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(54) BRUCELLOSIS, Q-FEVER, AND LYME DISEASE BIOMARKERS AND USES THEREOF

BIOMARKER FÜR BRUCELLOSE, Q-FIEBER UND LYME-KRANKHEIT SOWIE VERWENDUNGEN DAVON

BIOMARQUEURS DE LA BRUCELLOSE, DE LA FIÈVRE Q ET DE LA MALADIE DE LYME ET LEURS UTILISATIONS

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Description

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/803,857, filed March 21, 2013.

BACKGROUND OF THE INVENTION

[0002] A zoonosis is an infectious disease that is transmitted between species (sometimes by a vector) from animals other than humans to humans or from humans to other animals. Of the more than 1,400 pathogens kown to infect humans, greater than 60% of them are zoonotic (see, *e.g.,* Taylor et al. (2001) Philosophical Transactions of the Royal Society B 356(1411):983-9).

[0003] Brucellosis is a bacterial zoonosis of global magnitude. The causative agent is a gram negative alpha proteobacterium, *Brucella,* that infects a wide range of mammals, including essentially all economically important domestic mammals and many wild species. In humans, untreated brucellosis is a long lasting disease characterized by recurrent fever episodes and clinical manifestations that include spondylitis, severe headaches, joint or abdominal pain, endocarditis, and meningoencephalitis. In severe non-treated cases brucellosis can cause death (Moreno E, Moriyón I (2006). In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrant E, editors. Vol. 5. Part 1, section 3.1. The Prokaryotes. New York: Springer-Verlag. pp. 315-456; Ariza J, et al. (2007) PLOS medicine 4:1872-1878; Pappas G, et al. (2005) New Engl J Med. 352:2325-2336).

[0004] Q-fever is a zoonotic disease caused by infection with *Coxiella burnetii,* an obligate intracellular pathogenic bacterium that affects humans and other animals. This organism is found in cattle, sheep, goats and other domestic mammals, including cats and dogs. The infection results from inhalation of a spore-like small cell variant, and from contact with the milk, urine, feces, vaginal mucus, or semen of infected animals; ticks may also transmit the bacteria. Although most persons with acute Q fever infection recover, others may experience serious illness with complications that may include pneumonia, granulomatous hepatitis, myocarditis (inflammation of the heart tissue), central nervous system complications, and death. Pregnant women who are infected may be at risk for pre-term delivery or miscarriage.

[0005] Lyme disease, is a zoonotic disease caused by bacteria belonging to the genus *Borrelia.* Lyme disease is transmitted to humans by the bite of infected ticks belonging to a few species of the genus Ixodes ("hard ticks"). If Lyme disease is not detected and treated while early symptoms are present, or if symptoms are not present, the infection may affect the skin, joints, nervous system, and heart within weeks to months after the initial infection. Late disseminated infections may occur and result in joint inflammation, numbness and tingling in the hands, feet, or back, severe fatigue, partial facial nerve paralysis, neurologic changes, including problems with memory, mood, or sleep, and sometimes problems speaking.

[0006] Detection of the zoonotic bacterium *Brucella* by culture remains the gold standard for diagnosis (Moreno E, Moriyón I (2006). In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrant E, editors. Vol. 5. Part 1, section 3.1. The Prokaryotes. New York: Springer-Verlag. pp. 315-456). These methods are slow, and can require weeks to detect bacterial colonies. Further complicating this approach is that the bacteria are present in readily available body fluids intermittently at best. Diagnosis has therefore depended primarily on serology, mostly the detection of circulating immunoglobulins reactive against bacterial LPS and the closely related native hapten (NH) polysaccharides (Moreno E, Moriyón I (2006). In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrant E, editors. Vol. 5. Part 1, section 3.1. The Prokaryotes. New York: Springer-Verlag. pp. 315-456; Cutler SJ, et al. (2005) J Appl Microbiol 98:1270-1281). Early in disease, however, antibodies are not always generated or their titers may be intermittent (Baldi PC, et al. (2001) Scand J Infect Dis 33:200-205), hampering early diagnosis.

[0007] Furthermore, with respect to Brucella, antibodies against Brucella LPS may also cross react with the LPS of other bacteria, such as *Vibrio cholera,* and some *Yersinia* and *Salmonella* serotypes, resulting in false positives (Moreno E, Moriyón I (2006). In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrant E, editors. Vol. 5. Part 1, section 3.1. The Prokaryotes. New York: Springer-Verlag. pp. 315-456; Baldi PC, et al. (2001) Scand J Infect Dis 33:200-205). Antibodies against alternative Brucella antigens have been evaluated as serological diagnostic markers but with uncertain results (Rolán HG, et al. (2008) Clin Vaccine Immunol 15: 208-214; Contreras-Rodriguez A, et al. (2006) FEMS Immunol Med Microbiol 48:252-256) since it appears that the alternative antigens tend to induce lower immunoglobulin titers than the highly immunogenic LPS. Currently none of the alternative antigens are used for diagnosis of brucellosis.

[0008] The gold standard for diagnosis of Lyme Disease is serology, detection of host antibodies reactive to *Borrelia* proteins. Antibody titers typically do not develop in the first 2 weeks of disease, neccessitating diagnosis to be done based on the non-specific clinical symptoms and the likelihood of exposure to ticks. Furthermore, the accuracy of the test is low on the order of 60% in the US, and less than that in Europe (Robertson J and al. 2000. J Clin Microbiology. 38(6): 2097-2102. Nelson K, Masters C. 2007. Infectious Disease Epidemiology: Theory and Practice. 2nd Ed. Jones and Bartlett Publishers. Chaper 25: Lyme disease. P. 1063-1086).

[0009] Serology is also the gold standard for diagnosis of Q-fever. Analysis of two samples taken 2-4 weeks apart is recommended. The reason for this is that the antibody titer for this disease also does not develop imemdiately after infection, and since the Q fever pathogen is endemic in most areas of the world, a significant subset of the population already has Q fever reactive antibodies from prior exposure (Fournier PE, Marrie TJ, Raoult D. 1998. J Clin Microbiol. 36(7): 1823-1834).

[0010] PCR methods have also been evaluated as direct diagnostics (Baldi PC, et al. (2001) Scand J Infect Dis 33:200-205; Debeaumont C, et al. (2005) Eur J Clin Microbiol Infect Dis 24:842-845; Casañas MC, et al. (2001) Eur J Clin Micribiol Infect Dis 20:127-131; Elfaki MG, et al. (2005) Med Sci Monit 11:MT69-74). PCR offers considerable improvement in detection speed over culture methods, and has been used to distinguish bacterial species and strains (Baldi PC, et al. (2001) Scand J Infect Dis 33:200-205). Although faster, PCR is less sensitivity as a diagnostic than culture methods (Baldi PC, et al. (2001) Scand J Infect Dis 33:200-205; Elfaki MG, et al. (2005) Med Sci Monit 11:MT69-74).

[0011] The current approaches for diagnosis, therefore, rely primarily on products of the host's immune system or on the growth of viable pathogens in culture. Each approach has limitations that can translate into false negative diagnoses. Accordingly, there is a need in the art for novel biomarkers of zoonotic infections, such as *Brucella, Coxiella burnetii,* and *Borrelia* infection.

SUMMARY OF THE INVENTION

[0012] The present invention is defined in the appended claims.

[0013] The present invention is based, at least in part, on the discovery of markers that are associated with the presence of *Brucella* infection. Accordingly, the present invention provides sensitive and facile methods and kits for determining whether a subject has Brucellosis, as well as methods for identifying a compound that can treat Brucellosis, methods of monitoring the effectiveness of a therapy for treating Brucellosis in a subject, and methods for treating a subject having Brucellosis by measuring and identifying particular markers, or particular combinations of markers.

[0014] The present disclosure is also based, at least in part, on the discovery of markers that are associated with the presence of Lyme Disease. Accordingly, the present disclosure provides sensitive and facile methods and kits for determining whether a subject has Lyme Disease, as well as methods for identifying a compound that can treat Lyme Disease, methods of monitoring the effectiveness of a therapy for treating Lyme Disease in a subject, and methods for treating a subject having Lyme Disease by measuring and identifying particular markers, or particular combinations of markers.

[0015] In addition, the present invention is based, at least in part, on the discovery of markers that are associated with the presence of Q-Fever. Accordingly, the present invention provides sensitive and facile methods and kits for determining whether a subject has Q-Fever, as well as methods for identifying a compound that can treat Q-Fever, methods of monitoring the effectiveness of a therapy for treating Q-Fever in a subject, and methods for treating a subject having Q-Fever by measuring and identifying particular markers, or particular combinations of markers.

[0016] The present disclosure is also based, at least in part, on the discovery of markers that can be used to determine whether a subject has Brucellosis or Q-Fever, markers that can be used to determine whether a subject has Lyme Disease or Q-Fever, markers that can be used to determine whether a subejct has Brucellosis or Lyme Disease, and markers that can be used to determine whether a subejct has Brucellosis or Q-Fever or Lyme Disease and provides sensitive and facile methods and kits for determining whether a subject has Brucellosis or Q-Fever or Lyme Disease.

[0017] Accordingly, in one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of one or more markers listed in Table 1 in a sample(s) from the subject; comparing the level of the one or more markers in the subject sample(s) with a level of the one or more markers in a control sample(s), wherein a difference in the level of the one or more markers in the subject sample(s) as compared to the level of the one or more markers in the control sample(s) indicates that the subject has Brucellosis.

[0018] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining the level of one or more markers listed in Table 1 in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of one or more markers listed in Table 1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the one or more markers in the first sample(s) with a level of the one or more markers in the second sample(s), wherein a difference in the level of the one or more markers in the first sample(s) as compared to the level of the one or more markers in the second sample(s) indicates that the treatment is effective.

[0019] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of one or more markers of the invention in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of the one or more markers listed in Table 1 in an aliquot as compared to the level

and/or activity of the one or more markers of the invention in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0020] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of any one or more of the markers listed in Table 1, thereby treating the subject.

[0021] In one embodiment, the level in the subject sample(s) is determined by mass spectrometry. In one embodiment, the mass spectrometry is matrix assisted laser desorption/time of flight (MALDI/TOF) mass spectrometry, liquid chromatography quadruple ion trap electrospray (LCQ-MS), or surface enhanced laser desorption ionization/time of flight (SELDI/TOF) mass spectrometry.

[0022] In one embodiment, the level in the subject sample(s) is determined by immunoassay.

[0023] In one embodiment, the sample(s) from the subject is a fluid sample(s). In another embodiment, the sample(s) from the subject is a tissue sample(s).

[0024] In one embodiment, the level of the marker is an expression level and/or activity of the marker.

[0025] In one embodiment, the one or more markers is selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENOl, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0026] In one embodiment, the methods further comprise determining the level of one or more markers selected from the group consisting of SLC3A2, GOT1, GOT2, ACADVL, DBI, ACOX1, AHNAK, AIMP1, AKR1B1, ANPEP, ANXA2, ANXA5, ANXA6, AKR7A2, ARPC3, ASAH1, B4GALT1, BCAM, BLOC1S5, CALU, CAPG, CAPZB, CAPZA2, CBX1, CDC37, HSPE1, CHMP1A, CHMP1B, CHMP2A, CHMP4A, CHMP4B, CHMP5, CLIC1, CNDP2, CNPY2, COPA, COPB2, CORO1A, CORO1B, CORO1C, CPVL, C19orf10, CXorf26, CXCL10, CACYBP, DSP, HSD17B4, DLG1, DNASE2, DDT, DYNLL1, EPB41L3, EEF1A1, EEF1B2, EEF1D, EEF2, ENG, EHD1, ELAVL1, EMC2, EMR2, ENO1, ENO3, HSP90B1, ERO1L, ESYT2, EVL, ST13, F5, FKBP4, FLNA, FH, H6PD, GPI, GDI2, ARHGDIA, GGH, GBA, PRKCSH, GNS, HSPA9, HSPA5, GSTP1, GLT25D1, HIST1H1D, HIST1H1B, HIST3H2A, H2AFY, HIST2H2BE, HIST2H2BF, HIST3H3, HIST1H4A, HSD17B10, HEXA, HMGB3, HNRNPC, HNRNPD, HNRNPK, HNRNPR, HNRNPU, HSP90AA1, HSP90AB1, HSPA1A, HSPA8, ICAM1, EIF5A2, IL1B, IMPDH2, ISYNA1, ISOC1, ITGA5, ITGAM, ITPR1, KRT9, KRT7, KRT8, CAMK2D, PKM, LASP1, LDHB, LIMA1, LMNA, LMNB1, LMNB2, LRCH1, GAA, LYN, MDH1, MDH2, MESDC2, MAT2A, MYL12A, MMP9, RABIF, MYH10, MYH9, MYO1E, MYO6, PPP1R12A, NAGA, POR, NME1, NME2, NDRG1, NDUFA2, NAP1L1, NPM1, NUTF2, NCL, NUDC, OAS2, PAFAH1B2, PA2G4, PARK7, PARP1, PRCP, PDCD6IP, P4HB, PDIA5, PEBP1, PECAM1, PFDN1, PFDN2, PGAM1, PGK1, PIP4K2A, PIP5K1A, PLEKHO2, PLEC, PLOD3, LCP1, PNP, ALPP, PPIA, PPIB, PRDX1, PRDX2, PRDX3, PRDX6, PRPF19, PSMC6, PSMC3, PSMC2, PRSS8, NPEPPS, PSMA7, PSMB5, PSMD10, PSMD11, PSMD14, PSMD1, PSMD2, PSMD4, PTBP3, PAICS, ATIC, PXMP2, RAB6A, RANGAP1, RAI14, RAN, RANBP1, RBBP4, RAD23B, RPL14, RPL23A, RPL24, RPL26, RPL29, RPL3, RPL31, RPL7A, RPL8, RPLP2, MRPL39, HNRNPA1, HNRNPA2B1, RPS23, RPS8, RSAD2, RPSA, RUVBL1, RUVBL2, S100P, S100A6, S100A11, SEC61B, SEPT11, SEPT2, SEPT6, SEPT7, SEPT9, PHGDH, SF3B3, SFPQ, SIAE, SNRPD3, NAPA, SOD2, SORT1, SGSH, SPRED1, SPTBN1, SPG11, SPTAN1, SRSF3, SRSF8, STIP1, STMN1, STRAP, STX11, SUMO3, EPRS, IARS2, TAGLN2, TALDO1, TUBA4A, TUBB4B, TUBB, TBL2, CCT4, CCT8, TDRD6, VCP, TKT, TLN1, TMOD3, TPI1, TRA2B, TXNRD1, USP14, UGGT1, ATP6V1A, ATP6V1B2, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, VPS4A, XRCC6, and YBX1.

[0027] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of HISTH4 in a sample(s) from the subject; comparing the level of HISTH4 in the subject sample(s) with a level of HISTH4 in a control sample(s), wherein a difference in the level of HISTH4 in the subject sample(s) as compared to the level of HISTH4 in the control sample(s) indicates that the subject has Brucellosis.

[0028] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining the level of HISTH4 in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of HISTH4 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of HISTH4 in the first sample(s) with a level of HISTH4 in the second sample(s), wherein a difference in the level of HISTH4 in the first sample(s) as compared to the level of HISTH4 in the second sample(s) indicates that the treatment is effective.

[0029] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HISTH4 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HISTH4 in an aliquot as compared to the level and/or activity of HISTH4 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0030] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HISTH4, thereby treating the subject.

[0031] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of MAT2A in a sample(s) from the subject; comparing the level of MAT2A in the subject sample(s) with a level of MAT2A in a control sample(s), wherein a difference in the level of MAT2A in the subject sample(s) as compared to the level of MAT2A in the control sample(s) indicates that the subject has Brucellosis.

[0032] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining the level of MAT2A in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of MAT2A in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of MAT2A in the first sample(s) with a level of MAT2A in the second sample(s), wherein a difference in the level of MAT2A in the first sample(s) as compared to the level of MAT2A in the second sample(s) indicates that the treatment is effective.

[0033] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of MAT2A in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of MAT2A in an aliquot as compared to the level and/or activity of MAT2A in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0034] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of MAT2A, thereby treating the subject.

[0035] In one aspect the present invention provides methods for determining whether a subject has Brucellosis. The methods include determining the level of MDH1 in a sample(s) from the subject; comparing the level of MDH1 in the subject sample(s) with a level of MDH1 in a control sample(s), wherein a difference in the level of MDH1 in the subject sample(s) as compared to the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0036] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining the level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of MDH1 in the first sample(s) with a level of MDH1 in the second sample(s), wherein a difference in the level of MDH1 in the first sample(s) as compared to the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0037] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of MDH1 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of MDH1 in an aliquot as compared to the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0038] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of MDH1, thereby treating the subject.

[0039] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of LYN in a sample(s) from the subject; comparing the level of LYN in the subject sample(s) with a level of LYN in a control sample(s), wherein a difference in the level of LYN in the subject sample(s) as compared to the level of LYN in the control sample(s) indicates that the subject has Brucellosis.

[0040] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining the level of LYN in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of LYN in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of LYN in the first sample(s) with a level of LYN in the second sample(s), wherein a difference in the level of LYN in the first sample(s) as compared to the level of LYN in the second sample(s) indicates that the treatment is effective.

[0041] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of LYN in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of LYN in an aliquot as compared to the level and/or activity of LYN in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0042] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of LYN, thereby treating the subject.

[0043] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The

methods include determining the level of HIST4H4 and the level of LYN in a sample(s) from the subject; comparing the level of HIST4H4 and the level of LYN in the subject sample(s) with a level of HIST4H4 and a level of LYN in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of LYN in the subject sample(s) as compared to the level of HIST4H4 and the level of LYN in the control sample(s) indicates that the subject has Brucelosis.

[0044] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of HIST4H4 and a level of LYN in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4 and a level of LYN in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of HIST4H4 and the level of LYN in the first sample(s) with the level of HIST4H4 and the level of LYN in the second sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of LYN in the first sample(s) as compared to the level of the HIST4H4 and the level of LYN in the second sample(s) indicates that the treatment is effective.

[0045] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4 and the level and/or activity of LYN in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4 and the level and/or activity of LYN in an aliquot as compared to the level and/or activity of HIST4H4 and the level and/or activity of LYN in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0046] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4 and the level and/or activity of LYN, thereby treating the subject.

[0047] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of HIST4H4 and the level of MAT2A in a sample(s) from the subject; comparing the level of HIST4H4 and the level of MAT2A in the subject sample(s) with a level of HIST4H4 and a level of MAT2A in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MAT2A in the subject sample(s) as compared to the level of HIST4H4 and the level of MAT2A in the control sample(s) indicates that the subject has Brucelosis.

