through more than one peptide sequence. Table 2 lists the 158 unique protein markers (identified by Uniprot Protein ID and Gene symbol) that represent a condensed list of the protein markers of Table 1. The 16 highlighted proteins of Table 2 (i.e., AGXT, ALDOB, APCS, APOC4, C5, C6, C9, CES1, CFH, CRP, FBP1, HPCAL4, LCAT, OLFM1, SERPINA7, and SLC5A1) represent select proteins found to demonstrate the strongest statistical association with early Lyme disease diagnosis and/or the ability the predict those Lyme disease patients who will develop PTLDS.

Example 2: MiRNAs can be used to differentiate PTLDS patients from non-PTLDS patients.

[0081] Longitudinal serum microRNA (miRNA) profiles from 20 post-treatment Lyme disease syndrome (PTLDS) patients were analyzed in four different timepoints. This included 20 non-PTLDS patients (10 with and 10 without symptom at first visit, which served as the baseline) in four different timepoints, and 20 controls in 2 different timepoints by small RNA sequencing. The small RNA sequencing reads were mapped against a human miRNA database under perfect match (no mismatch allowed) using a tool developed at Institute for

Systems Biology - sRNAnalyzer found on the internet at srnanalyzer.systemsbiology.net. The mapped reads were normalized with read per million (RPM) of mapped reads and Log2-transformed before analysis. After removing low abundant miRNAs (at least one sample with read count > global mean value), 391 miRNAs from 2996 detected miRNAs were kept for further analyses.

[0082] Differential analysis was performed to identify affected miRNA during the development of Lyme disease with criteria: fold change (FC) ≥2, *p*-Value ≥0.05 and average concentration≥ 5 (normalized log2 transformed value) on various patient groups across all available time points. The affected miRNAs are listed in Tables 7-9.

Table 7 lists affected miRNA within each patient group (compared to baseline concentration in each group). ***. D:** represents decreased concentration, **U:** represents increased concentration in sample.

TABLE 7

Lyme patient, Symptom at baseline					Lyme patient, No Symptom at Baseline								
Baseline	**Visit 2**	**Visit 3**	**Visit 4**	*	**Baseline**	**Visit 2**	*	**Visit 3**	*	**Visit 4**	*
			hsa-let-7a-5p	D		hsa-let-7a-5p	U	hsa-let-7g-5p	U	hsa-let-7a-5p	U
			hsa-let-7f-5p	D		hsa-miR-144-3p	U	hsa-miR-1307-3p	U	hsa-let-7b-5p	U
			hsa-let-7g-5p	D		hsa-miR-185-5p	U	hsa-miR-134-5p	U	hsa-let-7f-5p	U
			hsa-let-7i-5p	D				hsa-miR-193a-5p	U	hsa-let-7g-5p	U
			hsa-miR-122-5p	D						hsa-miR-122-5p	U
			hsa-miR-126-3p	D						hsa-miR-134-5p	U
			hsa-miR-16-5p	D						hsa-miR-146a-5p	U
			hsa-miR-26a-5p	D						hsa-miR-16-5p	U
										hsa-miR-193a-5p	U
										hsa-miR-378a-3p	U

Post-Treatment Lyme Disease Syndrome (PLDS)					
Baseline	**Visit 2**	**Visit 3**	*****	**Visit 4**	*****
		hsa-miR-21-5p	U	hsa-let-7a-5p	U
		hsa-miR-486-5p	U	hsa-let-7i-5p	U
		hsa-miR-92a-3p	U	hsa-miR-101-3p	U
				hsa-miR-103a-3p	U
				hsa-miR-144-3p	U
				hsa-miR-146a-5p	U
				hsa-miR-191-5p	U
				hsa-miR-193a-5p	U
				hsa-miR-19b-3p	U
				hsa-miR-21-5p	U
				hsa-miR-221-3p	U
				hsa-miR-24-3p	U
				hsa-miR-26a-5p	U
				hsa-miR-27a-3p	U
				hsa-miR-30d-5p	U
				hsa-miR-30e-5p	U
				hsa-miR-363-3p	U
				hsa-miR-425-5p	U
				hsa-miR-451a-5p	U
				hsa-miR-486-5p	U
				hsa-miR-92a-3p	U

Table 8 lists affected miRNA compared between patient groups. ***. D:** represents decreased concentration, **U:** represents increased concentration in sample.