[0048] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of HIST4H4 and a level of MAT2A in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4 and a level of MAT2A in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of HIST4H4 and the level of MAT2A in the first sample(s) with the level of HIST4H4 and the level of MAT2A in the second sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MAT2A in the first sample(s) as compared to the level of the HIST4H4 and the level of MAT2A in the second sample(s) indicates that the treatment is effective.

[0049] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4 and the level and/or activity of MAT2A in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4 and the level and/or activity of MAT2A in an aliquot as compared to the level and/or activity of HIST4H4 and the level and/or activity of MAT2A in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0050] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4 and the level and/or activity of MAT2A, thereby treating the subject.

[0051] In one aspect the present invention provides methods for determining whether a subject has Brucellosis. The methods include determining the level of HIST4H4 and the level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4 and the level of MDH1 in the subject sample(s) with a level of HIST4H4 and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4 and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0052] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of HIST4H4 and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4 and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of HIST4H4 and the level of MDH1 in the first sample(s) with the level of HIST4H4 and the level of MDH1 in the second sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MDH1 in the first sample(s) as compared to the level of the HIST4H4 and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0053] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a

subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4 and the level and/or activity of MDH1 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4 and the level and/or activity of MDH1 in an aliquot as compared to the level and/or activity of HIST4H4 and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0054] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4 and the level and/or activity of MDH1, thereby treating the subject.

[0055] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of LYN and the level of MAT2A in a sample(s) from the subject; comparing the level of LYN and the level of MAT2A in the subject sample(s) with a level of LYN and a level of MAT2A in a control sample(s), wherein a difference in the level of LYN and a difference in the level of MAT2A in the subject sample(s) as compared to the level of LYN and the level of MAT2A in the control sample(s) indicates that the subject has Brucelosis.

[0056] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of LYN and a level of MAT2A in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of LYN and a level of MAT2A in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of LYN and the level of MAT2A in the first sample(s) with the level of LYN and the level of MAT2A in the second sample(s), wherein a difference in the level of LYN and a difference in the level of MAT2A in the first sample(s) as compared to the level of the LYN and the level of MAT2A in the second sample(s) indicates that the treatment is effective.

[0057] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of LYN and the level and/or activity of MAT2A in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of LYN and the level and/or activity of MAT2A in an aliquot as compared to the level and/or activity of LYN and the level and/or activity of MAT2A in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0058] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of LYN and the level and/or activity of MAT2A, thereby treating the subject.

[0059] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of LYN and the level of MDH1 in a sample(s) from the subject; comparing the level of LYN and the level of MDH1 in the subject sample(s) with a level of LYN and a level of MDH1 in a control sample(s), wherein a difference in the level of LYN and a difference in the level of MDH1 in the subject sample(s) as compared to the level of LYN and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0060] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of LYN and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of LYN and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of LYN and the level of MDH1 in the first sample(s) with the level of LYN and the level of MDH1 in the second sample(s), wherein a difference in the level of LYN and a difference in the level of MDH1 in the first sample(s) as compared to the level of the LYN and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0061] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of LYN and the level and/or activity of MDH1 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of LYN and the level and/or activity of MDH1 in an aliquot as compared to the level and/or activity of LYN and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0062] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of LYN and the level and/or activity of MDH1, thereby treating the subject.

[0063] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining the level of MAT2A and the level of MDH1 in a sample(s) from the subject; comparing the level of MAT2A and the level of MDH1 in the subject sample(s) with a level of MAT2A and a level of MDH1 in a control sample(s), wherein a difference in the level of MAT2A and a difference in the level of MDH1 in the subject sample(s) as compared to the level of MAT2A and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0064] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level of MAT2A and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of MAT2A and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of MAT2A and the level of MDH1 in the first sample(s) with the level of MAT2A and the level of MDH1 in the second sample(s), wherein a difference in the level of MAT2A and a difference in the level of MDH1 in the first sample(s) as compared to the level of the MAT2A and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0065] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of MAT2A and the level and/or activity of MDH1 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of MAT2A and the level and/or activity of MDH1 in an aliquot as compared to the level and/or activity of MAT2A and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0066] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of MAT2A and the level and/or activity of MDH1, thereby treating the subject.

[0067] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining a level of HIST4H4, a level of LYN, and a level of MAT2A in a sample(s) from the subject; comparing the level of HIST4H4, the level of LYN, and the level of MAT2A in the subject sample(s) with a level of HIST4H4, a level of LYN, and a level of MAT2A in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of LYN, and a difference in the level of MAT2A in the subject sample(s) as compared to the level of HIST4H4, the level of LYN, and the level of MAT2A in the control sample(s) indicates that the subject has Brucellosis.

[0068] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level HIST4H4, a level of LYN, and a level of MAT2A in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4, a level of LYN, and a level of MAT2A in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the HIST4H4, the level of LYN, and the level of MAT2A with the level of HIST4H4, the level of LYN, and the level of MAT2A in the second sample(s), wherein a difference in the level of HIST4H4, the level of LYN, and the level of MAT2A in the first sample(s) as compared to the level of HIST4H4, the level of LYN, and the level of MAT2A in the second sample(s) indicates that the treatment is effective.

[0069] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MAT2A of the invention in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4, the level and/or the activity of LYN, and the level and/or the activity of MAT2A in an aliquot as compared to the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MAT2A in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0070] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MAT2A, thereby treating the subject.

[0071] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining a level of HIST4H4, a level of LYN, and a level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4, the level of LYN, and the level of MDH1 in the subject sample(s) with a level of HIST4H4, a level of LYN, and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of LYN, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4, the level of LYN, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0072] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level HIST4H4, a level of LYN, and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4, a level of LYN, and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the HIST4H4, the level of LYN, and the level of MDH1 with the level of HIST4H4, the level of LYN, and the level of MDH1 in the second sample(s), wherein a difference in the level of HIST4H4, the level of LYN, and the level of MDH1 in the first sample(s) as compared to the level of HIST4H4, the level of LYN, and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0073] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member

of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MDH1 of the invention in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4, the level and/or the activity of LYN, and the level and/or the activity of MDH1 in an aliquot as compared to the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0074] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4, the level and/or activity of LYN, and the level and/or activity of MDH1, thereby treating the subject.

[0075] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the subject sample(s) with a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of MAT2A, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0076] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level HIST4H4, a level of MAT2A, and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the HIST4H4, the level of MAT2A, and the level of MDH1 with the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the second sample(s), wherein a difference in the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the first sample(s) as compared to the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0077] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4, the level and/or activity of MAT2A, and the level and/or activity of MDH1 of the invention in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4, the level and/or the activity of MAT2A, and the level and/or the activity of MDH1 in an aliquot as compared to the level and/or activity of HIST4H4, the level and/or activity of MAT2A, and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0078] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4, the level and/or activity of MAT2A, and the level and/or activity of MDH1, thereby treating the subject.

[0079] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining a level of LYN, a level of MAT2A, and a level of MDH1 in a sample(s) from the subject; comparing the level of LYN, the level of MAT2A, and the level of MDH1 in the subject sample(s) with a level of LYN, a level of MAT2A, and a level of MDH1 in a control sample(s), wherein a difference in the level of LYN, a difference in the level of MAT2A, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of LYN, the level of MAT2A, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0080] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level LYN, a level of MAT2A, and a level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of LYN, a level of MAT2A, and a level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the LYN, the level of MAT2A, and the level of MDH1 with the level of LYN, the level of MAT2A, and the level of MDH1 in the second sample(s), wherein a difference in the level of LYN, the level of MAT2A, and the level of MDH1 in the first sample(s) as compared to the level of LYN, the level of MAT2A, and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0081] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of LYN, the level and/or activity of MAT2A, and the level and/or activity of MDH1 of the invention in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of LYN, the level and/or the activity of MAT2A, and the level and/or the activity of MDH1 in an aliquot as compared to the level and/or activity of LYN, the level and/or activity of MAT2A, and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0082] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods

include administering to the subject an effective amount of an agent that modulates the level and/or activity of LYN, the level and/or activity of MAT2A, and the level and/or activity of MDH1, thereby treating the subject.

[0083] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis. The methods include determining a level of HIST4H4, a level of MAT2A, a level of MDH1, and a level of LYN in a sample(s) from the subject; comparing the level of HIST4H4, the level of MAT2A, the level of MDH1, and a level of LYN in the subject sample(s) with a level of HIST4H4, a level of MAT2A, a level of MDH1, and a level of LYN in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of MAT2A, a difference in the level of MDH1, and a difference in the level of LYN in the subject sample(s) as compared to the level of HIST4H4, the level of MAT2A, the level of MDH1, and the level of LYN in the control sample(s) indicates that the subject has Brucellosis.

[0084] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Brucellosis. The methods include determining a level HIST4H4, a level of MAT2A, a level of MDH1, and a level of LYN in a first sample(s) from the subject prior to the initiation of the treatment; determining a level of HIST4H4, a level of MAT2A, a level of MDH1, and a level of LYN in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of the HIST4H4, the level of MAT2A, the level of MDH1, and the level of LYN with the level of HIST4H4, the level of MAT2A, the level of MDH1, and the level of LYN in the second sample(s), wherein a difference in the level of HIST4H4, the level of MAT2A, the level of MDH1, and the level of LYN in the first sample(s) as compared to the level of HIST4H4, the level of MAT2A, the level of MDH1, and the level of LYN in the second sample(s) indicates that the treatment is effective.

[0085] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Brucellosis. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of HIST4H4, the level and/or activity of MAT2A, the level and/or activity of MDH1, and the level and/or activity of LYN in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of HIST4H4, the level and/or the activity of MAT2A, the level and/or the activity of MDH1, and the level and/or activity of LYN in an aliquot as compared to the level and/or activity of HIST4H4, the level and/or activity of MAT2A, the level and/or activity of MDH1, and the level and/or activity of LYN in a control sample, thereby identifying a compound that is useful for treating a subject having Brucellosis.

[0086] In one aspect, the present disclosure provides methods for treating a subject having Brucellosis. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of HIST4H4, the level and/or activity of MAT2A, the level and/or activity of MDH1, and the level and/or activity of LYN, thereby treating the subject.

[0087] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of HIST4H4 in a sample(s) from the subject; comparing the level of HIST4H4 in the subject sample(s) with a level of HIST4H4 in a control sample(s), wherein a difference in the level of HIST4H4 in the subject sample(s) as compared to the level of HIST4H4 in the control sample(s) indicates that the subject has Brucellosis.

[0088] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of MAT2A in a sample(s) from the subject; comparing the level of MAT2A in the subject sample(s) with a level of MAT2A in a control sample(s), wherein a difference in the level of MAT2A in the subject sample(s) as compared to the level of MAT2A in the control sample(s) indicates that the subject has Brucellosis.

[0089] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of LDHB in a sample(s) from the subject; comparing the level of LDHB in the subject sample(s) with a level of LDHB in a control sample(s), wherein a difference in the level of LDHB in the subject sample(s) as compared to the level of LDHB in the control sample(s) indicates that the subject has Brucellosis.

[0090] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of MDH1 in a sample(s) from the subject; comparing the level of MDH1 in the subject sample(s) with a level of MDH1 in a control sample(s), wherein a difference in the level of MDH1 in the subject sample(s) as compared to the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0091] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of HIST4H4 and the level of LDHB in a sample(s) from the subject; comparing the level of HIST4H4 and the level of LDHB in the subject sample(s) with a level of HIST4H4 and a level of LDHB in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of LDHB in the subject sample(s) as compared to the level of HIST4H4 and the level of LDHB in the control sample(s) indicates that the subject has Brucelosis.

[0092] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of HIST4H4 and the level of MAT2A in a sample(s) from the subject; comparing the level of HIST4H4 and the level of MAT2A in the subject sample(s) with a level of HIST4H4 and a level

of MAT2A in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MAT2A in the subject sample(s) as compared to the level of HIST4H4 and the level of MAT2A in the control sample(s) indicates that the subject has Brucelosis.

[0093] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of HIST4H4 and the level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4 and the level of MDH1 in the subject sample(s) with a level of HIST4H4 and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4 and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0094] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of LDHB and the level of MAT2A in a sample(s) from the subject; comparing the level of LDHB and the level of MAT2A in the subject sample(s) with a level of LDHB and a level of MAT2A in a control sample(s), wherein a difference in the level of LDHB and a difference in the level of MAT2A in the subject sample(s) as compared to the level of LDHB and the level of MAT2A in the control sample(s) indicates that the subject has Brucelosis.

[0095] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of LDHB and the level of MDH1 in a sample(s) from the subject; comparing the level of LDHB and the level of MDH1 in the subject sample(s) with a level of LDHB and a level of MDH1 in a control sample(s), wherein a difference in the level of LDHB and a difference in the level of MDH1 in the subject sample(s) as compared to the level of LDHB and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0096] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining the level of MAT2A and the level of MDH1 in a sample(s) from the subject; comparing the level of MAT2A and the level of MDH1 in the subject sample(s) with a level of MAT2A and a level of MDH1 in a control sample(s), wherein a difference in the level of MAT2A and a difference in the level of MDH1 in the subject sample(s) as compared to the level of MAT2A and the level of MDH1 in the control sample(s) indicates that the subject has Brucelosis.

[0097] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of HIST4H4, a level of LDHB, and a level of MAT2A in a sample(s) from the subject; comparing the level of HIST4H4, the level of LDHB, and the level of MAT2A in the subject sample(s) with a level of HIST4H4, a level of LDHB, and a level of MAT2A in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of LDHB, and a difference in the level of MAT2A in the subject sample(s) as compared to the level of HIST4H4, the level of LDHB, and the level of MAT2A in the control sample(s) indicates that the subject has Brucellosis.

[0098] In one aspect the present invention provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of HIST4H4, a level of LDHB, and a level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4, the level of LDHB, and the level of MDH1 in the subject sample(s) with a level of HIST4H4, a level of LDHB, and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of LDHB, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4, the level of LDHB, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0099] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a sample(s) from the subject; comparing the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the subject sample(s) with a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of MAT2A, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of HIST4H4, the level of MAT2A, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0100] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever. The methods include determining a level of LDHB, a level of MAT2A, and a level of MDH1 in a sample(s) from the subject; comparing the level of LDHB, the level of MAT2A, and the level of MDH1 in the subject sample(s) with a level of LDHB, a level of MAT2A, and a level of MDH1 in a control sample(s), wherein a difference in the level of LDHB, a difference in the level of MAT2A, and a difference in the level of MDH1 in the subject sample(s) as compared to the level of LDHB, the level of MAT2A, and the level of MDH1 in the control sample(s) indicates that the subject has Brucellosis.

[0101] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of HIST4H4 in a sample(s) from the subject; comparing the level of HIST4H4 in the subject sample(s) with a level of HIST4H4 in a control sample(s), wherein a difference in the level of HIST4H4 in the subject sample(s) as compared to the level of HIST4H4 in the control sample(s) indicates that

the subject has Brucellosis.

[0102] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of MMP9 in a sample(s) from the subject; comparing the level of MMP9 in the subject sample(s) with a level of MMP9 in a control sample(s), wherein a difference in the level of MMP9 in the subject sample(s) as compared to the level of MMP9 in the control sample(s) indicates that the subject has Brucellosis.

[0103] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of LYN in a sample(s) from the subject; comparing the level of LYN in the subject sample(s) with a level of LYN in a control sample(s), wherein a difference in the level of LYN in the subject sample(s) as compared to the level of LYN in the control sample(s) indicates that the subject has Brucellosis.

[0104] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of ICAM1 in a sample(s) from the subject; comparing the level of ICAM1 in the subject sample(s) with a level of ICAM1 in a control sample(s), wherein a difference in the level of ICAM1 in the subject sample(s) as compared to the level of ICAM1 in the control sample(s) indicates that the subject has Brucellosis.

[0105] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of HIST4H4 and the level of ICAM1 in a sample(s) from the subject; comparing the level of HIST4H4 and the level of ICAM1 in the subject sample(s) with a level of HIST4H4 and a level of ICAM1 in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of ICAM1 in the subject sample(s) as compared to the level of HIST4H4 and the level of ICAM1 in the control sample(s) indicates that the subject has Brucelosis.

[0106] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of HIST4H4 and the level of LYN in a sample(s) from the subject; comparing the level of HIST4H4 and the level of LYN in the subject sample(s) with a level of HIST4H4 and a level of LYN in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of LYN in the subject sample(s) as compared to the level of HIST4H4 and the level of LYN in the control sample(s) indicates that the subject has Brucelosis.

[0107] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of HIST4H4 and the level of MMP9 in a sample(s) from the subject; comparing the level of HIST4H4 and the level of MMP9 in the subject sample(s) with a level of HIST4H4 and a level of MMP9 in a control sample(s), wherein a difference in the level of HIST4H4 and a difference in the level of MMP9 in the subject sample(s) as compared to the level of HIST4H4 and the level of MMP9 in the control sample(s) indicates that the subject has Brucelosis.

[0108] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of ICAM1 and the level of LYN in a sample(s) from the subject; comparing the level of ICAM1 and the level of LYN in the subject sample(s) with a level of ICAM1 and a level of LYN in a control sample(s), wherein a difference in the level of ICAM1 and a difference in the level of LYN in the subject sample(s) as compared to the level of ICAM1 and the level of LYN in the control sample(s) indicates that the subject has Brucelosis.

[0109] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of ICAM1 and the level of MMP9 in a sample(s) from the subject; comparing the level of ICAM1 and the level of MMP9 in the subject sample(s) with a level of ICAM1 and a level of MMP9 in a control sample(s), wherein a difference in the level of ICAM1 and a difference in the level of MMP9 in the subject sample(s) as compared to the level of ICAM1 and the level of MMP9 in the control sample(s) indicates that the subject has Brucelosis.

[0110] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of LYN and the level of MMP9 in a sample(s) from the subject; comparing the level of LYN and the level of MMP9 in the subject sample(s) with a level of LYN and a level of MMP9 in a control sample(s), wherein a difference in the level of LYN and a difference in the level of MMP9 in the subject sample(s) as compared to the level of LYN and the level of MMP9 in the control sample(s) indicates that the subject has Brucelosis.

[0111] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of HIST4H4, a level of ICAM1, and a level of LYN in a sample(s) from the subject; comparing the level of HIST4H4, the level of ICAM1, and the level of LYN in the subject sample(s) with a level of HIST4H4, a level of ICAM1, and a level of LYN in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of ICAM1, and a difference in the level of LYN in the subject sample(s) as compared to the level of HIST4H4, the level of ICAM1, and the level of LYN in the control sample(s) indicates that the subject has Brucellosis.

[0112] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of HIST4H4, a level of ICAM1, and a level of MMP9 in a

sample(s) from the subject; comparing the level of HIST4H4, the level of ICAM1, and the level of MMP9 in the subject sample(s) with a level of HIST4H4, a level of ICAM1, and a level of MMP9 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of ICAM1, and a difference in the level of MMP9 in the subject sample(s) as compared to the level of HIST4H4, the level of ICAM1, and the level of MMP9 in the control sample(s) indicates that the subject has Brucellosis.

[0113] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of HIST4H4, a level of LYN, and a level of MMP9 in a sample(s) from the subject; comparing the level of HIST4H4, the level of LYN, and the level of MMP9 in the subject sample(s) with a level of HIST4H4, a level of LYN, and a level of MMP9 in a control sample(s), wherein a difference in the level of HIST4H4, a difference in the level of LYN, and a difference in the level of MMP9 in the subject sample(s) as compared to the level of HIST4H4, the level of LYN, and the level of MMP9 in the control sample(s) indicates that the subject has Brucellosis.

[0114] In one aspect the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining a level of ICAM1, a level of LYN, and a level of MMP9 in a sample(s) from the subject; comparing the level of ICAM1, the level of LYN, and the level of MMP9 in the subject sample(s) with a level of ICAM1, a level of LYN, and a level of MMP9 in a control sample(s), wherein a difference in the level of ICAM1, a difference in the level of LYN, and a difference in the level of MMP9 in the subject sample(s) as compared to the level of ICAM1, the level of LYN, and the level of MMP9 in the control sample(s) indicates that the subject has Brucellosis.

[0115] In one embodiment, the methods further comprise determining the level of one or more additional markers listed in Table 1.

[0116] In another embodiment, the methods further comprise determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENOl, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0117] In one embodiment, the level of the marker is an expression level and/or activity of the marker.

[0118] In one embodiment, the level in the subject sample(s) is determined by mass spectrometry. In one embodiment, the mass spectrometry is matrix assisted laser desorption/time of flight (MALDI/TOF) mass spectrometry, liquid chromatography quadruple ion trap electrospray (LCQ-MS), or surface enhanced laser desorption ionization/time of flight (SELDI/TOF) mass spectrometry.

[0119] In one embodiment, the level in the subject sample(s) is determined by immunoassay.

[0120] In one embodiment, the sample(s) from the subject is a fluid sample(s). In another embodiment, the sample(s) from the subject is a tissue sample(s).

[0121] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of one or more markers listed in Table 1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0122] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of one or more markers listed in Table 1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0123] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of HISTH4 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0124] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HISTH4 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0125] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0126] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of MAT2A in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0127] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents determining the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0128] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0129] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0130] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of LYN in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0131] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0132] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of LYN in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0133] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0134] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of MAT2A in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0135] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0136] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4 and the level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0137] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of LYN and the level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0138] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of LYN and the level of MAT2A in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0139] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of LYN and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0140] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of LYN and the level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0141] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining the level of MAT2A and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0142] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of MAT2A and the level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0143] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining a level of HIST4H4, a level of LYN, and a level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0144] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4, a level of LYN, and a level of MAT2A in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0145] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining a level of HIST4H4, a level of LYN, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0146] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4, a level of LYN, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0147] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0148] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0149] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits

include reagents for determining a level of LYN, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0150] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of LYN, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0151] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis. The kits include reagents for determining a level of HIST4H4, a level of MAT2A, a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis.

[0152] In one aspect, the present invention provides kits for monitoring the effectiveness of a treatment in a subject having Brucellosis. The kits include reagents for determining the level of HIST4H4, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0153] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of HIST4H4 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0154] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0155] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of LDHB in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0156] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0157] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of HIST4H4 and the level of LDHB in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0158] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of HIST4H4 and the level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0159] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of HIST4H4 and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0160] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of LDHB and the level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0161] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of LDHB and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0162] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining the level of MAT2A and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0163] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of HIST4H4, a level of LDHB, and a level of MAT2A in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0164] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of HIST4H4, a level of LDHB, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0165] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of HIST4H4, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0166] In one aspect, the present invention provides kits for determining whether a subject has Brucellosis or Q-fever. The kits include reagents for determining a level of LDHB, a level of MAT2A, and a level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever.

[0167] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of HIST4H4 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0168] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of MMP9 in a subject sample(s) and instructions for use of

the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0169] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0170] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of ICAM1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0171] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of HIST4H4 and the level of ICAM1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0172] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of HIST4H4 and the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0173] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of HIST4H4 and the level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0174] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of ICAM1 and the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0175] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of ICAM1 and the level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0176] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining the level of LYN and the level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0177] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of HIST4H4, a level of ICAM1, and a level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0178] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of HIST4H4, a level of ICAM1, and a level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0179] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of HIST4H4, a level of LYN, and a level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0180] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Lyme Disease. The kits include reagents for determining a level of ICAM1, a level of LYN, and a level of MMP in a subject sample(s) and instructions for use of the kit to determine whether the subject has Brucellosis or Lyme Disease.

[0181] In one embodiment, the kits further include reagents for determining the level of any one or more of the markers listed in Table 1 in a sample(s) from the subject.

[0182] In another embodiment, the kits further include reagents for determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENOl, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0183] In one aspect, the present disclosure provides methods for identifying a Brucellosis marker. The methods include identifying proteins differentially expressed in a cell that is not infected with *Brcuella* and a cell that is infected with *Brucella,* thereby generating a provisional list of markers, determining the level of one or more of the provisional markers in a control sample; and determining the level of the one or more provisional markers in a test sample, wherein a difference in the level of a marker in the control sample as compared to the level in the test sample identifies the marker as a Brucellosis marker.

[0184] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1 in a sample(s) from the subject; comparing the level of B4GALT1 in the subject sample(s) with a level of B4GALT1 in a control sample(s), wherein a difference in the level of B4GALT1 in the subject sample(s) as compared to the level of B4GALT1 in the control sample(s) indicates that the subject has Q-Fever.

[0185] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of CALU in a sample(s) from the subject; comparing the level of CALU in the subject sample(s) with a level of CALU in a control sample(s), wherein a difference in the level of CALU in the subject sample(s) as compared to the level of CALU in the control sample(s) indicates that the subject has Q- Fever.

[0186] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of LYN in a sample(s) from the subject; comparing the level of LYN in the subject sample(s) with a level of LYN in a control sample(s), wherein a difference in the level of LYN in the subject sample(s) as compared to the level of LYN in the control sample(s) indicates that the subject has Q-Fever.

[0187] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of TPI1 in a sample(s) from the subject; comparing the level of TPI1 in the subject sample(s) with a level of TPI1 in a control sample(s), wherein a difference in the level of TPI1 in the subject sample(s) as compared to the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0188] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1 and the level of CALU in a sample(s) from the subject; comparing the level of B4GALT1 and the level of CALU in the subject sample(s) with a level of B4GALT1 and a level of CALU in a control sample(s), wherein a difference in the level of B4GALT1 and a difference in the level of CALU in the subject sample(s) as compared to the level of B4GALT1 and the level of CALU in the control sample(s) indicates that the subject has Q-Fever.

[0189] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of B4GALT1 and the level of LYN in a sample(s) from the subject; comparing the level of B4GALT1 and the level of LYN in the subject sample(s) with a level of B4GALT1 and a level of LYN in a control sample(s), wherein a difference in the level of B4GALT1 and a difference in the level of LYN in the subject sample(s) as compared to the level of B4GALT1 and the level of LYN in the control sample(s) indicates that the subject has Q-Fever.

[0190] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of B4GALT1 and the level of TPI1 in a sample(s) from the subject; comparing the level of B4GALT1 and the level of TPI1 in the subject sample(s) with a level of B4GALT1 and a level of TPI1 in a control sample(s), wherein a difference in the level of B4GALT1 and a difference in the level of TPI1 in the subject sample(s) as compared to the level of B4GALT1 and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0191] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1, a level of CALU, and a level of LYN in a sample(s) from the subject; comparing the level of B4GALT1, the level of CALU, and the level of LYN in the subject sample(s) with a level of B4GALT1, a level of CALU, and a level of LYN in a control sample(s), wherein a difference in the level of B4GALT1, a difference in the level of CALU, and a difference in the level of LYN in the subject sample(s) as compared to the level of B4GALT1, the level of CALU, and the level of LYN in the control sample(s) indicates that the subject has Q-Fever.

[0192] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1, a level of CALU, and a level of TPI1 in a sample(s) from the subject; comparing the level of B4GALT1, the level of CALU, and the level of TPI1 in the subject sample(s) with a level of B4GALT1, a level of CALU, and a level of TPI1 in a control sample(s), wherein a difference in the level of B4GALT1, a difference in the level of CALU, and a difference in the level of TPI1 in the subject sample(s) as compared to the level of B4GALT1, the level of CALU, and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0193] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1, a level of LYN, and a level of TPI1 in a sample(s) from the subject; comparing the level of B4GALT1, the level of LYN, and the level of TPI1 in the subject sample(s) with a level of B4GALT1, a level of LYN, and a level of TPI1 in a control sample(s), wherein a difference in the level of B4GALT1, a difference in the level of LYN, and a difference in the level of TPI1 in the subject sample(s) as compared to the level of B4GALT1, the level of LYN, and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0194] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of B4GALT1, a level of LYN, a level of CALU, and a level of TPI1 in a sample(s) from the subject; comparing the level of B4GALT1, the level of LYN, the level of CALU, and the level of TPI1 in the subject sample(s) with a level of B4GALT1, a level of LYN,, the level of CALU, and a level of TPI1 in a control sample(s), wherein a difference in the level of B4GALT1, a difference in the level of LYN,, a difference in the level of CALU, and a difference in the level of TPI1 in the subject sample(s) as compared to the level of B4GALT1, the level of LYN, the level of CALU, and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0195] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of CALU and the level of LYN in a sample(s) from the subject; comparing the level of CALU and the level of LYN in the subject sample(s) with a level of CALU and a level of LYN in a control sample(s), wherein a difference in the level of CALU and a difference in the level of LYN in the subject sample(s) as compared to the level of CALU and the level of LYN in the control sample(s) indicates that the subject has Q-Fever.

[0196] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of CALU and the level of TPI1 in a sample(s) from the subject; comparing the level of CALU and the level of TPI1 in the subject sample(s) with a level of CALU and a level of TPI1 in a control sample(s), wherein a difference in the level of CALU and a difference in the level of TPI1 in the subject sample(s) as compared to the level of CALU and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0197] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of LYN and the level of TPI1 in a sample(s) from the subject; comparing the level of LYN and the level of TPI1 in the subject sample(s) with a level of LYN and a level of TPI1 in a control sample(s), wherein a difference in the level of LYN and a difference in the level of TPI1 in the subject sample(s) as compared to the level of LYN and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0198] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining a level of CALU, a level of LYN, and a level of TPI1 in a sample(s) from the subject; comparing the level of CALU, the level of LYN, and the level of TPI1 in the subject sample(s) with a level of CALU, a level of LYN, and a level of TPI1 in a control sample(s), wherein a difference in the level of CALU, a difference in the level of LYN, and a difference in the level of TPI1 in the subject sample(s) as compared to the level of CALU, the level of LYN, and the level of TPI1 in the control sample(s) indicates that the subject has Q-Fever.

[0199] In one embodiment, the methods further comprise determining the level of one or more additional markers listed in Table 1.

[0200] In another embodiment, the methods further comprise determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENOl, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0201] In one embodiment, the level of the marker is an expression level and/or activity of the marker.

[0202] In one embodiment, the level in the subject sample(s) is determined by mass spectrometry. In one embodiment, the mass spectrometry is matrix assisted laser desorption/time of flight (MALDI/TOF) mass spectrometry, liquid chromatography quadruple ion trap electrospray (LCQ-MS), or surface enhanced laser desorption ionization/time of flight (SELDI/TOF) mass spectrometry.

[0203] In one embodiment, the level in the subject sample(s) is determined by immunoassay.

[0204] In one embodiment, the sample(s) from the subject is a fluid sample(s). In another embodiment, the sample(s) from the subject is a tissue sample(s).

[0205] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of B4GALT1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0206] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of CALU in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0207] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include eagents for determining a level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0208] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0209] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of B4GALT1 and the level of CALU in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0210] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of B4GALT1 and the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0211] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of B4GALT1 and the level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0212] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of B4GALT1, a level of CALU, and a level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0213] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of B4GALT1, a level of LYN, and a level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0214] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of B4GALT1, a level of CALU, and a level of TPI1 in a subject

sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0215] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of B4GALT1, a level of CALU, a level of LYN, and a level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0216] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of CALU and the level of LYN in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0217] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of CALU and the level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0218] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining a level of CALU, a level of LYN, and a level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0219] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever or Lyme Disease. The kits include reagents for determining the level of LYN and the level of TPI1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever or Lyme Disease.

[0220] In one embodiment, the kits further include reagents for determining the level of any one or more of the markers listed in Table 1 in a sample(s) from the subject.

[0221] In another embodiment, the kits further include reagents for determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0222] In one aspect, the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever or Lyme Disease. The methods include determining a level of HIST4H4, a level of LYN, a level of MAT2A, a level of MMP9, a level of CALU, a level of LDHB, and a level of B4GALT1 in a sample(s) from the subject; comparing the level of HIST4H4, the level of LYN, the level of MAT2A, the level of MMP9, the level of CALU, the level of LDHB, and the level of B4GALT1 in a control sample(s), wherein a higher level of HIST4H4, a higher level of LYN, a higher level of MAT2A, a higher level of MMP9, a higher level of CALU, a higher level of LDHB, and a higher level of B4GALT1 in a sample(s) from the subject as compared to the level of HIST4H4, the level of LYN, the level of MAT2A, the level of MMP9, the level of CALU, the level of LDHB, and the level of B4GALT1 in the control sample(s) indicates that the subject has Brucellosis, wherein a lower level of HIST4H4, a higher level of LYN, ahigher level of MAT2A, a lower level of MMP9, a lower level of CALU, a lower level of LDHB, and a lower level of B4GALT1 in a sample(s) from the subject as compared to the level of HIST4H4, the level of LYN, the level of MAT2A, the level of MMP9, the level of CALU, the level of LDHB, and the level of B4GALT1 in the control sample(s) indicates that the subject has Q-Fever, and wherein a lower level of HIST4H4, a higher level of LYN, a higher level of MAT2A, a higher level of MMP9, a higher level of CALU, a lower level of LDHB, and a lower level of B4GALT1 in a sample(s) from the subject as compared to the level of HIST4H4, the level of LYN, the level of MAT2A, the level of MMP9, the level of CALU, the level of LDHB, and the level of B4GALT1 in the control sample(s) indicates that the subject has Lyme Disease.

[0223] In one aspect, the present disclosure provides methods for determining whether a subject has Lyme Disease. The methods include determining the level of MMP9 in a sample(s) from the subject; comparing the level of MMP9 in the subject sample(s) with a level of MMP9 in a control sample(s), wherein a difference in the level of MMP9 in the subject sample(s) as compared to the level of MMP9 in the control sample(s) indicates that the subject has Lyme Disease.

[0224] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Lyme Disease. The methods include determining the level of MMP9 in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of MMP9 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of MMP9 in the first sample(s) with a level of MMP9 in the second sample(s), wherein a difference in the level of MMP9 in the first sample(s) as compared to the level of MMP9 in the second sample(s) indicates that the treatment is effective.

[0225] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Lyme Disease. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of MMP9 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of MMP9 in an aliquot as compared to the level and/or activity of MMP9 in a control sample, thereby identifying a compound that is useful for treating a subject having Lyme Disease.

[0226] In one aspect, the present disclosure provides methods for treating a subject having Lyme Disease. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of MMP9, thereby treating the subject.

[0227] In one aspect, the present disclosure provides methods for determining whether a subject has Lyme Disease. The methods include determining the level of MMP9 and the level of MDH1 in a sample(s) from the subject; comparing the level of MMP9 and the level of MDH1 in the subject sample(s) with a level of MMP9 and a level of MDH1 in a control sample(s), wherein a difference in the level of MMP9 and a difference in the level of MDH1 in the subject sample(s) as compared to the level of MMP9 and the level of MDH1 in the control sample(s) indicates that the subject has Lyme Disease.

[0228] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Lyme Disease. The methods include determining the level of MMP9 and the level of MDH1 in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of MMP9 and the level of MDH1 in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of MMP9 and the level of MDH1 in the first sample(s) with the level of MMP9 and the level of MDH1 in the second sample(s), wherein a difference in the level of MMP9 and a difference in the level of MDH1 in the first sample(s) as compared to the level of MMP9 and the level of MDH1 in the second sample(s) indicates that the treatment is effective.

[0229] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Lyme Disease. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of MMP9 and the level and/or activity of MDH1 in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of MMP9 and the level and/or activity of MDH1 in an aliquot as compared to the level and/or activity of MMP9 and the level and/or activity of MDH1 in a control sample, thereby identifying a compound that is useful for treating a subject having Lyme Disease.

[0230] In one aspect, the present disclosure provides methods for treating a subject having Lyme Disease. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of MMP9 and the level and/or activity of MDH1, thereby treating the subject.

[0231] In one aspect, the present disclosure provides methods for determining whether a subject has Q-Fever. The methods include determining the level of LYN and the level of LDHB in a sample(s) from the subject; comparing the level of LYN and the level of LDHB in the subject sample(s) with a level of LYN and a level of LDHB in a control sample(s), wherein a difference in the level of LYN and a difference in the level of LDHB in the subject sample(s) as compared to the level of LYN and the level of LDHB in the control sample(s) indicates that the subject has Q-Fever.

[0232] In one aspect, the present disclosure provides methods for monitoring the effectiveness of a treatment in a subject having Q-Fever. The methods include determining the level of LYN and the level of LDHB in a first sample(s) from the subject prior to the initiation of the treatment; determining the level of LYN and the level of LDHB in a second sample(s) from the subject after at least a portion of the treatment has been administered; comparing the level of LYN and the level of LDHB in the first sample(s) with the level of LYN and the level of LDHB in the second sample(s), wherein a difference in the level of LYN and a difference in the level of LDHB in the first sample(s) as compared to the level of LYN and the level of LDHB in the second sample(s) indicates that the treatment is effective.

[0233] In one aspect, the present disclosure provides methods for identifying a compound that is useful for treating a subject having Q-Fever. The methods include contacting an aliquot of a sample(s) from the subject with each member of a library of compounds; determining the effect of a member of the library of compounds on the level and/or activity of LYN and the level and/or activity of LDHB in each of the aliquots; and selecting a member of the library of compounds which modulates the level and/or the activity of LYN and the level and/or activity of LDHB in an aliquot as compared to the level and/or activity of LYN and the level and/or activity of LDHB in a control sample, thereby identifying a compound that is useful for treating a subject having Q-Fever.