TABLE 8

Lyme patient, Symptom at baseline		Lyme patient, No Symptom at Baseline		Post-Treatment Lyme Disease Syndrome (PLDS)		No Symptom at Baseline/Symptom at baseline					
Compare to control (no Lyme)						Compare between Lyme patient					
All time points	*****	**All time points**	*****	**All time points**	*****	Baseline	*	visit 2	visit 3	*	visit 4	*
hsa-let-7i-5p	D	hsa-let-7b-5p	D	hsa-let-7g-5p	D	hsa-let-7a-5p	D		hsa-miR-99a-5p	U	hsa-let-7f-5p	U
hsa-miR-101-3p	D	hsa-let-7g-5p	D	hsa-let-7i-5p	D	hsa-let-7f-5p	D				hsa-let-7g-5p	U
hsa-miR-122-5p	D	hsa-let-7i-5p	D	hsa-miR-101-3p	D	hsa-let-7g-5p	D				hsa-miR-126-3p	U
hsa-miR-1307-3p	D	hsa-miR-101-3p	D	hsa-miR-103a-3p	D	hsa-let-7i-5p	D				hsa-miR-148a-3p	U
hsa-miR-130b-5p	U	hsa-miR-103b-5p	U	hsa-miR-1180-3p	D	hsa-miR-103a-3p	D				hsa-miR-192-5p	U
hsa-miR-140-3p	D	hsa-miR-10a-5p	U	hsa-miR-122-5p	D	hsa-miR-122-5p	D				hsa-miR-363-3p	U
hsa-miR-146a-5p	D	hsa-miR-122-5p	D	hsa-miR-126-3p	D	hsa-miR-144-3p	D					
hsa-miR-183-5p	U	hsa-miR-1307-3p	D	hsa-miR-128-3p	D	hsa-miR-16-5p	D					
hsa-miR-184-3p	U	hsa-miR-130b-5p	U	hsa-miR-1307-3p	D							
hsa-miR-185-5p	D	hsa-miR-134-5p	D	hsa-miR-134-5p	D							
hsa-miR-186-5p	D	hsa-miR-140-3p	D	hsa-miR-140-3p	D							
hsa-miR-191-5p	D	hsa-miR-144-3p	D	hsa-miR-144-3p	D							
hsa-miR-193a-5p	D	hsa-miR-146a-5p	D	hsa-miR-146a-5p	D							
hsa-miR-19b-3p	D	hsa-miR-148a-3p	D	hsa-miR-148a-3p	D							
hsa-miR-21-5p	D	hsa-miR-148a-5p	U	hsa-miR-151a-3p	D							
hsa-miR-221-3p	D	hsa-miR-151a-3p	D	hsa-miR-16-5p	D							
hsa-miR-22-3p	D	hsa-miR-16-5p	D	hsa-miR-181a-5p	D							
hsa-miR-24-3p	D	hsa-miR-183-5p	U	hsa-miR-185-5p	D							
hsa-miR-27a-3p	D	hsa-miR-184-3p	U	hsa-miR-186-5p	D							
hsa-miR-30d-5p	D	hsa-miR-185-5p	D	hsa-miR-191-5p	D							
hsa-miR-30e-5p	D	hsa-miR-186-5p	D	hsa-miR-192-5p	D							
hsa-miR-320a-3p	D	hsa-miR-191-5p	D	hsa-miR-193a-5p	D							
hsa-miR-363-3p	D	hsa-miR-193a-5p	D	hsa-miR-19b-3p	D							
hsa-miR-370-3p	U	hsa-miR-19b-3p	D	hsa-miR-21-5p	D							
hsa-miR-378a-3p	D	hsa-miR-21-5p	D	hsa-miR-221-3p	D							
hsa-miR-378c-5p	U	hsa-miR-221-3p	D	hsa-miR-22-3p	D							
hsa-miR-423-3p	D	hsa-miR-22-3p	D	hsa-miR-24-3p	D							
hsa-miR-423-5p	D	hsa-miR-24-3p	D	hsa-miR-26a-5p	D							
hsa-miR-425-5p	D	hsa-miR-26a-5p	D	hsa-miR-27a-3p	D							
hsa-miR-451a-5p	D	hsa-miR-27a-3p	D	hsa-miR-30d-5p	D							
hsa-miR-485-5p	U	hsa-miR-30d-5p	D	hsa-miR-30e-5p	D							
hsa-miR-486-5p	D	hsa-miR-30e-5p	D	hsa-miR-320a-3p	D							
hsa-miR-615-3p	U	hsa-miR-320a-3p	D	hsa-miR-3615-3p	D							
hsa-miR-6852-5p	U	hsa-miR-363-3p	D	hsa-miR-363-3p	D							
hsa-miR-7974-3p	U	hsa-miR-370-3p	U	hsa-miR-378a-3p	D							
hsa-miR-92a-3p	D	hsa-miR-378a-3p	D	hsa-miR-423-3p	D							
hsa-miR-99a-5p	D	hsa-miR-423-3p	D	hsa-miR-423-5p	D							
		hsa-miR-423-5p	D	hsa-miR-425-5p	D							
		hsa-miR-425-5p	D	hsa-miR-451a-5p	D							
		hsa-miR-451a-5p	D	hsa-miR-483-5p	D							
		hsa-miR-485-5p	D	hsa-miR-486-5p	D							
		hsa-miR-486-5p	D	hsa-miR-532-5p	D							
		hsa-miR-615-3p	U	hsa-miR-589-5p	D							
		hsa-miR-6852-5p	U	hsa-miR-629-5p	D							
		hsa-miR-7974-3p	U	hsa-miR-744-5p	D							
		hsa-miR-92a-3p	D	hsa-miR-92a-3p	D							
		hsa-miR-99a-5p	D	hsa-miR-99a-5p	D							

Table 9 lists affected miRNA compared between patient groups. *. **D:** represents decreased concentration, **U:** represents increased concentration in sample.

TABLE 9

Post-Treatment Lyme Disease Syndrome (PLDS)/Lyme patient, Symptom at Baseline								Post-Treatment Lyme Disease Syndrome (PLDS)/Lyme patient, No Symptom at Baseline							
Compare PLDS to Lyme patient															
Baseline	*	visit 2	*	visit 3	*	visit 4	*	Baseline	visit 2	*	visit 3	*	visit 4	*	
hsa-let-7a-5p	D	hsa-miR-130b-5p	D	hsa-miR-130b-5p	D	hsa-let-7a-5p	U		hsa-miR-1180-3p	D	hsa-miR-101-3p	D	hsa-miR-130b-5p	D	
hsa-let-7f-5p	D	hsa-miR-140-3p	D	hsa-miR-183-5p	D	hsa-let-7b-5p	U		hsa-miR-130b-5p	D	hsa-miR-103b-5p	D	hsa-miR-183-5p	D	
hsa-let-7g-5p	D	hsa-miR-183-5p	D	hsa-miR-184-3p	D	hsa-let-7i-5p	U		hsa-miR-144-3p	D	hsa-miR-1180-3p	D	hsa-miR-485-5p	D	
hsa-let-7i-5p	D	hsa-miR-370-3p	D	hsa-miR-370-3p	D	hsa-miR-126-3p	U		hsa-miR-3615-3p	D	hsa-miR-128-3p	D	hsa-miR-615-3p	D	
hsa-miR-101-3p	D	hsa-miR-485-5p	D	hsa-miR-485-5p	D	hsa-miR-130b-5p	D		hsa-miR-485-5p	D	hsa-miR-130b-5p	D			
hsa-miR-103a-3p	D	hsa-miR-615-3p	D	hsa-miR-6852-5p	D	hsa-miR-191-5p	U		hsa-miR-615-3p	D	hsa-miR-143-3p	D			
hsa-miR-122-5p	D	hsa-miR-6852-5p	D	hsa-miR-99b-5p	D	hsa-miR-21-5p	U				hsa-miR-148a-3p	D			
hsa-miR-126-3p	D	hsa-miR-99b-5p	D			hsa-miR-26a-5p	U				hsa-miR-151a-3p	D			
hsa-miR-128-3p	D					hsa-miR-30d-5p	U				hsa-miR-183-5p	D			
hsa-miR-1307-3p	D					hsa-miR-485-5p	D				hsa-miR-192-5p	D			
hsa-miR-130b-5p	D					hsa-miR-92a-3p	U				hsa-miR-370-3p	D			
hsa-miR-143-3p	D										hsa-miR-485-5p	D			
hsa-miR-144-3p	D										hsa-miR-576-3p	D			
hsa-miR-16-5p	D										hsa-miR-589-5p	D			
hsa-miR-370-3p	D										hsa-miR-615-3p	D			
hsa-miR-451a-5p	D										hsa-miR-6852-5p	D			
hsa-miR-485-5p	D										hsa-miR-99b-5p	D			
hsa-miR-490-3p	D														
hsa-miR-615-3p	D														
hsa-miR-744-5p	D														
hsa-miR-7974-3p	D														
hsa-miR-99b-5p	D														

[0083] The results demonstrated that Hsa-miR-130b-5p concentration was found to be lower in patients with PTLDS compared to those without PTLDS (FIG. 5). In addition, Hsa-let-7a-5p concentration was found to be higher in Lyme patients with clinical symptom at baseline (first visit) compared to patients with no symptom at baseline (FIG. 6).