[0234] In one aspect, the present disclosure provides methods for treating a subject having Q-Fever. The methods include administering to the subject an effective amount of an agent that modulates the level and/or activity of LYN and the level and/or activity of LDHB, thereby treating the subject.

[0235] In one embodiment, the methods further include determining the level of one or more additional markers listed in Table 1.

[0236] In another embodiment, the methods further include determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

[0237] In one embodiment, the level of the marker is an expression level and/or activity of the marker.

[0238] In one embodiment, the level in the subject sample(s) is determined by mass spectrometry. In one embodiment, the mass spectrometry is matrix assisted laser desorption/time of flight (MALDI/TOF) mass spectrometry, liquid chromatography quadruple ion trap electrospray (LCQ-MS), or surface enhanced laser desorption ionization/time of flight (SELDI/TOF) mass spectrometry.

[0239] In one embodiment, the level in the subject sample(s) is determined by immunoassay.

[0240] In one embodiment, the sample(s) from the subject is a fluid sample(s). In another embodiment, the sample(s) from the subject is a tissue sample(s).

[0241] In one aspect, the present disclosure provides kits for determining whether a subject has Brucellosis or Q-

Fever or Lyme Disease. The kits include reagents for determining level of HIST4H4, a level of LYN, a level of MAT2A, a level of MMP9, a level of CALU, a level of LDHB, and a level of B4GALT1 in a sample(s) from the subject and instructions for use of the kit to determine whether the subject has Brucellosis or Q-Fever or Lyme Disease.

[0242] In one aspect, the present disclosure provides kits for determining whether a subject has Lyme Disease. The kits include reagents for determining the level of MMP9 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Lyme Disease.

[0243] In one aspect, the present disclosure provides kits monitoring the effectiveness of a treatment in a subject having Lyme Disease. The kits include reagents for determining the level of MMP9 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0244] In one aspect, the present disclosure provides kits for determining whether a subject has Lyme Disease. The kits include reagents for determining the level of MMP9 and the level of MDH1 in a subject sample(s) and instructions for use of the kit to determine whether the subject has Lyme Disease.

[0245] In one aspect, the present disclosure provides kits monitoring the effectiveness of a treatment in a subject having Lyme Disease. The kits include reagents for determining the level of MMP9 and the level of MDH1 in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0246] In one aspect, the present disclosure provides kits for determining whether a subject has Q-Fever. The kits include for determining the level of LYN and the level of LDHB in a subject sample(s) and instructions for use of the kit to determine whether the subject has Q-Fever.

[0247] In one aspect, the present disclosure provides kits monitoring the effectiveness of a treatment in a subject having Q-Fever. The kits include reagents for determining the level of LYN and the level of LDHB in a subject sample(s) and instructions for use of the kit to monitor the effectiveness of the treatment.

[0248] In one embodiment, the kits further include reagents for determining the level of any one or more of the markers listed in Table 1 in a sample(s) from the subject.

[0249] In another embodiment, the kits further include reagents for determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, CORO1A, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0250]

Figure 1 depicts the preparation of host cell subcellular fractions. (A) Immunofluorescence microscopy of THP-1 cells 24 hours after infection with B. abortus 2308 (A1) or B. abortus 544 (A2). Immunofluorescence microscopy of JAr trophoblast cells (A3) and AC-1M88 trophoblast cells (A4) 24 hours after infection with B. abortus 2308. Dark gray dots represent intracellular bacteria. (B) western blot analysis of increasing amounts of starting material (homogenate; μg) compared to secretory vesicle preparations (SPV) and secretory vesicle contents (SC) of host cell lines 24 hours following Brucella infection using markers of specific subcellular compartments. Na+/K+ ATPase reactivity was used a marker of plasma membrane, BIP and B2M as soluble potentially secreted proteins, P62, GS28, TIM23 as markers of nuclear, Golgi, and mitochondrial membranes, respectively, and actin as a marker of the cytoskeleton.

Figures 2-12 are boxplots of the normalized protein intensity of exemplary biomarkers of the invention (as indicated). In each boxplot, each subject is represented by a dot. The median protein concentration is indicated by a bold line, the 50% range by filed rectanges, and the 95% range by hatched lines. Size and description of each group is indicated on the x-axis, normalized protein intensity on the y-axis.

DETAILED DESCRIPTION OF THE INVENTION

[0251] The present invention is based, at least in part, on the discovery of markers that are associated with Brucellosis. In particular, biomarkers associated with Brucellosis have been discovered, prioritized, and validated in relevant *in vitro* experimental systems. The markers were identified as being expressed, e.g., essentially specifically expressed, in samples from subjects having Brucellosis as compared to healthy control subjects, subjects having Q Fever, and subjects having Lyme Disease.

[0252] Accordingly, the present disclosure provides sensitive and facile methods and kits for determining whether a subject has Brucellosis, methods and kits for determining whether a subject has Brucellosis or Q Fever, methods and kits for determining whether a subject has Brucellosis or Lyme Disease, methods for identifying a compound that is useful for treating Brucellosis, methods and kits for monitoring the effectiveness of a therapy for resting a subject having Brucellosis, and methods for treating a subject having Brucellosis by measuring and identifying particular markers, or

particular combinations of markers.

[0253] Various aspects of the invention are described in further detail in the following subsections:

I. Definitions

[0254] As used herein, each of the following terms has the meaning associated with it in this section.

[0255] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0256] A "marker" or "biomarker" is an organic biomolecule which is differentially present in a sample taken from a subject of one phenotypic status (*e.g.*, having a disease) as compared with another phenotypic status (*e.g.*, not having the disease). A biomarker is differentially present between different phenotypic statuses if the mean or median level, *e.g.,* expression level, of the biomarker in the different groups is calculated to be statistically significant. Common tests for statistical significance include, among others, t-test, ANOVA, Kruskal-Wallis, Wilcoxon, Mann- Whitney and odds ratio. Biomarkers, alone or in combination, provide measures of relative risk that a subject belongs to one phenotypic status or another. As such, they are useful as markers for, *e.g.,* disease (prognostics and diagnostics), therapeutic effectiveness of a drug (theranostics) and of drug toxicity.

[0257] In some embodiments, the accuracy of a marker(s) useful in the compositions and methods of the present invention may be characterized by a Receiver Operating Characteristic curve ("ROC curve"). An ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic marker(s). An ROC curve shows the relationship between sensitivity and specificity. That is, an increase in sensitivity will be accompanied by a decrease in specificity. The closer the curve follows the left axis and then the top edge of the ROC space, the more accurate the marker(s). Conversely, the closer the curve comes to the 45-degree diagonal of the ROC graph, the less accurate the marker(s). The area under the ROC is a measure of a marker(s) accuracy. The accuracy of the marker(s) depends on how well the marker(s) separates the group being tested into those with and without the disease in question. An area under the curve (referred to as "AUC") of 1 represents a perfect marker(s), while an area of 0.5 represents a less useful marker(s). Thus, in some embodiments, biomarkers and methods of the present invention have an AUC greater than about 0.50, an AUC greater than about 0.60, or an AUC greater than about 0.70.

[0258] A "zoonosis" is an infectious disease that is transmitted between species (sometimes by a vector) from animals other than humans to humans or from humans to other animals, such as Brucellosis, Q-Fever, and Lyme Disease.

[0259] "Brucellosis", also also called Bang's disease, Crimean fever, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, rock fever, or undulant fever, is a worldwide zoonosis caused by infection with the bacterial genus *Brucella.* These organisms, which are small aerobic intracellular coccobacilli, localize in the reproductive organs of host animals, causing abortions and sterility. They are shed in large numbers in the animal's urine, milk, placental fluid, and other fluids. Exposure to infected animals and animal products causes Brucellosis in humans. Transmission from human to human is rare but possible. There are seven currently recognized *Brucella* species: *B melitensis, B abortus, B suis, B. canis, B. ovis Sheep, B. neotomae,* and *B. pinnipediae* and *B. cetaceae,* four of which are known to cause disease in humans (*B. abortus, B. melitensis, B. canis, B. suis). B. pinnipediae* and *B. cetaceae* are distinctive species that typically affect marine animals; however, these strains were recently described to cause disease in humans.

[0260] The most common symptoms of Brucellosis are like those associated with many other febrile diseases and may include muscular pain and sweating. The duration of the disease can vary from a few weeks to many months or even years. In the first stage of the disease, septicaemia occurs and leads to the classic triad of undulant fevers, sweating, and migratory arthralgia and myalgia.

[0261] "Q fever" is a zonnoses caused by infection with *Coxiella burnetii,* an obligate gram-negative intracellular bacterium. This organism may be found in cattle, sheep, goats and other domestic mammals, including cats and dogs. The infection results from inhalation of a spore-like small cell variant, and from contact with the milk, urine, feces, vaginal mucus, or semen of infected animals. These products contain large numbers of bacteria that become aerosolized after drying.

[0262] The most common manifestation is mild flu-like symptoms with abrupt onset of fever, malaise, profuse perspiration, severe headache, myalgia (muscle pain), joint pain, loss of appetite, upper respiratory problems, dry cough, pleuritic pain, chills, confusion and gastrointestinal symptoms, such as nausea, vomiting and diarrhea. The fever typically lasts seven to 14 days.

[0263] "Lyme Disease" is a zonnoses caused by infection with the spirochete, *Borrelia.* There are at least three species of bacteria belonging to the genus *Borrelia* that cause infection in humans: *Borrelia burgdorferi sensu stricto, Borrelia afzelii, and Borrelia garinii.* The bacteria are inoculated into the skin by a tick bite, from ticks of the genus *Ixodes.*

[0264] The manifestations of Lyme disease have been divided into 3 stages: localized, disseminated, and persistent. However, in individual patients, no rigid cutoffs exist between stages. The first 2 stages are part of the early infection, whereas persistent disease is considered late infection.Stage 3 disease may occur within 1 year of infection, not many years later.

[0265] In some instances, subjects with early localized Lyme disease develop erythema migrans, a rash characteristic of Lyme Disease. However, since many subjects do not develop erythema migrans or because erythema migrans was not recognized by the patient or correctly diagnosed by the physician, and/or the subject was unaware that they have been bitten by a tick, the main presentation of subjects having Lyme Disease is undifferentiated febrile illness.

[0266] A "level of a marker" or "the level of a biomarker" refers to an amount of a marker present in a sample being tested. A level of a marker may be either in absolute level or amount (*e.g.,* μg/ml) or a relative level or amount (*e.g.,* relative intensity of signals).

[0267] A "higher level" or an "increase in the level" of marker refers to a level of a marker in a test sample that is greater than the standard error of the assay employed to assess the level of the marker, and is preferably at least twice, and more preferably three, four, five, six, seven, eight, nine, or ten or more times the level of marker in a control sample (*e.g.,* a sample from a healthy subject, a subject having Q-Fever, a subject having Lyme Disease, and/or, the average level of the marker in several control samples).

[0268] A "lower level" or a "decrease in the level" of a marker refers to a level of the marker in a test sample that is less than the standard error of the assay employed to assess the level of the marker, and preferably at least twice, and more preferably three, four, five, six, seven, eight, nine, or ten or more times less than the level of the marker in a control sample (*e.g.,* a sample from a healthy subject, a subject having Q-Fever, a subject having Lyme Disease, and/or, the average level of the marker in several control samples).

[0269] The term "known standard level" or "control level" refers to an accepted or predetermined level of a marker which is used to compare the level of the marker in a sample derived from a subject. In one embodiment, the control level of a marker is based the level of the marker in a sample(s) from a healthy subject(s), and/or, the average level of the marker in several control samples. In another embodiment, the control level of a marker is based the level of the marker in a sample(s) from a subject(s) having Brucellosis, and/or, the average level of the marker in several control samples. In another embodiment, the control level of a marker is based the level of the marker in a sample(s) from a subject(s) having Q-Fever, and/or, the average level of the marker in several control samples. In another embodiment, the control level of a marker is based the level of the marker in a sample(s) from a subject(s) having Lyme Disease, and/or, the average level of the marker in several control samples. In another embodiment, the control level of a marker is based the level of the marker in a sample(s) from a subject(s) having Salmonellosis (*i.e.,* infection with a gram-negative motile bacilli from the genus *Salmonella*), and/or, the average level of the marker in several control samples. In one embodiment, the control level of a marker in a sample from a subject is a level of the marker previously determined in a sample(s) from the subject. In yet another embodiment, the control level of a marker is based on the level of the marker in a sample from a subject(s) prior to the administration of a therapy for Brucellosis. In another embodiment, the control level of a marker is based on the level of the marker in a sample from a subject(s) prior to the administration of a therapy for Q-Fever. In another embodiment, the control level of a marker is based on the level of the marker in a sample from a subject(s) prior to the administration of a therapy for Lyme Disease. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Brucellosis that is not contacted with a test compound. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Q-Fever that is not contacted with a test compound. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Lyme Disease that is not contacted with a test compound. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Brucellosis that is contacted with a test compound. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Q-Fever that is contacted with a test compound. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from a subject(s) having Lyme Diease that is contacted with a test compound. In one embodiment, the control level of a marker is based on the level of the marker in a sample(s) from an *in vitro* model of Brucellosis infection. In one embodiment, the control level of a marker is based on the level of the marker in a sample(s) from an animal model of Brucellosis, a cell, or a cell line derived from the animal model of Brucellosis. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from an animal model of Q-Fever, a cell, or a cell line derived from the animal model of Q-Fever. In another embodiment, the control level of a marker is based on the level of the marker in a sample(s) from an animal model of Lyme Disease, a cell, or a cell line derived from the animal model of Lyme Disease.

[0270] Alternatively, and particularly as further information becomes available as a result of routine performance of the methods described herein, population-average values for "control" level of expression of a marker may be used. In other embodiments, the "control" level of a marker may be determined by determining the level of a marker in a subject sample obtained from a subject before infection with *Brucella, Coxiella burnetii,* and/or *Burrelia* from archived subject samples, and the like.

[0271] As used herein, the terms "patient" or "subject" refer to human and non-human animals, e.g., veterinary patients. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dog, cat, horse, cow, chickens, amphibians, and reptiles. In one embodiment, the subject is a human.

[0272] The term "sample" as used herein refers to a collection of similar cells or tissue isolated from a subject, as well as tissues, cells and fluids present within a subject. The term "sample" includes any body fluid (*e.g.,* blood fluids, lymph, gynecological fluids, cystic fluid, urine, ocular fluids and fluids collected by bronchial lavage and/or peritoneal rinsing), or a cell from a subject. In one embodiment, the tissue or cell is removed from the subject. In another embodiment, the tissue or cell is present within the subject. Other subject samples, include tear drops, serum, cerebrospinal fluid, feces, sputum and cell extracts. In one embodiment the sample is a blood sample. In another embodiment, the sample is a serum sample. In one embodiment, the biological sample contains protein molecules from the test subject. In another embodiment, the biological sample may contain mRNA molecules from the test subject or genomic DNA molecules from the test subject.

[0273] The term "determining" means methods which include detecting the presence or absence of marker(s) in the sample, quantifying the amount of marker(s) in the sample, and/or qualifying the type of biomarker. Measuring can be accomplished by methods known in the art and those further described herein.

[0274] As used herein, the various forms of the term "modulate" are intended to include stimulation (*e.g.,* increasing or upregulating a particular response or activity) and inhibition (*e.g.,* decreasing or downregulating a particular response or activity).

[0275] A kit is any manufacture (*e.g.* a package or container) comprising at least one reagent, *e.g.* a probe, a primer, or an antibody, for specifically detecting a marker of the invention, the manufacture being promoted, distributed, or sold as a unit for performing the methods of the present invention. In certain embodiments, a lit may include a substrate, *e.g.,* a substrate comprising a capture reagent for one or more markers of the invention and/or a capture reagent bound to one or more markers of the invention. In some embodiments, such kits comprise instructions for determining the level of a marker(s) using mass spectrometry.

II. Markers of the Invention

[0276] The present invention is based upon the discovery of markers that are essentially specifically expressed in samples from subjects having a zoonosis, such as Brucellosis. Q-Fever, or Lyme Disease (Table 1). The markers in Table 1 have been shown to be differentially present in samples of subjects having Brucellosis and control subjects, samples from subjects having Q-Fever and control subjects, and/or samples from subjects having Lyme Disease and control subjects.

[0277] The present invention is also based upon the discovery of markers that are essentially specifically expressed in samples from subjects having Brucellosis (Table 1) and can distinguish a disorder presenting as a fever of unknown origin, *i.e.,* Brucellosis, from other diseases presenting as fever of unknown origin, such as, for example, Q-fever and/or Lyme Disease. These markers have been shown to be differentially present in samples of subjects having Brucellosis and subjects having Q-fever and/or Lyme Disease.

[0278] Accordingly, the level of any one marker or any combination of markers listed in Table 1 and found in a test sample compared to a control, or the presence or absence of one marker or combination of markers listed in Table 1 in the test sample may be used in the methods and kits of the present invention.

[0279] The markers of the invention are listed in Table 1. The nucleotide and amino acid sequences of the markers are known in the art and may be found in, for example, the GenBank Accession numbers listed in Table 1.

Table 1. Markers of the Invention.

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
SLC3A2	4F2 cell-surface antigen heavy chain	4F2_HUMAN	P08195	NP_ 001012680.1 NP_ 001012682.1 NP_ 001013269.1 NP_002385.3 NM_ 001012662.2 NM_ 001012664.2 NM_ 001013251.2 NM_002394.5
GOT1	Aspartate aminotransferase, cytoplasmic	AATC_HUMAN	P17174	NP_002070.1 NM_002079.2
GOT2	Aspartate aminotransferase, mitochondrial precursor	AATM_HUMAN	P00505	NP_002071.2 NM_002080.2
ACADVL	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor	ACADV_HUMAN	P49748	NP_000009.1 NP_ 001029031.1 NP_ 001257376.1 NM_000018.3 NM_ 001033859.2 NM_ 001270447.1

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
DBI	Acyl-CoA-binding protein	ACBP_HUMAN	P07108	NP_001073331.1 NP_001073332.1 NP_001171488.1 NP_001171512.1 NP_001171513.1 NP_001171514.1 NP_065438.1 NM_001079862.1 NM_001079863.1 NM_001178017.1 NM_001178041.1 NM_001178042.1 NM_001178043.1 NM_020548.6
ACOX1	Peroxisomal acyl-coenzyme A oxidase 1	ACOX1_HUMAN	Q15067	NP_001171968.1 NP_004026.2 NP_009223.2 NM_001185039.1 NM_004035.6 NM_007292.5
AHNAK	Neuroblast differentiation-associated protein AHNAK	AHNK_HUMAN	Q09666	NP_001611.1 NP_076965.2 NM_001620.2 NM_024060.3
AIMP1	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1	AIMP1_HUMAN	Q12904	NP_001135887.1 NP_001135888.1 NP_004748.2 NM_001142415.1 NM_001142416.1 NM_004757.3

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
AKR1B1	Aldose reductase	ALDR_HUMAN	P15121	NP_001619.1 NM_001628.2
ANPEP	Aminopeptidase N	AMPN_HUMAN	P15144	NP_001141.2 NM_001150.2
ANXA2	Annexin A2	ANXA2_HUMAN	P07355	NP_001002857.1 NP_001002858.1 NP_001129487.1 NP_004030.1 NM_001002857.1 NM_001002858.2 NM_001136015.2 NM_004039.2
ANXA5	Annexin A5	ANXA5_HUMAN	P08758	NP_001145.1 NM_001154.3
ANXA6	Annexin A6	ANXA6_HUMAN	P08133	NP_001146.2 NP_001180473.1 NM_001155.4 NM_001193544.1
AKR7A2	Aflatoxin B1 aldehyde reductase member 2	ARK72_HUMAN	043488	NP_003680.2 NM_003689.3
ARPC3	Actin-related protein 2/3 complex subunit 3	ARPC3_HUMAN	015145	NP_005710.1 NM_005719.2
ASAH1	Acid ceramidase precursor	ASAH1_HUMAN	Q13510	NP_001120977.1 NP_004306.3 NP_808592.2 NM_001127505.1 NM_004315.4 NM_177924.3
B4GALT1	Beta-1,4-galactosyltransferase 1	B4GT1_HUMAN	P15291	NP_001488.2 NM_001497.3
BCAM	Basal cell adhesion molecule precursor	BCAM_HUMAN	P50895	NP_001013275.1 NP_005572.2 NM_001013257.2 NM_005581.4

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
BLOC1S5	Biogenesis of lysosome-related organelles complex 1 subunit 5	BL1S5_HUMAN	Q8TDH9	NP_958437.1 NM_201280.2
CALU	Calumenin precursor	CALU_HUMAN	O43852	NP_001124146.1 NP_001186600.1 NP_001186601.1 NP_001186602.1 NP_001186603.1 NP_001210.1 NM_001130674.2 NM_001199671.1 NM_001199672.1 NM_001199673.1 NM_001199674.1 NM_001219.4
CAPG	Macrophage-capping protein	CAPG_HUMAN	P40121	NP_001243068.1 NP_001738.2 NM_001256139.1 NM_001747.3
CAPZB	F-actin-capping protein subunit beta	CAPZB_HUMAN	P47756	NP_001193469.1 NP_004921.1 NM_001206540.1 NM_004930.3
CAPZA2	F-actin-capping protein subunit alpha-2	CAZA2_HUMAN	P47755	NP_006127.1 NM_006136.2
CBX1	Chromobox protein homolog 1	CBX1_HUMAN	P83916	NP_001120700.1 NP_006798.1 NM_001127228.1 NM_006807.4
CDC37	Hsp90 co-chaperone Cdc37	CDC37_HUMAN	Q16543	NP_008996.1 NM_007065.3

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
HSPE1	10 kDa heat shock protein, mitochondrial	CH10_HUMAN	P61604	NP_002148.1 NM_002157.2
CHMP1A	Charged multivesicular body protein 1a	CHM1A_HUMAN	Q9HD42	NP_002759.2 NM_002768.3
CHMP1B	Charged multivesicular body protein 1b	CHM1B_HUMAN	Q7LBR1	NP_065145.2 NM_020412.4
CHMP2A	Charged multivesicular body protein 2a	CHM2A_HUMAN	043633	NP_055268.1 NP_940818.1 NM_014453.2 NM_198426.1
CHMP4A	Charged multivesicular body protein 4a	CHM4A_HUMAN	Q9BY43	NP_054888.2 NM_014169.3
CHMP4B	Charged multivesicular body protein 4b	CHM4B_HUMAN	Q9H444	NP_789782.1 NM_176812.4
CHMP5	Charged multivesicular body protein 5	CHMP5_HUMAN	Q9NZZ3	NP_ 001182465.1 NP_057494.3 NM_ 001195536.1 NM_016410.5
CLIC1	Chloride intracellular channel protein 1	CLIC1_HUMAN	000299	NP_001279.2 NM_001288.4
CNDP2	Cytosolic nonspecific dipeptidase	CNDP2_HUMAN	Q96KP4	NP_ 001161971.1 NP_060705.2 NM_ 001168499.1 NM_018235.2
CNPY2	Protein canopy homolog 2 precursor	CNPY2_HUMAN	Q9Y2B0	NP_ 001177920.1 NP_055070.1 NM_ 001190991.1 NM_014255.5
COPA	Coatomer subunit alpha	COPA_HUMAN	P53621	NP_ 001091868.1 NP_004362.2 NM_ 001098398.1 NM_004371.3
COPB2	Coatomer subunit beta’	COPB2_HUMAN	P35606	NP_004757.1 NM_004766.2

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
CORO1A	Coronin-1A	COR1A_HUMAN	P31146	NP_001180262.1 NP_009005.1 NM_001193333.2 NM_007074.3
CORO1B	Coronin-1B	COR1B_HUMAN	Q9BR76	NP_001018080.1 NP_065174.1 NM_001018070.2 NM_020441.2
CORO1C	Coronin-1C	COR1C_HUMAN	Q9ULV4	NP_055140.1 NM_014325.2
CPVL	Probable serine carboxypeptidase CPVL precursor	CPVL_HUMAN	Q9H3G5	NP_061902.2 NP_112601.3 NM_019029.2 NM_031311.3
C19orf10	UPF0556 protein C19orf10 precursor	CS010_HUMAN	Q969H8	NP_061980.1 NM_019107.3
CXorf26	UPF0368 protein Cxorf26	CX026_HUMAN	Q9BVG4	NP_057584.2 NM_016500.3
CXCL10	C-X-C motif chemokine 10 precursor	CXL10_HUMAN	P02778	NP_001556.2 NM_001565.3
CACYBP	Calcyclin-binding protein	CYBP_HUMAN	Q9HB71	NP_001007215.1 NP_055227.1 NM_001007214.1 NM_014412.2
DSP	Desmoplakin	DESP_HUMAN	P15924	NP_001008844.1 NP_004406.2 NM_001008844.1 NM_004415.2
HSD17B4	Peroxisomal multifunctional enzyme type 2	DHB4_HUMAN	P51659	NP_000405.1 NP_001186220.1 NP_001186221.1 NM_000414.3 NM_001199291.1 NM_001199292.1

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
DLG1	Disks large homolog 1	DLG1_HUMAN	Q12959	NP_ 001091894.1 NP_ 001191315.1 NP_ 001191316.1 NP_ 001191317.1 NP_004078.2 NM_ 001098424.1 NM_ 001204386.1 NM_ 001204387.1 NM_ 001204388.1 NM_004087.2
DNASE2	Deoxyribonuclea se-2-alpha precursor	DNS2A_HUMAN	000115	NP_001366.1 NM_001375.2
DDT	D-dopachrome decarboxylase	DOPD_HUMAN	P30046	NP_ 001077861.1 NP_001346.1 NM_ 001084392.1 NM_001355.3
DYNLL1	Dynein light chain 1, cytoplasmic	DYL1_HUMAN	P63167	NP_ 001032583.1 NP_ 001032584.1 NP_003737.1 NM_ 001037494.1 NM_ 001037495.1 NM_003746.2
EPB41L3	Band 4.1-like protein 3	E41L3_HUMAN	Q9Y2J2	NP_036439.2 NM_012307.2
EEF1A1	Elongation factor 1-alpha 1	EF1A1_HUMAN	P68104	NP_001393.1 NM_001402.5
EEF1B2	Elongation factor 1-beta	EF1B_HUMAN	P24534	NP_ 001032752.1 NP_001950.1 NP_066944.1 NM_ 001037663.1 NM_001959.3 NM_021121.3

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
EEF1D	Elongation factor 1-delta	EF1D_HUMAN	P29692	NP_ 001123525.2 NP_ 001123527.1 NP_ 001123528.1 NP_ 001123529.1 NP_001951.2 NP_115754.3 NM_ 001130053.2 NM_ 001130055.2 NM_ 001130056.2 NM_ 001130057.2 NM_001960.4 NM_032378.4
EEF2	Elongation factor 2	EF2_HUMAN	P13639	NP_001952.1 NM_001961.3
ENG	Endoglin precursor	EGLN_HUMAN	P17813	NP_000109.1 NP_ 001108225.1 NM_000118.2 NM_ 001114753.1
EHD1	EH domain-containing protein 1	EHD1_HUMAN	Q9H4M9	NP_006786.2 NM_006795.2
ELAVL1	ELAV-like protein 1	ELAV1_HUMAN	Q15717	NP_001410.2 NM_001419.2
EMC2	ER membrane protein complex subunit 2	EMC2_HUMAN	Q15006	NP_055488.1 NM_014673.3
EMR2	EGF-like module-containing mucin-like hormone receptor-like 2 precursor	EMR2_HUMAN	Q9UHX3	NP_038475.2 NM_013447.3
ENO1	Alpha-enolase	ENOA_HUMAN	P06733	NP_ 001188412.1 NP_001419.1 NM_ 001201483.1 NM_001428.3

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
ENO3	Beta-enolase	ENOB_HUMAN	P13929	NP_ 001180432.1 NP_001967.3 NP_443739.3 NM_ 001193503.1 NM_001976.4 NM_053013.3
HSP90B1	Endoplasmin precursor	ENPL_HUMAN	P14625	NP_003290.1 NM_003299.2
ERO1L	ERO1-like protein alpha precursor	ERO1A_HUMAN	Q96HE7	NP_055399.1 NM_014584.1
ESYT2	Extended synaptotagmin-2	ESYT2_HUMAN	A0FGR8	NP_065779.1 NM_020728.2
EVL	Ena/VASP-like protein	EVL_HUMAN	Q9UI08	NP_057421.1 NM_016337.2
ST13	Hsc70-interacting protein	F10A1_HUMAN	P50502	NP_003923.2 NM_003932.3
F5	Coagulation factor V precursor	FA5_HUMAN	P12259	NP_000121.2 NM_000130.4
FKBP4	Peptidyl-prolyl cis-trans isomerase FKBP4	FKBP4_HUMAN	Q02790	NP_002005.1 NM_002014.3
FLNA	Filamin-A	FLNA_HUMAN	P21333	NP_ 001104026.1 NP_001447.2 NM_ 001110556.1 NM_001456.3
FH	Fumarate hydratase, mitochondrial precursor	FUMH_HUMAN	P07954	NP_000134.2 NM_000143.3
H6PD	GDH/6PGL endoplasmic bifunctional protein precursor	G6PE_HUMAN	O95479	NP_004276.2 NM_004285.3
GPI	Glucose-6-phosphate isomerase	G6PI_HUMAN	P06744	NP_000166.2 NP_ 001171651.1 NM_000175.3 NM_ 001184722.1

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
GDI2	Rab GDP dissociation inhibitor beta	GDIB_HUMAN	P50395	NP_ 001108628.1 NP_001485.2 NM_ 001115156.1 NM_001494.3
ARHGDIA	Rho GDP-dissociation inhibitor 1	GDIR1_HUMAN	P52565	NP_ 001172006.1 NP_ 001172007.1 NP_ _004300.1 NM_ 001185077.1 NM_ 001185078.1 NM_004309.4
GGH	Gamma-glutamyl hydrolase precursor	GGH_HUMAN	Q92820	NP_ 003869.1 NM_003878.2
GBA	Glucosylceramidase precursor	GLCM_HUMAN	P04062	NP_000148.2 NP_ 001005741.1 NP_ 001005742.1 NM_000157.3
				NM_ 001005741.2 NM_ 001005742.2
PRKCSH	Glucosidase 2 subunit beta precursor	GLU2B_HUMAN	P14314	NP_ 001001329.1 NP_002734.2 NM_ 001001329.1 NM_002743.2
GNS	N-acetylglucosamine-6-sulfatase precursor	GNS_HUMAN	P15586	NP_002067.1 NM_002076.3
HSPA9	Stress-70 protein, mitochondrial precursor	GRP75_HUMAN	P38646	NP_004125.3 NM_004134.6
HSPA5	78 kDa glucose-regulated protein precursor	GRP78_HUMAN	P11021	NP_005338.1 NM_005347.4
GSTP1	Glutathione S-transferase P	GSTP1_HUMAN	P09211	NP_000843.1 NM_000852.3
GLT25D1	Procollagen galactosyltransferase 1 precursor	GT251_HUMAN	Q8NBJ5	NP_078932.2 NM_024656.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
HIST1H1D	Histone H1.3	H13_HUMAN	P16402	NP_005311.1 NM_005320.2
HIST1H1B	Histone H1.5	H15_HUMAN	P16401	NP_005313.1 NM_005322.2
HIST3H2A	Histone H2A type 3	H2A3_HUMAN	Q7L7L0	NP_254280.1 NM_033445.2
H2AFY	Core histone macro-H2A.1	H2AY_HUMAN	O75367	NP_ 001035248.1 NP_004884.1 NP_613075.1 NP_613258.2 NM_ 001040158.1 NM_004893.2 NM_138609.2 NM_138610.2
HIST2H2BE	Histone H2B type 2-E	H2B2E_HUMAN	Q16778	NP_003519.1 NM_003528.2
HIST2H2BF	Histone H2B type 2-F	H2B2F_HUMAN	Q5QNW6	NP_ 001019770.1 NP_ 001154806.1 NM_ 001024599.3 NM_ 001161334.1
HIST3H3	Histone H3.1t	H31T_HUMAN	Q16695	NP_003484.1 NM_003493.2
HIST1H4A	Histone H4	H4_HUMAN	P62805	NP_ 001029249.1 NP_003486.1 NP_003529.1 NP_003530.1 NP_003531.1 NP_003532.1 NP_003533.1 NP_003534.1 NP_003535.1 NP_003536.1 NP_003537.1 NP_003539.1 NP_068803.1 NP_778224.1 NM_ 001034077.4 NM_003495.2 NM_003538.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
				NM_003539.3 NM_003540.3 NM_003541.2 NM_003542.3 NM_003543.3 NM_003544.2 NM_003545.3 NM_003546.2 NM_003548.2 NM_021968.3 NM_175054.2
HSD17B10	3-hydroxyacyl-CoA dehydrogenase type-2	HCD2_HUMAN	Q99714	NP_001032900.1 NP_004484.1 NM_001037811.2 NM_004493.2
HEXA	Beta-hexosaminidase subunit alpha precursor	HEXA_HUMAN	P06865	NP_000511.2 NM_000520.4
HMGB3	High mobility group protein B3	HMGB3_HUMAN	O15347	NP_005333.2 NM_005342.2
HNRNPC	Heterogeneous nuclear ribonucleoproteins C1/C2	HNRPC_HUMAN	P07910	NP_001070910.1 NP_001070911.1 NP_004491.2 NP_112604.2 NM_001077442.1 NM_001077443.1 NM_004500.3 NM_031314.2
HNRNPD	Heterogeneous nuclear ribonucleoprotein D0	HNRPD_HUMAN	Q14103	NP_001003810.1 NP_002129.2 NP_112737.1 NP_112738.1 NM_001003810.1 NM_002138.3 NM_031369.2 NM_031370.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
HNRNPK	Heterogeneous nuclear ribonucleoprotein K	HNRPK_HUMAN	P61978	NP_002131.2 NP_112552.1 NP_112553.1 NM_002140.3 NM_031262.2 NM_031263.2
HNRNPR	Heterogeneous nuclear ribonucleoprotein R	HNRPR_HUMAN	O43390	NP_ 001095867.1 NP_ 001095868.1 NP_005817.1 NM_ 001102397.1 NM_ 001102398.1 NM_005826.3
HNRNPU	Heterogeneous nuclear ribonucleoprotein U	HNRPU_HUMAN	Q00839	NP_004492.2 NP_114032.2 NM_004501.3 NM_031844.2
HSP90AA1	Heat shock protein HSP 90-alpha	HS90A_HUMAN	P07900	NP_ 001017963.2 NP_005339.3 NM_ 001017963.2 NM_005348.3
HSP90AB1	Heat shock protein HSP 90-beta	HS90B_HUMAN	P08238	NP_031381.2 NM_007355.2
HSPA1A	Heat shock 70 kDa protein 1A/1B	HSP71_HUMAN	P08107	NP_005336.3 NP_005337.2 NM_005345.5 NM_005346.4
HSPA8	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	P11142	NP_006588.1 NP_694881.1 NM_006597.4 NM_153201.2
ICAM1	Intercellular adhesion molecule 1 precursor	ICAM1_HUMAN	P05362	NP_000192.2 NM_000201.2
EIF5A2	Eukaryotic translation initiation factor 5A-2	IF5A2_HUMAN	Q9GZV4	NP_065123.1 NM_020390.5
IL1B	Interleukin-1 beta precursor	IL1B_HUMAN	P01584	NP _000567.1 NM_000576.2
IMPDH2	Inosine-5'-monophosphate dehydrogenase 2	IMDH2_HUMAN	P12268	NP_000875.2 NM_000884.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
ISYNA1	Inositol-3-phosphate synthase 1	INO1_HUMAN	Q9NPH2	NP_001164409.1 NP_001240318.1 NP_057452.1 NM_001170938.1 NM_001253389.1 NM_016368.4
ISOC1	Isochorismatase domain-containing protein 1	ISOC1_HUMAN	Q96CN7	NP_057132.2 NM_016048.2
ITGA5	Integrin alpha-5 precursor	ITA5_HUMAN	P08648	NP_002196.2 NM_002205.2
ITGAM	Integrin alpha-M precursor	ITAM_HUMAN	P11215	NP_000623.2 NP_001139280.1 NM_000632.3 NM_001145808.1
ITPR1	Inositol 1,4,5-trisphosphate receptor type 1	ITPR1_HUMAN	Q14643	NP_001093422.2 NP_002213.5 NM_001099952.2 NM_002222.5
KRT9	Keratin, type I cytoskeletal 9	K1C9_HUMAN	P35527	NP_000217.2 NM_000226.3
KRT7	Keratin, type II cytoskeletal 7	K2C7_HUMAN	P08729	NP_005547.3 NM_005556.3
KRT8	Keratin, type II cytoskeletal 8	K2C8_HUMAN	P05787	NP_001243222.1 NP_002264.1 NM_001256293.1 NM_002273.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
CAMK2D	Calcium/calmod ulin-dependent protein kinase type II subunit delta	KCC2D_HUMAN	Q13557	NP_001212.2 NP_742112.1 NP_742113.1 NP_742125.1 NP_742126.1 NP_742127.1 NM_001221.3 NM_172114.1 NM_172115.2 NM_172127.2 NM_172128.2 NM_172129.1
PKM	Pyruvate kinase	KPYM_HUMAN	P14618	NP_ 001193725.1 NP_ 001193727.1 NP_ 001193728.1 NP_002645.3 NP_872270.1 NP_872271.1 NM_ 001206796.1 NM_ 001206797.1 NM_ 001206798.1 NM_ 001206799.1 NM_002654.4 NM_182470.2 NM_182471.2
LASP1	LIM and SH3 domain protein 1	LASP1_HUMAN	Q14847	NP_ 001258537.1 NP_006139.1 NM_ 001271608.1 NM_006148.3
LDHB	L-lactate dehydrogenase B chain	LDHB_HUMAN	P07195	NP_ 001167568.1 NP_002291.1 NM_ 001174097.1 NM_002300.6