[0084] These results demonstrate that certain miRNAs can be used to diagnose Lyme disease subtype. For example, by using four miRNAs (miR-423-5p, miR-21-5p, miR-130b-5p, and miR-615-3p) (*see* Tables 8 and 9), an average of 89% diagnostic accuracy was

achieved (20,000 times 5-fold cross-validation) in differentiating PTLDS patients from non-PTLDS patients including all visit time points. In another example, by using the four-miRNA panel of miR-130b-5p, miR-485-5p, miR-615-3p, and miR-423-5p, an average of 88.0% diagnostic accuracy (2,000 times 5-fold cross-validation) in differentiating PTLDS patients (20 individuals) from non-PTLDS patients (20 individuals), including all visit time points, can be achieved (*see* FIG. 8). Certain miRNAs can also be used for early diagnosis by differentiating Lyme disease patients from healthy controls. In one example, by using the four miRNAs of miR-130b-5p, miR-19b-3p, miR-485-5p, and miR-193a-5p, an average of 91.8% diagnostic accuracy (2,000 times 5-fold cross-validation) in differentiating Lyme disease patients (40 individuals) from controls (20 individuals) at baseline can be achieved (*see* FIG. 7).

Example 3: Protein markers can be used as biomarkers for early diagnosis of Lyme Disease and for distinguishing subjects who are likely to develop PTLDS from those who are not likely to develop PTLDS.

[0085] Multivariate analysis and *t* test analysis was performed to identify protein markers that may be used for diagnostic purposes and for predicting the likelihood that a subject would later develop PTLDS.

[0086] Using *t* test analysis, 10 proteins were identified that have significantly perturbed levels in patient serum after infection as compared to control subjects ($p < 0.005$). These 10 proteins were the proteins with the gene symbols of AFM, ALDOB, APOA4, C9, CRP, CST6, FBP1, ITIH2, PGLYRP2, and S100A9. Of these 10 proteins, 7 of them presented altered serum levels predominantly in patients who later developed PTLDS. These 7 proteins were the proteins with the gene symbols of AFM, ALDOB, APOA4, CST6, FBP1, ITIH2, and PGLYRP2.

[0087] Multivariate analysis also revealed 10 peptides representing 10 proteins that were highly cooperative in classifying Lyme disease patients. These 10 proteins were the proteins with the gene symbols of ALDOB, APOB, C9, CFHR1, CRP, CST6, F9, GC, ITIH4, and PF4.

[0088] By using both *t* test and multivariate analysis, 4 proteins were identified, which includes the proteins with the gene symbols of ALDOB, C9, CRP, and CST6.

[0089] It was also determined that a panel of 4 proteins, consisting of the proteins with the gene symbols of AFM, ALDOB, CST6, and PGLYRP2, may provide high predictive value to distinguish subjects who later develop PTLDS from those who would return to health after treatment.

[0090] The ratio of protein pairs was also determined and analyzed for predictive value. The protein pair ratios of C9/CST6, FBP1/CST6, and ALDOB/CST6 were identified as among the best performing ratios for distinguishing Lyme disease patients in the early phase of the disease after tick bite, and also for predicting the development of PTLDS.

[0091] In summary, 16 proteins were identified in this set of analyses that may serve as potential biomarkers for early diagnosis of Lyme disease. These 16 proteins are the proteins with the gene symbols of AFM, ALDOB, APOA4, APOB, C9, CFHR1, CRP, CST6, F9, FBP1, GC, ITIH2, ITIH4, PF4, PGLYRP2, and S100A9, which are mainly acute phase and innate immune system response proteins and proteins highly enriched in *B. burgdorferi* affected organs. A panel of 4 of these proteins (AFM, ALDOB, CST6, and PGLYRP2) may possess high predictive value to distinguish individuals who later develop PTLDS from those who return to health after treatment.

CLAIMS

1. A single panel for determining in a test subject,

(a) the presence or absence of Lyme disease;

(b) the probability that the subject will develop chronic Lyme disease symptoms;

(c) the probability that the subject will respond to a Lyme disease treatment; or

(d) the probability that the subject has PTLDS.

wherein the single panel comprises, in an organized array on a single solid support, one or more detection reagents selected from the group consisting of antibodies, aptamers, oligonucleotide probes and combinations thereof that detect one or more miRNA markers of Lyme disease and/or one or more protein markers of Lyme disease, and wherein the detection of one or more miRNA markers and/or one or more protein markers correlates to (a), (b), (c) or (d) when detected at a level different from a control level;

wherein the one or more miRNA markers comprises one or more miRNA markers selected from the group consisting of hsa-miR-423, hsa-miR-21, hsa-miR-130b, hsa-miR-615, hsa-miR-19b, hsa-miR-485, and hsa-miR-193a, and

wherein the one or more protein markers comprises one or more protein markers selected from the group consisting of proteins or peptides derived from genes with the gene symbols of ACO1, ACY1, AFM, AGXT, ALDH1A1, ALDOB, AMBP, ANKRD65, APCS, APOA4, APOB, APOC2, APOC4, ApoE, APOF, APOM, ASH1L, ASTN1, BHMT, C1QB, C1QC, C1QL1, C1QL4, C1R, C1S, C2, C3, C4A, C4B, C4BPA, C4BPB, C5, C6, C7, C8A, C8B, C8G, C9, CA1, CD59, CD5L, CD93, CES1, CFB, CFD, CFH, CFHR1, CFHR5, CFI, CLSTN3, CNDP1, CNTFR, CPB2, CPN2, CRP, CSPG5, CST6, CTSB, CTSS, CTTNBP2, DDT, DEFA1, DEFA1B, DLG5, DMGDH, EEF1G, EPHA4, F10, F12, F9, FBP1, FOXN2, FSCN1, FTL, GABRA5, GC, GOLGB1, GOT1, GP6, GPR180, GPT, GSG2, GSTM2, GSTO1, HABP2, HBB, HGFAC, HMGXB4, HP, HPCAL4, HSPA9, IFNA2, IGFALS, IL1RAP, IQGAP1, ITGA2B, ITIH2, ITIH4, KIAA1462, KNG1, KRT9, LAP3, LCAT, LPA, LTBP3, LTN1, MAP2, MYL4, MYL6, NQO1, OIT3, OLFM1, PARVB, PF4, PGLYRP2, PHLDB3, PKLR, PLG, PLXDC1, POLR3H, PON1, PPP1R13L, PSMA1, PSMA4, PSMA5, PSMA7, PSMB1, PSMB4, PSME1, PYGL, RCN1, RRBP1, RUNDC3A, RYR2, S100A9, SAA1, SEC23A, SELL, SERPINA12, SERPINA7, SERPINF2, SKAP2, SLC5A1, SMC1A, SOD2, SRCIN1, SSC5D, STK40, TRANK1, TRAP1, UNC45A, VTN, WDR48, ZBED6, ZNF238, ASL, HPD, MECOM, and MYH6.