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
LIMA1	LIM domain and actin-binding protein 1	LIMA1_HUMAN	Q9UHB6	NP_001107018.1 NP_001107019.1 NP_001230704.1 NP_057441.1 NM_001113547.1 NM_001243775.1 NM_016357.4
LMNA	Prelamin-A/C precursor	LMNA_HUMAN	P02545	NP_005563.1 NP_733821.1 NP_733822.1 NM_005572.3 NM_170707.3 NM_170708.3
LMNB1	Lamin-B1 precursor	LMNB1_HUMAN	P20700	NP_005564.1 NM_005573.3
LMNB2	Lamin-B2 precursor	LMNB2_HUMAN	Q03252	NP_116126.3 NM_032737.3
LRCH1	Leucine-rich repeat and calponin homology domain-containing protein 1	LRCH1_HUMAN	Q9Y2L9	NP_001157683.1 NP_001157685.1 NP_055931.1 NM_001164211.1 NM_001164213.1 NM_015116.2
GAA	Lysosomal alpha-glucosidase precursor	LYAG_HUMAN	P10253	NP_000143.2 NP_001073271.1 NP_001073272.1 NM_000152.3 NM_001079803.1 NM_001079804.1
LYN	Tyrosine-protein kinase Lyn	LYN_HUMAN	P07948	NP_001104567.1 NP_002341.1 NM_001111097.2 NM_002350.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
MDH1	Malate dehydrogenase, cytoplasmic	MDHC_HUMAN	P40925	NP_ 001186040.1 NP_ 001186041.1 NP_005908.1 NM_ 001199111.1 NM_ 001199112.1 NM_005917.3
MDH2	Malate dehydrogenase, mitochondrial precursor	MDHM_HUMAN	P40926	NP_005909.2 NM_005918.2
MESDC2	LDLR chaperone MESD precursor	MESD_HUMAN	Q14696	NP_055969.1 NM_015154.1
MAT2A	S-adenosylmethionine synthase isoform type-2	METK2_HUMAN	P31153	NP_005902.1 NM_005911.5
MYL12A	Myosin regulatory light chain 12A	ML12A_HUMAN	P19105	NP_006462.1 NM_006471.2
MMP9	Matrix metalloproteinase-9 precursor	MMP9_HUMAN	P14780	NP_004985.2 NM_004994.2
RABIF	Guanine nucleotide exchange factor MSS4	MSS4_HUMAN	P47224	NP_002862.2 NM_002871.4
MYH10	Myosin-10	MYH10_HUMAN	P35580	NP_ 001242941.1 NP_005955.3 NM_ 001256012.1 NM_005964.3
MYH9	Myosin-9	MYH9_HUMAN	P35579	NP_002464.1 NM_002473.4
MYO1E	Unconventional myosin-Ie	MYO1E_HUMAN	Q12965	NP_004989.2 NM_004998.3
MYO6	Unconventional myosin-VI	MYO6_HUMAN	Q9UM54	NP_004990.3 NM_004999.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PPP1R12A	Protein phosphatase 1 regulatory subunit 12A	MYPT1_HUMAN	O14974	NP_ 001137357.1 NP_ 001137358.1 NP_ 001231919.1 NP_ 001231921.1 NP_002471.1 NM_ 001143885.1 NM_ 001143886.1 NM_ 001244990.1 NM_ 001244992.1 NM_002480.2
NAGA	Alpha-N-acetylgalactosaminidase precursor	NAGAB_HUMAN	P17050	NP_000253.1 NM_000262.2
POR	NADPH-cytochrome P450 reductase	NCPR_HUMAN	P16435	NP_000932.3 NM_000941.2
NME1	Nucleoside diphosphate kinase A	NDKA_HUMAN	P15531	NP_000260.1 NP_937818.1 NM_000269.2 NM_198175.1
NME2	Nucleoside diphosphate kinase B	NDKB_HUMAN	P22392	NP_ 001018146.1 NP_ 001018147.1 NP_ 001018148.1 NP_ 001018149.1 NP_002503.1 NM_ 001018136.2 NM_ 001018137.2 NM_ 001018138.1 NM_ 001018139.2 NM_002512.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
NDRG1	Protein NDRG1	NDRG1_HUMAN	Q92597	NP_ 001128714.1 NP_ 001245361.1 NP_ 001245362.1 NP_006087.2 NM_ 001135242.1 NM_ 001258432.1 NM_ 001258433.1 NM_006096.3
NDUFA2	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2	NDUA2_HUMAN	O43678	NP_002479.1 NM_002488.4
NAP1L1	Nucleosome assembly protein 1-like 1 precursor	NP1L1_HUMAN	P55209	NP_004528.1 NP_631946.1 NM_004537.4 NM_139207.2
NPM1	Nucleophosmin	NPM_HUMAN	P06748	NP_ 001032827.1 NP_002511.1 NP_954654.1 NM_ 001037738.2 NM_002520.6 NM_199185.3
NUTF2	Nuclear transport factor 2	NTF2_HUMAN	P61970	NP_005787.1 NM_005796.1
NCL	Nucleolin	NUCL_HUMAN	P19338	NP_005372.2 NM_005381.2
NUDC	Nuclear migration protein nudC	NUDC_HUMAN	Q9Y266	NP_006591.1 NM_006600.3
OAS2	2'-5'-oligoadenylate synthase 2	OAS2_HUMAN	P29728	NP_ 001027903.1 NP_002526.2 NP_058197.2 NM_ 001032731.1 NM_002535.2 NM_016817.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PAFAH1B2	Platelet-activating factor acetylhydrolase IB subunit beta	PA1B2_HUMAN	P68402	NP_001171675.1 NP_001171676.1 NP_001171677.1 NP_002563.1 NM_001184746.1 NM_001184747.1 NM_001184748.1 NM_002572.3
PA2G4	Proliferation-associated protein 2G4	PA2G4_HUMAN	Q9UQ80	NP_006182.2 NM_006191.2
PARK7	Protein DJ-1 precursor	PARK7_HUMAN	Q99497	NP_001116849.1 NP_009193.2 NM_001123377.1 NM_007262.4
PARP1	Poly [ADP-ribose] polymerase 1	PARP1_HUMAN	P09874	NP_001609.2 NM_001618.3
PRCP	Lysosomal Pro-X carboxypeptidase precursor	PCP_HUMAN	P42785	NP_005031.1 NP_955450.2 NM_005040.2 NM_199418.2
PDCD61P	Programmed cell death 6-interacting protein	PDC6I_HUMAN	Q8WUM4	NP_001155901.1 NP_037506.2 NM_001162429.2 NM_013374.5
P4HB	Protein disulfide-isomerase precursor	PDIA1_HUMAN	P07237	NP_000909.2 NM_000918.3
PDIA5	Protein disulfide-isomerase A5 precursor	PDIA5_HUMAN	Q14554	NP_006801.1 NM_006810.3
PEBP1	Phosphatidylethanolamine-binding protein 1	PEBP1_HUMAN	P30086	NP_002558.1 NM_002567.2
PECAM1	Platelet endothelial cell adhesion molecule precursor	PECA1_HUMAN	P16284	NP_000433.4 NM_000442.4
PFDN1	Prefoldin subunit 1	PFD1_HUMAN	O60925	NP_002613.2 NM_002622.4
PFDN2	Prefoldin subunit 2	PFD2_HUMAN	Q9UHV9	NP_036526.2 NM_012394.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PGAM1	Phosphoglycerate mutase 1	PGAM1_HUMAN	P18669	NP_002620.1 NM_002629.2
PGK1	Phosphoglycerate kinase 1	PGK1_HUMAN	P00558	NP_000282.1 NM_000291.3
PIP4K2A	Phosphatidylino sitol 5-phosphate 4-kinase type-2 alpha	PI42A_HUMAN	P48426	NP_005019.2 NM_005028.4
PIP5K1A	Phosphatidylino sitol 4-phosphate 5-kinase type-1 alpha	PI51A_HUMAN	Q99755	NP_001129108.1 NP_001129109.1 NP_001129110.1 NP_003548.1 NM_001135636.1 NM_001135637.1 NM_001135638.1 NM_003557.2
PLEKHO2	Pleckstrin homology domain-containing family O member 2	PKHO2_HUMAN	Q8TD55	NP_001181988.1 NP_079477.2 NM_001195059.1 NM_025201.4
PLEC	Plectin	PLEC_HUMAN	Q15149	NP_000436.2 NP_958780.1 NP_958781.1 NP_958782.1 NP_958783.1 NP_958784.1 NP_958785.1 NP_958786.1 NM_000445.3 NM_201378.2 NM_201379.1 NM_201380.2 NM_201381.1 NM_201382.2 NM_201383.1 NM_201384.1
PLOD3	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3 precursor	PLOD3_HUMAN	O60568	NP_001075.1 NM_001084.4
LCP1	Plastin-2	PLSL_HUMAN	P13796	NP_002289.2 NM_002298.4

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PNP	Purine nucleoside phosphorylase	PNPH_HUMAN	P00491	NP_000261.2 NM_000270.3
ALPP	Alkaline phosphatase, placental type precursor	PPB1_HUMAN	P05187	NP_001623.3 NM_001632.3
PPIA	Peptidyl-prolyl cis-trans isomerase A	PPIA_HUMAN	P62937	NP_066953.1 NM_021130.3
PPIB	Peptidyl-prolyl cis-trans isomerase B precursor	PPIB_HUMAN	P23284	NP_000933.1 NM_000942.4
PRDX1	Peroxiredoxin-1	PRDX1_HUMAN	Q06830	NP_ 001189360.1 NP_002565.1 NP_859047.1 NP_859048.1 NM_ 001202431.1 NM_002574.3 NM_181696.2 NM_181697.2
PRDX2	Peroxiredoxin-2	PRDX2_HUMAN	P32119	NP_005800.3 NP_859428.1 NM_005809.4 NM_181738.1
PRDX3	Thioredoxin-dependent peroxide reductase, mitochondrial precursor	PRDX3_HUMAN	P30048	NP_006784.1 NP_054817.2 NM_006793.2 NM_014098.2
PRDX6	Peroxiredoxin-6	PRDX6_HUMAN	P30041	NP_004896.1 NM_004905.2
PRPF19	Pre-mRNA-processing factor 19	PRP19_HUMAN	Q9UMS4	NP_055317.1 NM_014502.4
PSMC6	26S protease regulatory subunit 10B	PRS10_HUMAN	P62333	NP_002797.3 NM_002806.3
PSMC3	26S protease regulatory subunit 6A	PRS6A_HUMAN	P17980	NP_002795.2 NM_002804.4
PSMC2	26S protease regulatory subunit 7	PRS7_HUMAN	P35998	NP_ 001191382.1 NP_002794.1 NM_ 001204453.1 NM_002803.3
PRSS8	Prostasin precursor	PRSS8_HUMAN	Q16651	NP_002764.1 NM_002773.3
NPEPPS	Puromycin-sensitive aminopeptidase	PSA_HUMAN	P55786	NP_006301.3 NM_006310.3

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PSMA7	Proteasome subunit alpha type-7	PSA7_HUMAN	O14818	NP_002783.1 NM_002792.3
PSMB5	Proteasome subunit beta type-5 precursor	PSB5_HUMAN	P28074	NP_ 001124197.1 NP_ 001138404.1 NP_002788.1 NM_ 001130725.1 NM_ 001144932.1 NM_002797.3
PSMD10	26S proteasome non-ATPase regulatory subunit 10	PSD10_HUMAN	O75832	NP_002805.1 NP_736606.1 NM_002814.3 NM_170750.2
PSMD11	26S proteasome non-ATPase regulatory subunit 11	PSD11_HUMAN	O00231	NP_ 001257411.1 NP_002806.2 NM_ 001270482.1 NM_002815.3
PSMD14	26S proteasome non-ATPase regulatory subunit 14	PSDE_HUMAN	O00487	NP_005796.1 NM_005805.5
PSMD1	26S proteasome non-ATPase regulatory subunit 1	PSMD1_HUMAN	Q99460	NP_ 001177966.1 NP_002798.2 NM_ 001191037.1 NM_002807.3
PSMD2	26S proteasome non-ATPase regulatory subunit 2	PSMD2_HUMAN	Q13200	NP_002799.3 NM_002808.3
PSMD4	26S proteasome non-ATPase regulatory subunit 4	PSMD4_HUMAN	P55036	NP_002801.1 NM_002810.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
PTBP3	Polypyrimidine tract-binding protein 3	PTBP3_HUMAN	O95758	NP_ 001157260.1 NP_ 001157262.1 NP_ 001231826.1 NP_ 001231827.1 NP_005147.3 NM_ 001163788.2 NM_ 001163790.2 NM_ 001244897.1 NM_ 001244898.1 NM_005156.6
PAICS	Multifunctional protein ADE2	PUR6_HUMAN	P22234	NP_ 001072992.1 NP_ 001072993.1 NP_006443.1 NM_ 001079524.1 NM_ 001079525.1 NM_006452.3
ATIC	Bifunctional purine biosynthesis protein PURH	PUR9_HUMAN	P31939	NP_004035.2 NM_004044.6
PXMP2	Peroxisomal membrane protein 2	PXMP2_HUMAN	Q9NR77	NP_061133.1 NM_018663.1
RAB6A	Ras-related protein Rab-6A	RAB6A_HUMAN	P20340	NP_002860.2 NP_942599.1 NM_002869.4 NM_198896.1
RANGAP1	Ran GTPase-activating protein 1	RAGP1_HUMAN	P46060	NP_002874.1 NM_002883.2

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Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
RAI14	Ankycorbin	RAI14_HUMAN	Q9P0K7	NP_001138992.1 NP_001138993.1 NP_001138994.1 NP_001138995.1 NP_001138997.1 NP_056392.2 NM_001145520.1 NM_001145521.1 NM_001145522.1 NM_001145523.1 NM_001145525.1 NM_015577.2
RAN	GTP-binding nuclear protein Ran	RAN_HUMAN	P62826	NP_006316.1 NM_006325.3
RANBP1	Ran-specific GTPase-activating protein	RANG_HUMAN	P43487	NP_002873.1 NM_002882.2
RBBP4	Histone-binding protein RBBP4	RBBP4_HUMAN	Q09028	NP_001128727.1 NP_001128728.1 NP_005601.1 NM_001135255.1 NM_001135256.1 NM_005610.2
RAD23B	UV excision repair protein RAD23 homolog B	RD23B_HUMAN	P54727	NP_002865.1 NM_002874.4
RPL14	60S ribosomal protein L14	RL14_HUMAN	P50914	NP_001030168.1 NP_003964.3 NM_001034996.2 NM_003973.4
RPL23A	60S ribosomal protein L23a	RL23A_HUMAN	P62750	NP_000975.2 NM_000984.5

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
RPL24	60S ribosomal protein L24	RL24_HUMAN	P83731	NP _000977.1 NM_000986.3
RPL26	60S ribosomal protein L26	RL26_HUMAN	P61254	NP _000978.1 NM_000987.3
RPL29	60S ribosomal protein L29	RL29_HUMAN	P47914	NP_000983.1 NM_000992.2
RPL3	60S ribosomal protein L3	RL3_HUMAN	P39023	NP_000958.1 NM_000967.3
RPL31	60S ribosomal protein L31	RL31_HUMAN	P62899	NP_000984.1 NP_ 001092047.1 NP _001093163.1 NM_000993.4 NM_ 001098577.2 NM_ 001099693.1
RPL7A	60S ribosomal protein L7a	RL7A_HUMAN	P62424	NP _000963.1 NM_000972.2
RPL8	60S ribosomal protein L8	RL8_HUMAN	P62917	NP _000964.1 NP_150644.1 NM_000973.3 NM_033301.1
RPLP2	60S acidic ribosomal protein P2	RLA2_HUMAN	P05387	NP_000995.1 NM_001004.3
MRPL39	39S ribosomal protein L39, mitochondrial	RM39_HUMAN	Q9NYK5	NP_059142.2 NP_542984.2 NM_017446.3 NM_080794.3
HNRNPA1	Heterogeneous nuclear ribonucleoprotei n A1	ROA1_HUMAN	P09651	NP_002127.1 NP_112420.1 NM_002136.2 NM_031157.2
HNRNPA2B 1	Heterogeneous nuclear ribonucleoprotei ns A2/B1	ROA2_HUMAN	P22626	NP_002128.1 NP_112533.1 NM_002137.3 NM_031243.2
RPS23	40S ribosomal protein S23	RS23_HUMAN	P62266	NP_001016.1 NM_001025.4
RPS8	40S ribosomal protein S8	RS8_HUMAN	P62241	NP_001003.1 NM_001012.1
RSAD2	Radical S-adenosyl methionine domain-containing protein 2	RSAD2_HUMAN	Q8WXG1	NP_542388.2 NM_080657.4

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
RPSA	40S ribosomal protein SA	RSSA_HUMAN	P08865	NP_001012321.1 NP_002286.2 NM_001012321.1 NM_002295.4
RUVBL1	RuvB-like 1	RUVB1_HUMAN	Q9Y265	NP_003698.1 NM_003707.2
RUVBL2	RuvB-like 2	RUVB2_HUMAN	Q9Y230	NP_006657.1 NM_006666.1
S100P	Protein S100-P	S100P_HUMAN	P25815	NP_005971.1 NM_005980.2
S100A6	Protein S100-A6	S10A6_HUMAN	P06703	NP_055439.1 NM_014624.3
S100A11	Protein S100-A11	S10AB_HUMAN	P31949	NP_005611.1 NM_005620.1
SEC61B	Protein transport protein Sec61 subunit beta	SC61B_HUMAN	P60468	NP_006799.1 NM_006808.2
SEPT11	Septin-11	SEP11_HUMAN	Q9NVA2	NP_060713.1 NM_018243.2
SEPT2	Septin-2	SEPT2_HUMAN	Q15019	NP_001008491.1 NP_001008492.1 NP_004395.1 NP_006146.1 NM_001008491.1 NM_001008492.1 NM_004404.3 NM_006155.1
SEPT6	Septin-6	SEPT6_HUMAN	Q14141	NP_055944.2 NP_665798.1 NP_665799.1 NP_665801.1 NM_015129.5 NM_145799.3 NM_145800.3 NM_145802.3
SEPT7	Septin-7	SEPT7_HUMAN	Q16181	NP_001779.3 NM_001788.5

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
SEPT9	Septin-9	SEPT9_HUMAN	Q9UHD8	NP_001106963.1 NP_001106964.1 NP_001106965.1 NP_001106966.1 NP_001106967.1 NP_001106968.1 NP_006631.2 NM_001113491.1 NM_001113492.1 NM_001113493.1 NM_001113494.1 NM_001113495.1 NM_001113496.1 NM_006640.4
PHGDH	D-3-phosphoglycerate dehydrogenase	SERA_HUMAN	O43175	NP_006614.2 NM_006623.3
SF3B3	Splicing factor 3B subunit 3	SF3B3_HUMAN	Q15393	NP_036558.3 NM_012426.4
SFPQ	Splicing factor, proline- and glutamine-rich	SFPQ_HUMAN	P23246	NP_005057.1 NM_005066.2
SIAE	Sialate O-acetylesterase precursor	SIAE_HUMAN	Q9HAT2	NP_001186851.1 NP_733746.1 NM_001199922.1 NM_170601.4
SNRPD3	Small nuclear ribonucleoprotein Sm D3	SMD3_HUMAN	P62318	NP_004166.1 NM_004175.3
NAPA	Alpha-soluble NSF attachment protein	SNAA_HUMAN	P54920	NP_003818.2 NM_003827.3

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
SOD2	Superoxide dismutase [Mn], mitochondrial precursor	SODM_HUMAN	P04179	NP_000627.2 NP_001019636.1 NP_001019637.1 NM_000636.2 NM_001024465.1 NM_001024466.1
SORT1	Sortilin precursor	SORT_HUMAN	Q99523	NP_001192157.1 NP_002950.3 NM_001205228.1 NM_002959.5
SGSH	N-sulphoglucosamine sulphohydrolase precursor	SPHM_HUMAN	P51688	NP_000190.1 NM_000199.3
SPRED1	Sprouty-related, EVH1 domain-containing protein 1	SPRE1_HUMAN	Q7Z699	NP_689807.1 NM_152594.2
SPTBN1	Spectrin beta chain, non-erythrocytic 1	SPTB2_HUMAN	Q01082	NP_003119.2 NP_842565.2 NM_003128.2 NM_178313.2
SPG11	Spatacsin	SPTCS_HUMAN	Q96JI7	NP_001153699.1 NP_079413.3 NM_001160227.1 NM_025137.3
SPTAN1	Spectrin alpha chain, non-erythrocytic 1	SPTN1_HUMAN	Q13813	NP_001123910.1 NP_001182461.1 NP_003118.2 NM_001130438.2 NM_001195532.1 NM_003127.3
SRSF3	Serine/arginine-rich splicing factor 3	SRSF3_HUMAN	P84103	NP_003008.1 NM_003017.4
SRSF8	Serine/arginine-rich splicing factor 8	SRSF8_HUMAN	Q9BRL6	NP_115285.1 NM_032102.2
STIP1	Stress-induced-phosphoprotein 1	STIP1_HUMAN	P31948	NP_006810.1 NM_006819.2

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
STMN1	Stathmin	STMN1_HUMAN	P16949	NP_001138926.1 NP_005554.1 NP_981944.1 NP_981946.1 NM_001145454.1 NM_005563.3 NM_203399.1 NM_203401.1
STRAP	Serine-threonine kinase receptor-associated protein	STRAP_HUMAN	Q9Y3F4	NP_009109.3 NM_007178.3
STX11	Syntaxin-11	STX11_HUMAN	O75558	NP_003755.2 NM_003764.3
SUMO3	Small ubiquitin-related modifier 3 precursor	SUMO3_HUMAN	P55854	NP_008867.2 NM_006936.2
EPRS	Bifunctional glutamate/proline--tRNA ligase	SYEP_HUMAN	P07814	NP_004437.2 NM_004446.2
IARS2	Isoleucine--tRNA ligase, mitochondrial precursor	SYIM_HUMAN	Q9NSE4	NP_060530.3 NM_018060.3
TAGLN2	Transgelin-2	TAGL2_HUMAN	P37802	NP_003555.1 NM_003564.1
TALDO1	Transaldolase	TALDO_HUMAN	P37837	NP_006746.1 NM_006755.1
TUBA4A	Tubulin alpha-4A chain	TBA4A_HUMAN	P68366	NP_005991.1 NM_006000.1
TUBB4B	Tubulin beta-4B chain	TBB4B_HUMAN	P68371	NP_006079.1 NM_006088.5
TUBB	Tubulin beta chain	TBB5_HUMAN	P07437	NP_821133.1 NM_178014.2
TBL2	Transducin beta-like protein 2	TBL2_HUMAN	Q9Y4P3	NP_036585.1 NM_012453.2
CCT4	T-complex protein 1 subunit delta	TCPD_HUMAN	P50991	NP_006421.2 NM_006430.3
CCT8	T-complex protein 1 subunit theta	TCPQ_HUMAN	P50990	NP_006576.2 NM_006585.2
TDRD6	Tudor domain-containing protein 6	TDRD6_HUMAN	O60522	NP_001010870.1 NP_001161831.1 NM_001010870.2 NM_001168359.1

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
VCP	Transitional endoplasmic reticulum ATPase	TERA_HUMAN	P55072	NP _009057.1 NM_007126.3
TKT	Transketolase	TKT_HUMAN	P29401	NP_001055.1 NP_ 001128527.1 NP_ 001244957.1 NM_001064.3 NM_ 001135055.2 NM_ 001258028.1
TLN1	Talin-1	TLN1_HUMAN	Q9Y490	NP_006280.3 NM_006289.3
TMOD3	Tropomodulin-3	TMOD3_HUMAN	Q9NYL9	NP_055362.1 NM_014547.4
TPI1	Triosephosphate isomerase	TPIS_HUMAN	P60174	NP_000356.1 NP_ 001152759.1 NM_000365.5 NM_ 001159287.1
TRA2B	Transformer-2 protein homolog beta	TRA2B_HUMAN	P62995	NP_ 001230808.1 NP_004584.1 NM_ 001243879.1 NM_004593.2
TXNRD1	Thioredoxin reductase 1, cytoplasmic	TRXR1_HUMAN	Q16881	NP_ 001087240.1 NP_ 001248374.1 NP_ 001248375.1 NP_003321.3 NP_877393.1 NP_877419.1 NP_877420.1 NM_ 001093771.2 NM_ 001261445.1 NM_ 001261446.1 NM_003330.3 NM_182729.2 NM_182742.2 NM_182743.2

(continued)

Marker Name	Protein Description	UNIPROT_ID	UNIPROT ACCESSION	GENBANK ACCESSION
USP14	Ubiquitin carboxyl-terminal hydrolase 14	UBP14_HUMAN	P54578	NP_001032411.1 NP_005142.1 NM_001037334.1 NM_005151.3
UGGT1	UDP-glucose:glycoprotein glucosyltransferase 1 precursor	UGGG1_HUMAN	Q9NYU2	NP_064505.1 NM_020120.3
ATP6V1A	V-type proton ATPase catalytic subunit A	VATA_HUMAN	P38606	NP_001681.2 NM_001690.3
ATP6V1B2	V-type proton ATPase subunit B, brain isoform	VATB2_HUMAN	P21281	NP_001684.2 NM_001693.3
ATP6V1D	V-type proton ATPase subunit D	VATD_HUMAN	Q9Y5K8	NP_057078.1 NM_015994.3
ATP6V1E1	V-type proton ATPase subunit E1	VATE1_HUMAN	P36543	NP_001034455.1 NP_001034456.1 NP_001687.1 NM_001039366.1 NM_001039367.1 NM_001696.3
ATP6V1F	V-type proton ATPase subunit F	VATF_HUMAN	Q16864	NP_001185838.1 NP_004222.2 NM_001198909.1 NM_004231.3
ATP6V1G1	V-type proton ATPase subunit G1	VATG1_HUMAN	O75348	NP_004879.1 NM_004888.3
VPS4A	Vacuolar protein sorting-associated protein 4A	VPS4A_HUMAN	Q9UN37	NP_037377.1 NM_013245.2
XRCC6	X-ray repair cross-complementing protein 6	XRCC6_HUMAN	P12956	NP_001460.1 NM_001469.3
YBX1	Nuclease-sensitive element-binding protein 1	YBOX1_HUMAN	P67809	NP_004550.2 NM_004559.3

[0280] In certain aspects of the invention, a single marker (e.g., any one of the markers listed in Table 1) may be used in the methods and compositions of the invention. For example, in one embodiment, the marker for use in the methods and compositions of the invention is LYN. In one embodiment, the marker is MAT2A. In one embodiment, the marker is B4GALT1. In one embodiment, the marker is HIST3H3. In one embodiment, the marker is ICAM1. In one embodiment, the marker is LDHB. In one embodiment, the marker is MDH1. In one embodiment, the marker is CALU. In one embodiment, the marker is CORO1A. In one embodiment, the marker is ENO1. In one embodiment, the marker is EPB41L3. In one embodiment, the marker is FLNA. In one embodiment, the marker is GSTP1. In one embodiment, the marker is H6PD. In one embodiment, the marker is HISTH2BE. In one embodiment, the marker is HIST4H4. In one embodiment,

the marker is ITGAM. In one embodiment, the marker is MMP9. In one embodiment, the marker is PRKCSH. In one embodiment, the marker is RPSA. In one embodiment, the marker is TKT. In one embodiment, the marker is TPI1. In some embodiments, the methods (and kits) may further comprise determining the level of a marker selected from the group consisting of the markers listed in Table 1. In other embodiments, the methods may further comprise determining the level of one or more markers selected from the group consisting of SLC3A2, GOT1, GOT2, ACADVL, DBI, ACOX1, AHNAK, AIMP1, AKR1B1, ANPEP, ANXA2, ANXA5, ANXA6, AKR7A2, ARPC3, ASAH1, B4GALT1, BCAM, BLOC1S5, CALU, CAPG, CAPZB, CAPZA2, CBX1, CDC37, HSPE1, CHMP1A, CHMP1B, CHMP2A, CHMP4A, CHMP4B, CHMP5, CLIC1, CNDP2, CNPY2, COPA, COPB2, CORO1A, CORO1B, CORO1C, CPVL, C19orf10, CXorf26, CXCL10, CACYBP, DSP, HSD17B4, DLG1, DNASE2, DDT, DYNLL1, EPB41L3, EEF1A1, EEF1B2, EEF1D, EEF2, ENG, EHD1, ELAVL1, EMC2, EMR2, ENO1, ENO3, HSP90B1, ERO1L, ESYT2, EVL, ST13, F5, FKBP4, FLNA, FH, H6PD, GPI, GDI2, ARHGDIA, GGH, GBA, PRKCSH, GNS, HSPA9, HSPA5, GSTP1, GLT25D1, HIST1H1D, HIST1H1B, HIST3H2A, H2AFY, HIST2H2BE, HIST2H2BF, HIST3H3, HIST1H4A, HSD17B10, HEXA, HMGB3, HNRNPC, HNRNPD, HNRNPK, HNRNPR, HNRNPU, HSP90AA1, HSP90AB1, HSPA1A, HSPA8, ICAM1, EIF5A2, IL1B, IMPDH2, ISYNA1, ISOC1, ITGA5, ITGAM, ITPR1, KRT9, KRT7, KRT8, CAMK2D, PKM, LASP1, LDHB, LIMA1, LMNA, LMNB1, LMNB2, LRCH1, GAA, LYN, MDH1, MDH2, MESDC2, MAT2A, MYL12A, MMP9, RABIF, MYH10, MYH9, MYO1E, MYO6, PPP1R12A, NAGA, POR, NME1, NME2, NDRG1, NDUFA2, NAP1L1, NPM1, NUTF2, NCL, NUDC, OAS2, PAFAH1B2, PA2G4, PARK7, PARP1, PRCP, PDCD6IP, P4HB, PDIA5, PEBP1, PECAM1, PFDN1, PFDN2, PGAM1, PGK1, PIP4K2A, PIP5K1A, PLEKHO2, PLEC, PLOD3, LCP1, PNP, ALPP, PPIA, PPIB, PRDX1, PRDX2, PRDX3, PRDX6, PRPF19, PSMC6, PSMC3, PSMC2, PRSS8, NPEPPS, PSMA7, PSMB5, PSMD10, PSMD11, PSMD14, PSMD1, PSMD2, PSMD4, PTBP3, PAICS, ATIC, PXMP2, RAB6A, RANGAP1, RAI14, RAN, RANBP1, RBBP4, RAD23B, RPL14, RPL23A, RPL24, RPL26, RPL29, RPL3, RPL31, RPL7A, RPL8, RPLP2, MRPL39, HNRNPA1, HNRNPA2B1, RPS23, RPS8, RSAD2, RPSA, RUVBL1, RUVBL2, S100P, S100A6, S100A11, SEC61B, SEPT11, SEPT2, SEPT6, SEPT7, SEPT9, PHGDH, SF3B3, SFPQ, SIAE, SNRPD3, NAPA, SOD2, SORT1, SGSH, SPRED1, SPTBN1, SPG11, SPTAN1, SRSF3, SRSF8, STIP1, STMN1, STRAP, STX11, SUMO3, EPRS, IARS2, TAGLN2, TALDO1, TUBA4A, TUBB4B, TUBB, TBL2, CCT4, CCT8, TDRD6, VCP, TKT, TLN1, TMOD3, TPI1, TRA2B, TXNRD1, USP14, UGGT1, ATP6V1A, ATP6V1B2, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, VPS4A, XRCC6, and YBX1.

[0281] In other aspects of the invention, more than one marker, *e.g.,* a plurality of markers, *e.g.,* two, three, four, five, six, seven, eight, nine, ten, eleven, or more markers, may be used in the methods and compositions of the invention. For example, in one embodiment, the markers for use in the methods and compositions of the invention include HIST4H4 and LYN. In one embodiment, the markers include HIST4H4 and MAT2A. In one embodiment, the markers include HIST4H4 and MDH1. In one embodiment, the markers include HIST4H4, LYN, and MAT2A. In one embodiment, the markers include HIST4H4, LYN, and MDH1. In one embodiment, the markers include HIST4H4, MAT2A, and MDH1. In one embodiment, the markers include LYN and MAT2A. In one embodiment, the markers include LYN and MDH1. In one embodiment, the markers include LYN, AMY2A, and MDH1. In one embodiment, the markers include MAT2A, and MDH1. In one embodiment, the markers include HIST4H4, MDH1, MAT2A, and LYN. In one embodiment, the markers include HIST4H4 and LDHB. In one embodiment, the markers include HIST4H4 and MAT2A. In one embodiment, the markers include HIST4H4 and MDH1. In one embodiment, the markers include HIST4H4, LDHB, and MAT2A. In one embodiment, the markers include HIST4H4, LDHB, and MDH1. In one embodiment, the markers include HIST4H4, MAT2A, and MDH1. In one embodiment, the markers include LDHB and MDH1. In one embodiment, the markers include LDHB, MAT2A, and MDH1. In one embodiment, the markers include HIST4H4 and ICAM1. In one embodiment, the markers include HIST4H4 and MMP9. In one embodiment, the markers include HIST4H4, ICAM1, and LYN. In one embodiment, the markers include HIST4H4, ICAM1, and MMP9. In one embodiment, the markers include HIST4H4, LYN and MMP9. In one embodiment, the markers include ICAM1 and LYN. In one embodiment, the markers include ICAM1 and MMP9. In one embodiment, the markers include ICAM1, LYN, and MMP9. In one embodiment, the markers include LYN and MMP9. In one embodiment, the markers include B4GALT1 and CALU. In one embodiment, the markers include B4GALT1 and LYN. In one embodiment, the markers include B4GALT1 and TPI1. In one embodiment, the markers include B4GALT1, CALU, and LYN. In one embodiment, the markers include B4GALT1, CALU, and TPI1. In one embodiment, the markers include B4GALT1, LYN, and TPI1. In one embodiment, the markers include B4GALT1, LYN, CALU, and TPI1. In one embodiment, the markers include CALU and LYN. In one embodiment, the markers include CALU and TPI1. In one embodiment, the markers include LYN and TPI1. In one embodiment, the markers include CALU, LYN, and TPI1. In one embodiment, the markers include HIST4H4, LYN, MAT2A, MMP9, CALU, LDHB, and B4GALT1. In one embodiment, the markers include MMP9 and MDH1. In one embodiment, the markers include LYN and LDHB.

[0282] In some embodiments, the methods may further comprise determining the level of a marker selected from the group consisting of the markers listed in Table 1. In other embodiments, the methods may further comprise determining the level of one or more markers selected from the group consisting of B4GALT1, HIST3H3, CALU, CORO1A, ENO1, EPB41L3, FLNA, GSTP1, H6PD, ITGAM, PRKCSH, RPSA, TKT, and TPI1.

III. Methods of the Invention

A. Diagnostic Methods

[0283] In certain aspects, the present invention provides diagnostic methods. For example, in one aspect, the present invention provides methods for determining whether a subject has Brucellosis. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Brucellosis.

[0284] In another aspect, the present invention provides methods for determining whether a subject has Q-fever. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Q-Fever.

[0285] In another aspect, the present disclosure provides methods for determining whether a subject has Lyme Disease. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Lyme Disease.

[0286] In another aspect, the present invention provides methods for determining whether a subject has Brucellosis or Q-fever. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Brucellosis.

[0287] In another aspect, the present disclosure provides methods for determining whether a subject has Brucellosis or Lyme Disease. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Brucellosis.

[0288] In another aspect, the present disclosure provides methods for determining whether a subject has Q-Fever or Lyme Disease. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates that the subject has Q-Fever.

[0289] In another aspect, the present disclosure provides methods for determining whether a subject has Brucellosis or Q-Fever or Lyme Disease. The methods include determining the level of one or more markers of the invention in a sample(s) from the subject with a level of the one or more markers in a control sample(s). A difference in the level (*e.g.,* higher or lower) of the one or more markers in the sample(s) from the subject as compared to the level of the one or more markers in the control sample indicates wherther the subject has Brucellosis or Q-Fever or Lyme Disease.