2. The single panel of claim 1, further comprising one or more detection reagents that detect the one or more protein markers and one or more miRNA markers.

3. The single panel of claim 1, wherein the one or more detection reagents detect:

(a) one or more peptides of the one or more protein markers;

(b) one or more mRNA that encode the one or more protein markers; or

(c) one or more peptides of the one or more protein markers and detect one or more mRNA that encode the one or more protein markers.

4. A method to diagnose a subject for:

(a) the presence or absence of Lyme disease;

(b) the probability that the subject will develop chronic Lyme disease symptoms;

(c) the probability that the subject will respond to a Lyme disease treatment; or

(d) the probability that the subject has PTLDS.

which method comprises:

(i) contacting a biological sample obtained from the test subject with the single panel of any one of claims 1-3; and

(ii) assessing the level of interaction between the one or more detection reagents on said single panel, and said biological sample, wherein a difference in the level of said interaction in said test subject as compared to one or more corresponding biological sample(s) from one or more subsets of Lyme disease indicates that the test subject is diagnosed according to (a), (b), (c) or (d).

5. The method of claim 4, wherein the biological sample is obtained from the test subject prior to diagnosis of Lyme disease, at or at the time of diagnosis of Lyme disease, or at a time point following diagnosis of Lyme disease.

6. A method to diagnose a subject for:

(a) the presence or absence of Lyme disease;

(b) the probability that the subject will develop chronic Lyme disease symptoms; or

(c) the probability that the subject will respond to a Lyme disease treatment; or

(d) the probability that the subject has PTLDS.

which method comprises:

(i) assessing the level of one or more miRNA markers of Lyme disease and/or protein markers of Lyme disease in a biological sample obtained from a test subject, and comparing said level to that biological sample(s) in control subject(s) wherein a difference in the level of said interaction in said test subject as compared to one or more corresponding biological sample(s) from one or more control subjects indicates that the test subject is diagnosed according to (a), (b), (c) or (d).

7. The method of claim 6, wherein the one or more miRNA markers of Lyme disease comprises one or more miRNA markers from the group consisting of hsa-miR-423, hsa-miR-21, hsa-miR-130b, hsa-miR-615, hsa-miR-19b, hsa-miR-485, and hsa-miR-193a.

8. The method of claim 6, wherein the one or more protein markers of Lyme disease is selected from the group consisting of proteins with the gene symbols of ACO1, ACY1, AFM, AGXT, ALDH1A1, ALDOB, AMBP, ANKRD65, APCS, APOA4, APOB, APOC2, APOC4, ApoE, APOF, APOM, ASH1L, ASTN1, BHMT, C1QB, C1QC, C1QL1, C1QL4, C1R, C1S, C2, C3, C4A, C4B, C4BPA, C4BPB, C5, C6, C7, C8A, C8B, C8G, C9, CA1, CD59, CD5L, CD93, CES1, CFB, CFD, CFH, CFHR1, CFHR5, CFI, CLSTN3, CNDP1, CNTFR, CPB2, CPN2, CRP, CSPG5, CST6, CTSB, CTSS, CTTNBP2, DDT, DEFA1, DEFA1B, DLG5, DMGDH, EEF1G, EPHA4, F10, F12, F9, FBP1, FOXN2, FSCN1, FTL, GABRA5, GC, GOLGB1, GOT1, GP6, GPR180, GPT, GSG2, GSTM2, GSTO1, HABP2, HBB, HGFAC, HMGXB4, HP, HPCAL4, HSPA9, IFNA2, IGFALS, IL1RAP, IQGAP1, ITGA2B, ITIH2, ITIH4, KIAA1462, KNG1, KRT9, LAP3, LCAT, LPA, LTBP3, LTN1, MAP2, MYL4, MYL6, NQO1, OIT3, OLFM1, PARVB, PF4, PGLYRP2, PHLDB3, PKLR, PLG, PLXDC1, POLR3H, PON1, PPP1R13L, PSMA1, PSMA4, PSMA5, PSMA7, PSMB1, PSMB4, PSME1, PYGL, RCN1, RRBP1, RUNDC3A, RYR2, S100A9, SAA1, SEC23A, SELL, SERPINA12, SERPINA7, SERPINF2, SKAP2, SLC5A1, SMC1A, SOD2, SRCIN1, SSC5D, STK40, TRANK1, TRAP1, UNC45A, VTN, WDR48, ZBED6, ZNF238, ASL, HPD, MECOM, and MYH6.

9. The method of claim 6, wherein the one or more markers comprise:

(a) one or more peptides of the one or more protein markers; and/or

(b) one or more mRNA that encode the one or more protein markers; and/or

(c) one or more peptides of the one or more protein markers and one or more mRNA that encode the one or more protein markers.

10. The method of any one of claims 6-9, wherein the biological sample is obtained from the test subject prior to diagnosis of Lyme disease, at or at the time of diagnosis of Lyme disease, or a time point following diagnosis of Lyme disease.

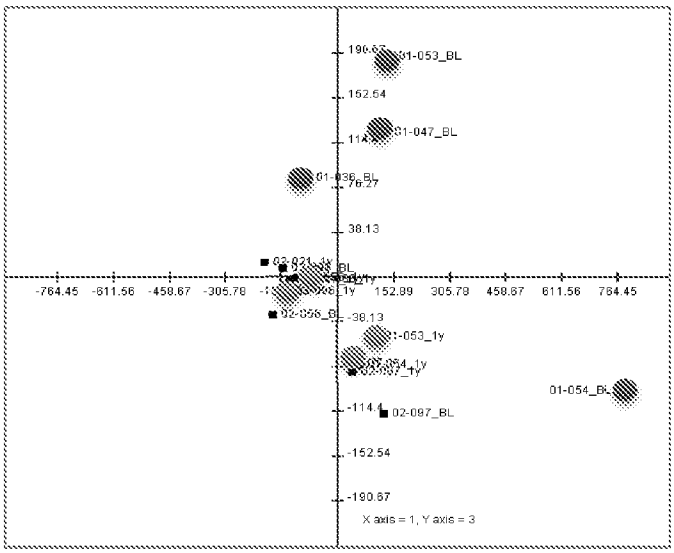


FIGURE 1

Figure 1. PCA analysis of 562 proteins quantified in all 16 samples showing that all 4 Lyme patients were clearly distinguished from the controls at time of diagnosis (i.e., baseline). RED big dots: 4 Lyme disease patients at baseline, BLUE big dots: 4 Lyme disease patients at 1 year post initial diagnosis, **Black** small squares: 4 controls at baseline and 1 year later.

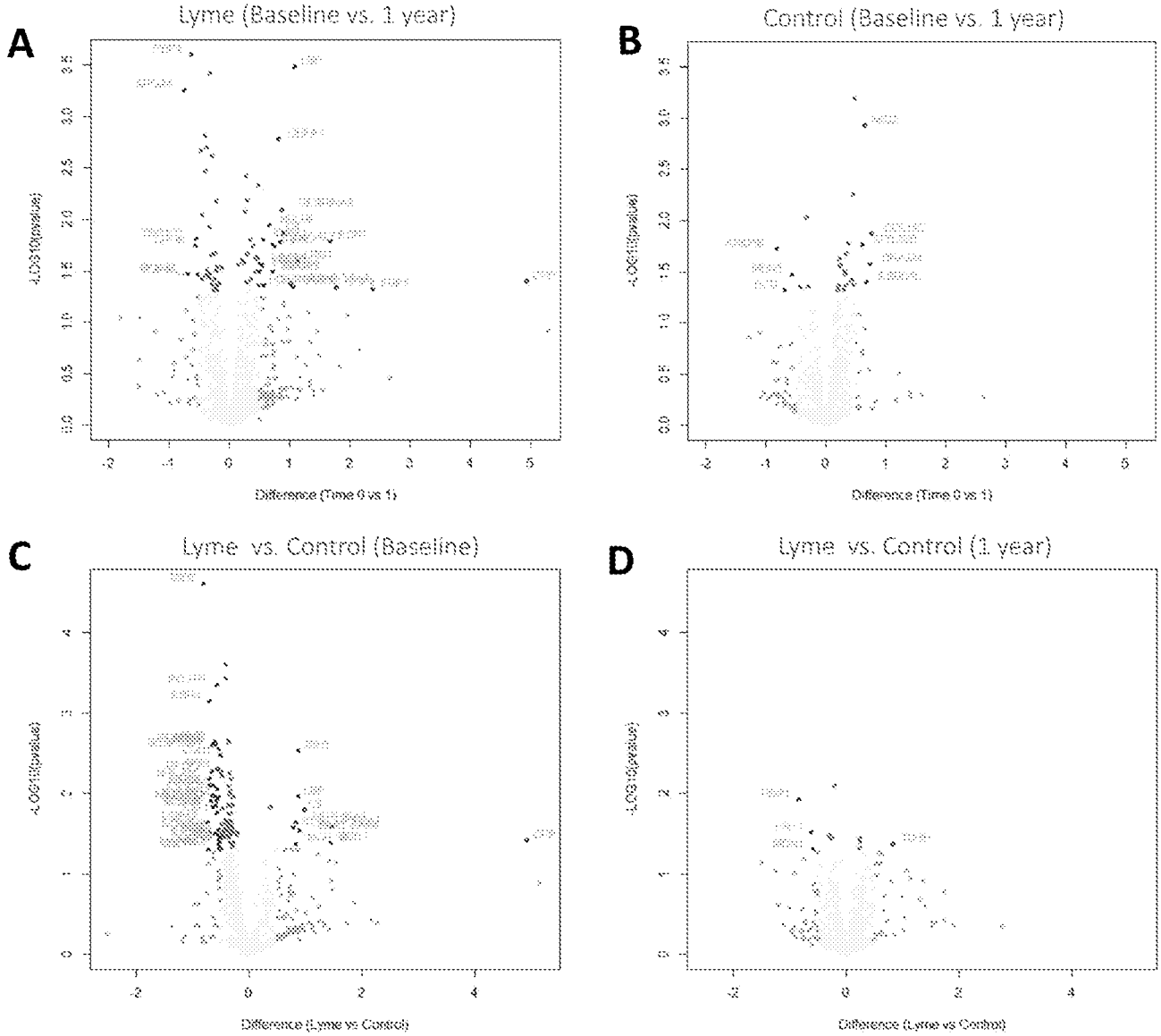


FIGURE 2

Figure 2. Volcano plotting of the 562 proteins that were quantifiable in all 16 samples shows differentially expressed proteins (DEPs) in Lyme sera compared to controls at baseline and 1-year after diagnosis (Blue). **A**: Lyme patients (Baseline vs. 1-year later), **B**: Controls (Baseline vs. 1-year later), **C**: Lyme patients vs. Controls at Baseline, **D**: Lyme patients vs. Controls at 1-year after initial infection. Dot colors: Blue and red colors denote proteins with p value <0.05 between 2 conditions (Y axis), Orange and Blue colors denote proteins with levels >0.5-fold difference between 2 conditions (X axis).