[0290] The methods of the present invention can be practiced in conjunction with any other method(s) used by the skilled practitioner to diagnose, prognose, and/or monitor Brucellosis, Q-Fever, and/or Lyme Disease. For example, the methods of the invention may be performed in conjunction with any clinical measurement of Brucellosis, Q-Fever, and/or Lyme Disease known in the art including serological, cytological, radiological, and/or detection (and quantification, if appropriate) of other molecular markers.

[0291] In any of the methods (and kits) of the invention, the level of a marker(s) of the invention in a sample obtained from a subject may be determined by any of a wide variety of well-known techniques and methods, which transform a marker of the invention within the sample into a moiety that can be detected and quantified. Non-limiting examples of such methods include analyzing the sample using immunological methods for detection of proteins, protein purification methods, protein function or activity assays, nucleic acid hybridization methods, nucleic acid reverse transcription methods, and nucleic acid amplification methods, immunoblotting, Western blotting, Northern blotting, electron microscopy, mass spectrometry, *e.g.,* MALDI-TOF and SELDI-TOF, immunoprecipitations, immunofluorescence, immunohistochemistry, enzyme linked immunosorbent assays (ELISAs), *e.g.,* amplified ELISA, quantitative blood based assays, *e.g.,* serum ELISA, quantitative urine based assays, flow cytometry, Southern hybridizations, array analysis, and the like, and combinations or sub-combinations thereof.

[0292] For example, an mRNA sample may be obtained from the sample from the subject (*e.g.,* blood, serum, bronchial lavage, mouth swab, biopsy, or peripheral blood mononuclear cells, by standard methods) and expression of mRNA(s) encoding a marker of the invention in the sample may be detected and/or determined using standard molecular biology

techniques, such as PCR analysis. A preferred method of PCR analysis is reverse transcriptase-polymerase chain reaction (RT-PCR). Other suitable systems for mRNA sample analysis include microarray analysis (*e.g.,* using Affymetrix's microarray system or Illumina's BeadArray Technology).

[0293] It will be readily understood by the ordinarily skilled artisan that essentially any technical means established in the art for detecting the level a marker of the invention at either the nucleic acid or protein level, can be used to determine the level a marker of the invention as discussed herein.

[0294] In one embodiment, the level of a marker of the invention in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA, or cDNA, of a marker of the invention gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton et al., Nuc. Acids Res. 12:7035), Northern blotting, *in situ* hybridization, and microarray analysis.

[0295] In one embodiment, the level of a marker of the invention is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific marker of the invention. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

[0296] Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to a marker mRNA. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 250 or about 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to marker genomic DNA.

[0297] In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of a marker of the invention mRNA.

[0298] An alternative method for determining the level of a marker of the invention in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.,* by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189-193), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of a marker of the invention is determined by quantitative fluorogenic RT-PCR (*i.e.,* the TaqMan™ System). Such methods typically utilize pairs of oligonucleotide primers that are specific for a marker of the invention. Methods for designing oligonucleotide primers specific for a known sequence are well known in the art.

[0299] The level of a marker of the invention mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934. The determination of a level of a marker of the invention may also comprise using nucleic acid probes in solution.

[0300] In one embodiment of the invention, microarrays are used to detect the level of a marker of the invention. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, *e.g.,* U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample.

[0301] In certain situations it may be possible to assay for the level of a marker of the invention at the protein level, using a detection reagent that detects the protein product encoded by the mRNA of a marker of the invention. For example, if an antibody reagent is available that binds specifically to a marker of the invention protein product to be detected, and not to other proteins, then such an antibody reagent can be used to detect the expression of a marker of the invention in a cellular sample from the subject, or a preparation derived from the cellular sample, using standard

antibody-based techniques known in the art, such as FACS analysis, and the like.

[0302] Other known methods for detecting a marker of the invention at the protein level include methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, or various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and Western blotting.

[0303] Proteins from samples can be isolated using techniques that are well known to those of skill in the art. The protein isolation methods employed can, for example, be those described in Harlow and Lane (Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

[0304] In one embodiment, antibodies, or antibody fragments, are used in methods such as Western blots or immunofluorescence techniques to detect the expressed proteins. Antibodies for determining the expression of a marker of the invention are commercially available and one of ordinary skill in the art can readily identify appropriate antibodies for use in the methods of the invention.

[0305] It is generally preferable to immobilize either the antibody or proteins on a solid support for Western blots and immunofluorescence techniques. Suitable solid phase supports or carriers include any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.

[0306] One skilled in the art will know many other suitable carriers for binding antibody or antigen, and will be able to adapt such support for use with the present invention. For example, protein isolated from cells can be run on a polyacrylamide gel electrophoresis and immobilized onto a solid phase support such as nitrocellulose. The support can then be washed with suitable buffers followed by treatment with the detectably labeled antibody. The solid phase support can then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on the solid support can then be detected by conventional means. Means of detecting proteins using electrophoretic techniques are well known to those of skill in the art (see generally, R. Scopes (1982) Protein Purification, Springer-Verlag, N.Y.; Deutscher, (1990) Methods in Enzymology Vol. 182: Guide to Protein Purification, Academic Press, Inc., N.Y.).

[0307] Other standard methods include immunoassay techniques which are well known to one of ordinary skill in the art and may be found in Principles And Practice Of Immunoassay, 2nd Edition, Price and Newman, eds., MacMillan (1997) and Antibodies, A Laboratory Manual, Harlow and Lane, eds., Cold Spring Harbor Laboratory, Ch. 9 (1988).

[0308] Antibodies used in immunoassays to determine the level of a marker of the invention, may be labeled with a detectable label. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.,* physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

[0309] In one embodiment, the antibody is labeled, *e.g.* a radio-labeled, chromophore-labeled, fluorophore-labeled, or enzyme-labeled antibody. In another embodiment, an antibody derivative (*e.g.* an antibody conjugated with a substrate or with the protein or ligand of a protein-ligand pair {*e.g.* biotin-streptavidin}), or an antibody fragment (*e.g.* a single-chain antibody, an isolated antibody hypervariable domain, *etc*.) which binds specifically with a marker of the invention.

[0310] In one embodiment of the invention, proteomic methods, *e.g.,* mass spectrometry, are used. Mass spectrometry is an analytical technique that consists of ionizing chemical compounds to generate charged molecules (or fragments thereof) and measuring their mass-to-charge ratios. In a typical mass spectrometry procedure, a sample is obtained from a subject, loaded onto the mass spectrometry, and its components (*e.g.,* a marker of the invention) are ionized by different methods (*e.g.,* by impacting them with an electron beam), resulting in the formation of charged particles (ions). The mass-to-charge ratio of the particles is then calculated from the motion of the ions as they transit through electromagnetic fields.

[0311] For example, matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) which involves the application of a biological sample, such as serum, to a protein-binding chip (Wright, G.L., Jr., et al. (2002) Expert Rev Mol Diagn 2:549; Li, J., et al. (2002) Clin Chem 48:1296; Laronga, C., et al. (2003) Dis Markers 19:229; Petricoin, E.F., *et al.* (2002) 359:572; Adam, B.L., et al. (2002) Cancer Res 62:3609; Tolson, J., et al. (2004) Lab Invest 84:845; Xiao, Z., et al. (2001) Cancer Res 61:6029) can be used to determine the level of a marker of the invention.

[0312] Furthermore, *in vivo* techniques for determination of the level of a marker of the invention include introducing into a subject a labeled antibody directed against a marker of the invention, which binds to and transforms a marker of the invention into a detectable molecule. As discussed above, the presence, level, or even location of the detectable marker of the invention in a subject may be detected determined by standard imaging techniques.

[0313] In general, it is preferable that the difference between the level of a marker of the invention in a sample from a subject and the amount of a marker of the invention in a control sample, is as great as possible. Although this difference can be as small as the limit of detection of the method for determining the level of a marker it is preferred that the

difference be at least greater than the standard error of the assessment method, and preferably a difference of at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 25-, 100-, 500-, 1000-fold or greater than the standard error of the assessment method.

B. Methods for Monitoring the Effectiveness of a Treatment

[0314] The present invention also provides methods for monitoring the effectiveness of a therapy or treatment regimen or any other therapeutic approach useful for treating a subject having a zoonosis, *e.g.,* Brucellosis and/or inhibiting relapse of the zoonosis, *e.g.,* Brucellosis, inhibiting chronic zoonotic infection, *e.g., Brucella* infection, the progression of the zoonosis, ie.g., Brucellosis to other organs (or a complication associated with involvement of other organ systems (*e.g.,* sacroiliitis, spondylitis, osteomyelitis, endocarditis (a primary cause of death), myocarditis, pericarditis, infections of the central nervous system, such as meningitis, encepahlitis, and meningoencephalitis, hepatitis, hepatic abscess, colitis, spontaneous peritonitis, orchitis, epididymo-orchitis, heaptomegaly, pneumonia, optic neuritis, and uveitis)) in a subject having Brucellosis.

[0315] The present invention also provides methods for monitoring the effectiveness of a therapy or treatment regimen or any other therapeutic approach useful for treating a subject having Q-Fever and/or inhibiting relapse of Q-Fever, inhibiting chronic *Coxiella burnetii* infection, the progression of Q-Fever to other organs (or a complication associated with involvement of other organ systems (*e.g.,* endocarditis , chronic hepatitis without endocarditis, osteomyelitis, osteoarthritis, and pneumonitis) in a subject having Q-Fever.

[0316] The present invention also provides methods for monitoring the effectiveness of a therapy or treatment regimen or any other therapeutic approach useful for treating a subject having Q-Fever and/or inhibiting relapse of Lyme Disease, inhibiting chronic *Borrelia* infection, the progression of Lyme Disease to other organs (or a complication associated with involvement of other organ systems (*e.g.,* paralysis of the facial nerves, recurring headaches or fainting, poor memory and reduced ability to concentrate, conjunctivitis, heart palpitations, endocarditis,
Inflammation of the joints, numbness and tingling in the hands, feet, or back, severe fatigue. Partial facial nerve paralysis, which usually occurs within the first few months after the tick bite, neurologic changes, including problems with memory, mood, or sleep, and sometimes problems speaking) in a subject having Lyme Disease.

[0317] In these methods the level of one or more markers of the invention in a pair of samples (a first sample not subjected to the treatment regimen and a second sample subjected to at least a portion of the treatment regimen) is assessed. A modulation in the level of expression of the one or more markers in the first sample, relative to the second sample, is an indication that the therapy is effective for for treating a subject having the zoonosis and/or inhibiting relapse of th zoonosis, inhibiting chronic zoonotic infection, the progression of the zoonosis to other organs (or a complication thereof) in a subject having the zoonosis.

C. Screening Methods

[0318] Using the methods described herein, a variety of molecules, particularly molecules sufficiently small to be able to cross the cell membrane, may be screened in order to identify molecules which modulate, *e.g.,* decrease or increase, the level and/or activity of a marker(s) of the invention. Compounds so identified can be administered to a subject in order to for treating a subject having a zoonosis, *e.g.,* Brucellosis, Q-Fever, or Lyme Disease, and/or inhibiting relapse of the zoonosis, inhibiting chronic zoonotic infection, the progression of the zoonosis to other organs (or a complication associated with involvement of other organ systems in a subject having the zoonosis.

[0319] Accordingly, in one embodiment, the invention provides methods for identifying modulators, *i.e.,* candidate or test compounds or agents (*e.g.,* enzymes, peptides, peptidomimetics, small molecules, ribozymes, or marker antisense molecules) which bind to a marker polypeptide; have a stimulatory or inhibitory effect on a marker expression; marker processing; marker post-translational modification (*e.g.,* glycosylation, ubiquitinization, or phosphorylation); marker activity; and/or have a stimulatory or inhibitory effect on the expression, processing or activity of a marker target molecule.

[0320] Methods for identifying a compound that can modulate the level and/or activity of a marker in a cell (*in vitro* and/or *in vivo*), for treating a subject having a zoonosis and/or inhibiting relapse of the zoonosis, inhibiting chronic zoonotic infection, the progression of the zoonosis to other organs (or a complication associated with involvement of other organ systems) (also referred to herein as screening assays) include separately contacting an aliquot of a sample (*e.g.,* a sample from the subject) with each member of a library of compounds; determining the effect of a member of the library of compounds on the level of one or more marker(s) of the invention (or the activity of one or more marker(s) of the invention) in each of the aliquots; and selecting a member of the library of compounds which modulates the level of and/or the activity of the one or more marker(s) of the invention in an aliquot as compared to the level and/or activity of the one or more marker(s) of the invention in a control sample, thereby identifying a compound that can modulate the level and/or activity of a marker in a cell, for treating a subject having the zoonosis and/or inhibiting relapse of the zoonosis, inhibiting chronic zoonotic infection, the progression of the zoonosis to other organs (or a complication asso-

ciated with involvement of other organ systems).

[0321] As used interchangeably herein, the terms "marker activity" and "biological activity of a marker" include activities exerted by a marker(s) protein on marker responsive cell or tissue, or on marker(s) nucleic acid molecule or protein target molecule, as determined *in vivo,* and/or *in vitro,* according to standard techniques. A marker(s) activity can be a direct activity, such as an association with a marker-target molecule. Alternatively, marker(s) activity is an indirect activity, such as a downstream biological event mediated by interaction of the marker(s) protein with a marker-target molecule or other molecule in a signal-transduction pathway involving the marker(s). The biological activities of the markers of the invention are known in the art and can be found at, for example, www.uniprot.org. The Uniprot Accession Numbers for each of the markers of the invention are provided in Table 1. The entire contents of each of these Uniprot records are hereby incorporated by reference. Methods for determining the effect of a compound on the level and/or activity of marker are known in the art and/or described herein.

[0322] A variety of test compounds can be evaluated using the screening assays described herein. The term "test compound" includes any reagent or test agent which is employed in the assays of the invention and assayed for its ability to influence the expression and/or activity of a marker. More than one compound, *e.g.,* a plurality of compounds, can be tested at the same time for their ability to modulate the expression and/or activity of a marker in a screening assay. The term "screening assay" preferably refers to assays which test the ability of a plurality of compounds to influence the readout of choice rather than to tests which test the ability of one compound to influence a readout. Preferably, the subject assays identify compounds not previously known to have the effect that is being screened for. In one embodiment, high throughput screening can be used to assay for the activity of a compound.

[0323] Candidate/test compounds include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, *e.g.,* Lam, K.S. et al. (1991) Nature 354:82-84; Houghten, R. et al. (1991) Nature 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L- configuration amino acids; 2) phosphopeptides (*e.g.,* members of random and partially degenerate, directed phosphopeptide libraries, see, *e.g.,* Songyang, Z. et al. (1993) Cell 72:767-778); 3) antibodies (*e.g.,* polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, $F(ab')_2$, Fab expression library fragments, and epitope-binding fragments of antibodies); 4) small organic and inorganic molecules (*e.g.,* molecules obtained from combinatorial and natural product libraries); 5) enzymes (*e.g.,* endoribonucleases, hydrolases, nucleases, proteases, synthatases, isomerases, polymerases, kinases, phosphatases, oxido-reductases and ATPases), 6) mutant forms of marker(s) molecules, *e.g.,* dominant negative mutant forms of the molecules, 7) nucleic acids, 8) carbohydrates, and 9) natural product extract compounds.

[0324] Test compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) Anticancer Drug Des. 12:145).

[0325] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994) J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al. (1994) J. Med. Chem. 37:1233.

[0326] Libraries of compounds can be presented in solution (*e.g.,* Houghten (1992) Biotechniques 13:412-421), or on beads (Lam (1991) Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) Proc Natl Acad Sci USA 89:1865-1869) or phage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. 87:6378-6382; Felici (1991) J. Mol. Biol. 222:301-310; Ladner *supra*.).

[0327] Compounds identified in the screening assays can be used in methods of modulating one or more of the biological responses regulated by a marker. It will be understood that it may be desirable to formulate such compound(s) as pharmaceutical compositions prior to contacting them with cells.

[0328] Once a test compound is identified by one of the variety of methods described hereinbefore, the selected test compound (or "compound of interest") can then be further evaluated for its effect on cells, for example by contacting the compound of interest with cells either *in vivo* (*e.g.,* by administering the compound of interest to a subject or animal model) or *ex vivo* (*e.g.,* by isolating cells from the subject or animal model and contacting the isolated cells with the compound of interest or, alternatively, by contacting the compound of interest with a cell line) and determining the effect of the compound of interest on the cells, as compared to an appropriate control (such as untreated cells or cells treated with a control compound, or carrier, that does not modulate the biological response).

[0329] Computer-based analysis of a marker with a known structure can also be used to identify molecules which will bind to a marker of the invention. Such methods rank molecules based on their shape complementary to a receptor site. For example, using a 3-D database, a program such as DOCK can be used to identify molecules which will bind. See

DesJarlias et al. (1988) J. Med. Chem. 31:722; Meng et al. (1992) J. Computer Chem. 13:505; Meng et al. (1993) Proteins 17:266; Shoichet et al. (1993) Science 259:1445. In addition, the electronic complementarity of a molecule to a marker can be analyzed to identify molecules which bind to the marker. This can be determined using, for example, a molecular mechanics force field as described in Meng et al. (1992) J. Computer Chem. 13:505 and Meng et al. (1993) Proteins 17:266. Other programs which can be used include CLIX which uses a GRID force field in docking of putative ligands. See Lawrence et al. (1992) Proteins 12:31; Goodford et al. (1985) J. Med. Chem. 28:849; Boobbyer et al. (1989) J. Med. Chem. 32:1083.

[0330] The instant invention also pertains to compounds identified using the foregoing screening assays.

D. Methods for Modulating the Expression And/Or Activity of a Biomarker of the Invention

[0331] Yet another aspect of the invention pertains to methods of modulating expression and/or activity of a marker in a cell. The modulatory methods of the invention involve contacting the cell with an agent that modulates the expression and/or activity of a marker such that the expression and/or activity of a marker in the cell is modulated. In order for the expression and/or activity of a marker to be modulated in a cell, the cell is contacted with a modulatory agent in an amount sufficient to modulate the expression and/or activity of a marker.

[0332] A "modulator" or "modulatory agent" is a compound or molecule that modulates, and may be, *e.g.,* an agonist, antagonist, activator, stimulator, suppressor, or inhibitor. As used herein, the term "modulator" refers to any moiety which modulates activity of a marker(s), including moieties which modulates marker(s) expression or modulates marker(s) function. The modulator may act by modulating the activity of a marker