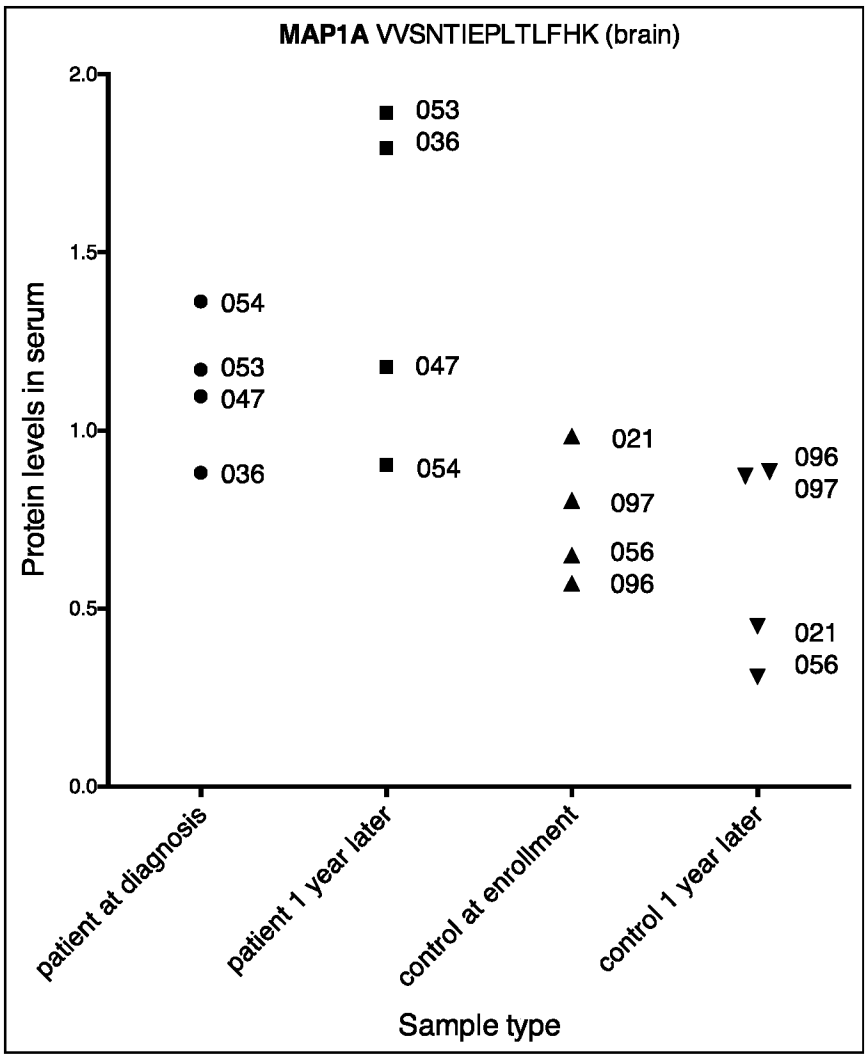


FIGURE 3

Figure 3. OSPs from several other organs, e.g., brain, kidney, skin and skeletal muscle were investigated. Five brain OSPs (NTM, IFNA2, ASTN1, CLSTN3 and MAP1A) showed altered serum levels in one or two of the 4 patients at different time points. The figure shows MAP1A as an example of a brain protein that increased at least one-fold 1 year after infection in two of the 4 patients (Patients #053 and #036) as compared to controls. Also observed were one skin OSP (SerpinA12) with higher serum concentration in patient 054 at diagnosis, and one skeletal muscle OSP (IGFN1) that increased in patient #036 at both diagnosis and one year later (not shown).

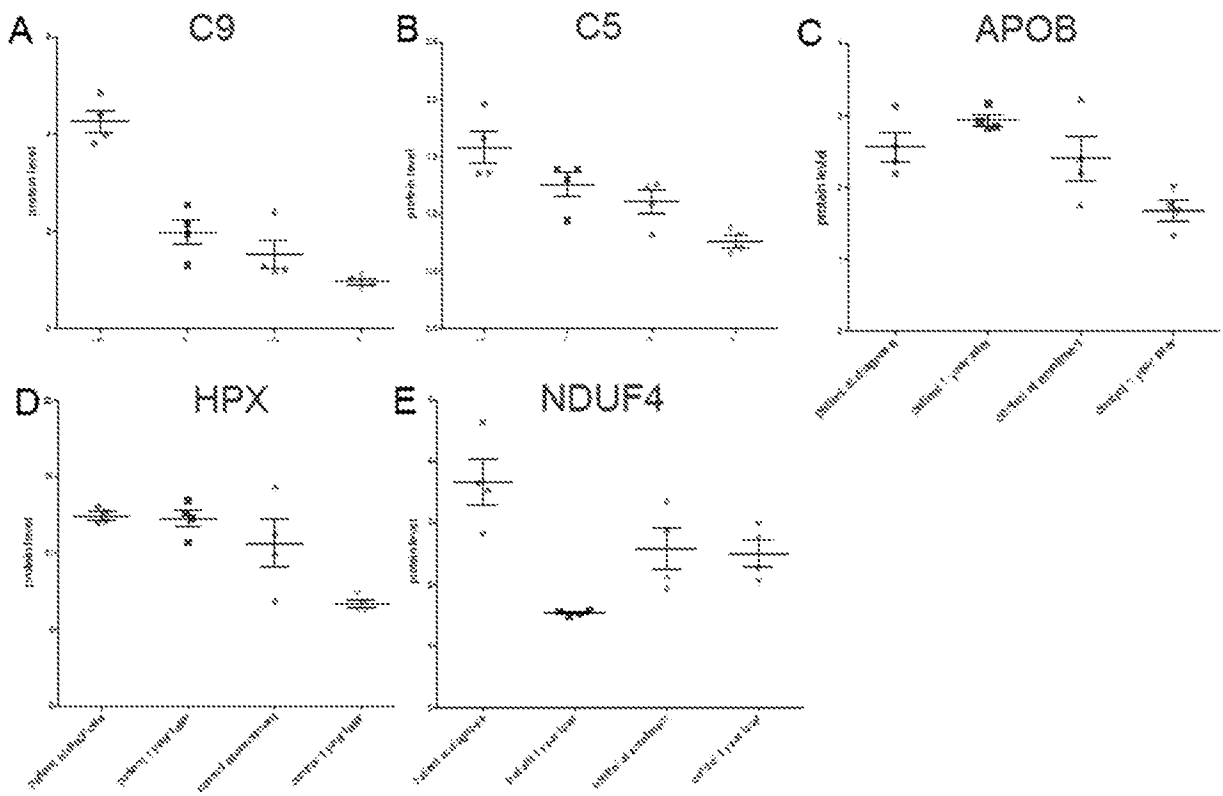


FIGURE 4

Figure 4. Relative abundances of representative organ-specific proteins measured by SRM in Lyme and control sera from baseline and 1-year time points. All proteins shown here are liver-enriched.

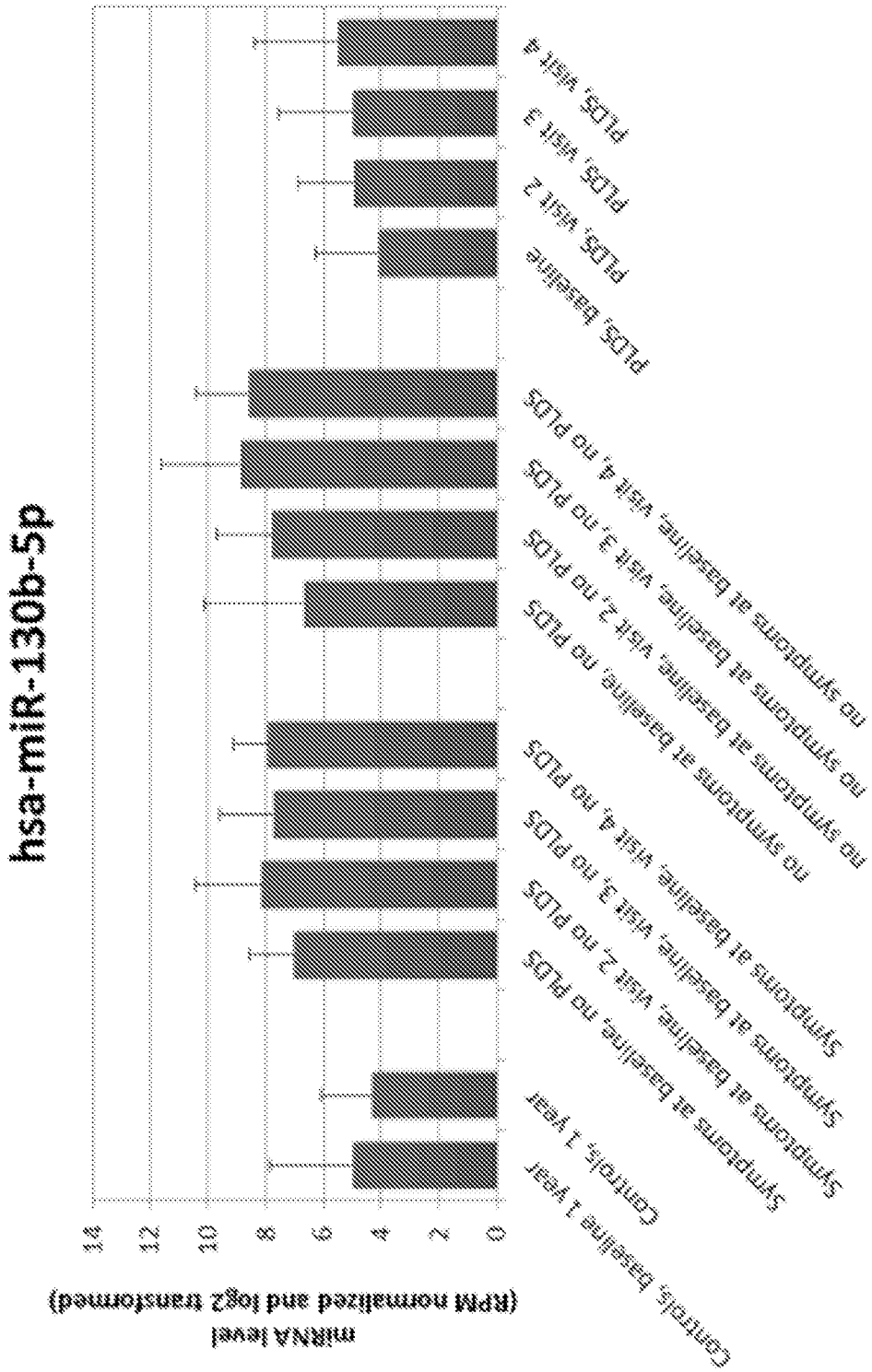


FIGURE 5

Figure 5. Hsa-miR-130b-5p concentration is lower in Lyme patients with PTLDS compared to patients without PTLDS.

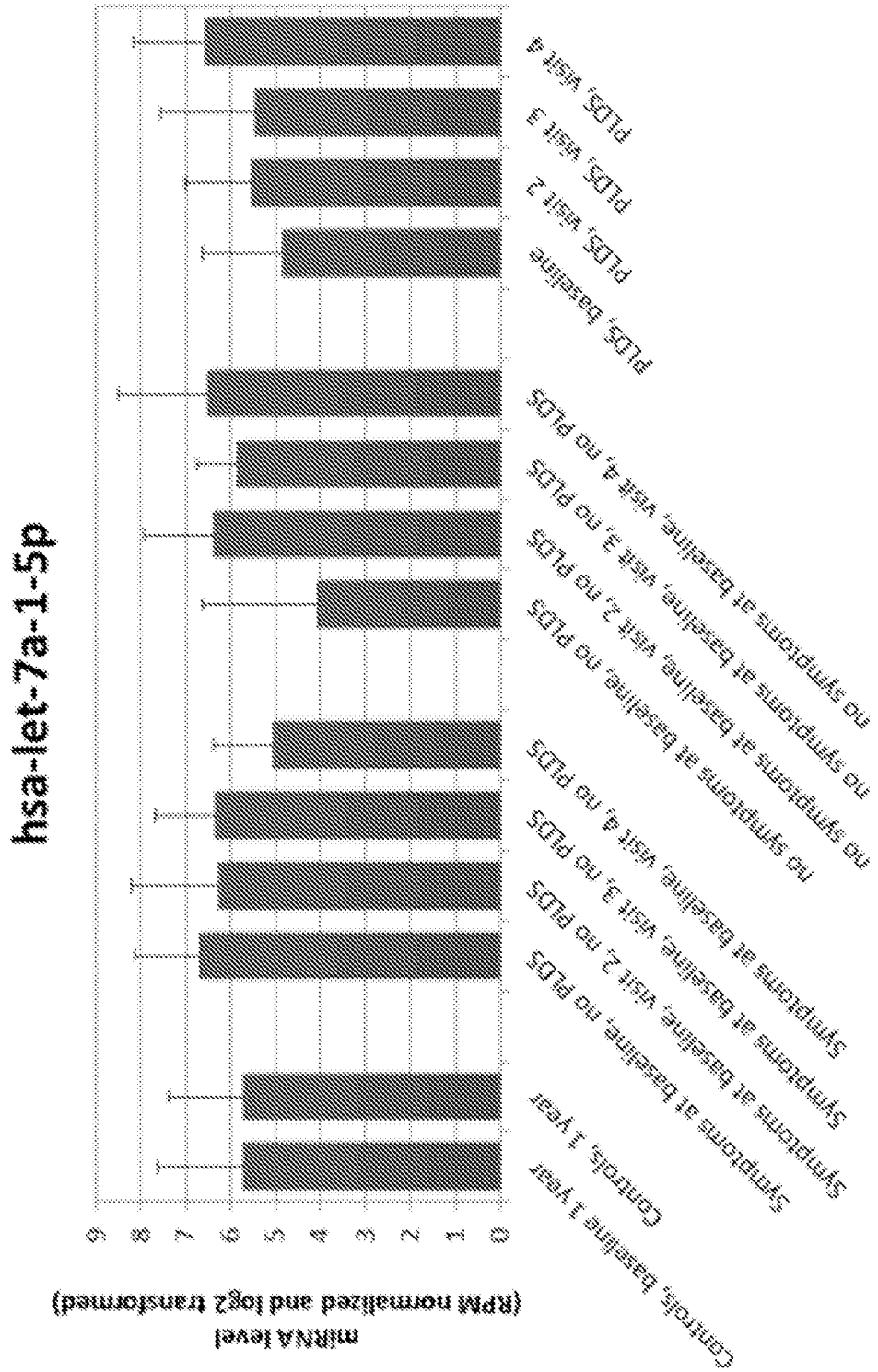


FIGURE 6

Figure 6. Hsa-let-7a-5p concentration is higher in Lyme patients with clinical symptom at baseline (i.e., first visit) compared to Lyme patients with no symptom at baseline.

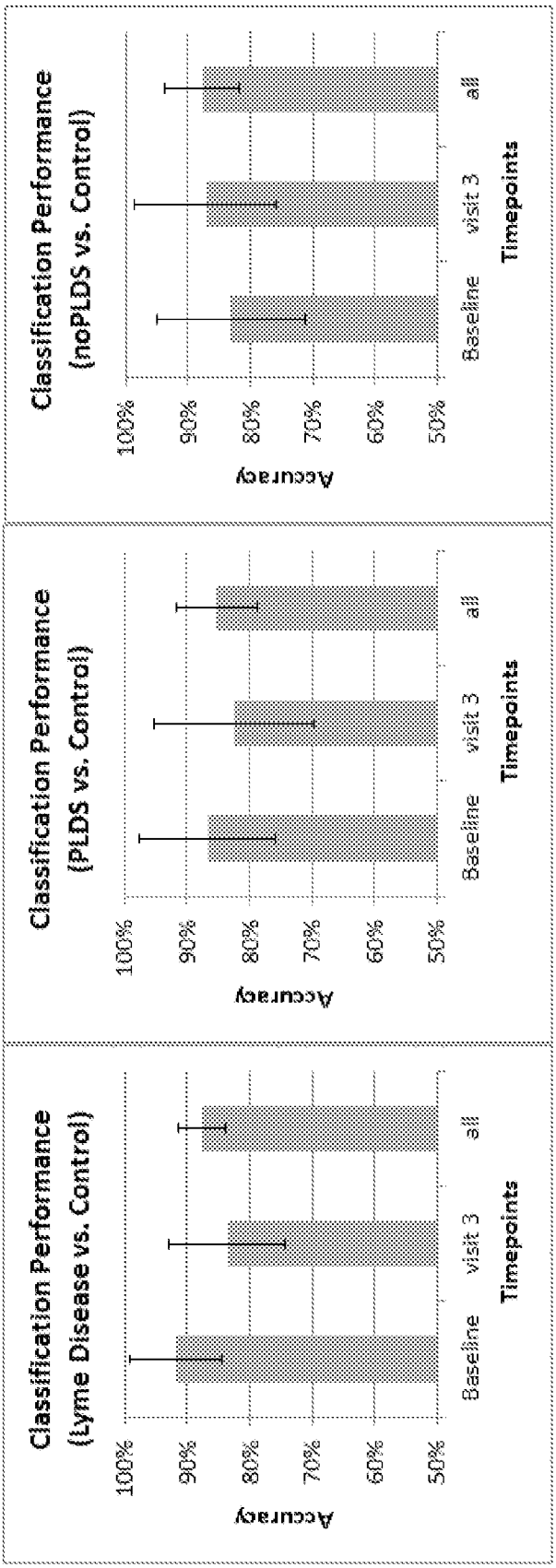


FIGURE 7

Figure 7. Classification performances for diagnosis of Lyme disease onset, using miR-130b-5p, miR-19b-3p, miR-485-5p, and miR-193a-5p. A) All Lyme disease patients vs. controls, B) PTLDS patients vs. controls, and C) Non-PTLDS patients vs. controls.

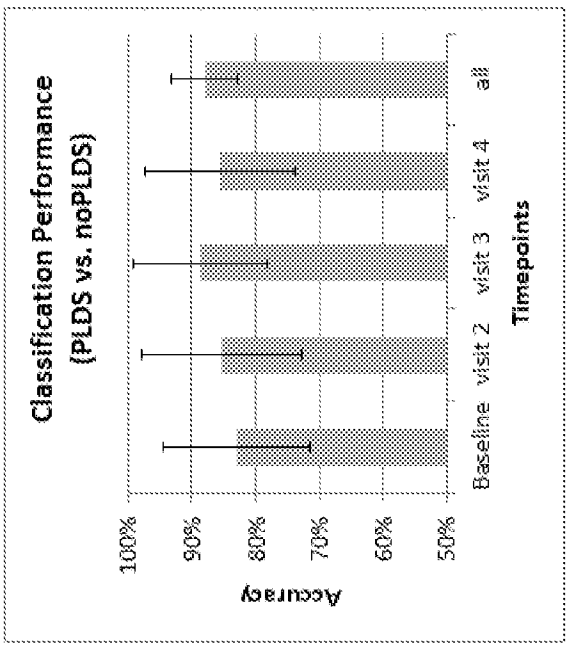


FIGURE 8

Figure 8. Classification performances for diagnosis of PTLDS from non-PTLDS, using miR-130b-5p, miR-485-5p, miR-615-3p, and miR-423-5p.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/67176

A. CLASSIFICATION OF SUBJECT MATTER
IPC - G01N 21/64, 33/82, 33/569 (2019.01)
CPC - G01N 21/64, 33/82, 33/569, 33/56911; Y02A 50/57

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.
Y	US 2008/0171667 A1 (BROWN et al.) 17 July 2008; abstract; paragraphs [0033], [0055]-[0057], [0225]	6, 8-9, 10/6, 10/8-9
Y	US 2012/0142023 A1 (ASCOLI et al.) 07 June 2012; abstract; paragraphs [0027], [0042]	6, 8-9, 10/6, 10/8-9
A	LOCHHEAD et al. 'MicroRNA Expression Shows Inflammatory Dysregulation and Tumor-Like Proliferative Responses in Joints of Patients With Postinfectious Lyme Arthritis' Arthritis & Rheumatology, 2017, Vol. 69, No. 5, pp. 1100-1110; page 1101, column 1, second paragraph	1-3, 4/1-3, 5/4/1-3, 7

☐ 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
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"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 April 2019 (02.04.2019)	15 APR 2019
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/67176

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: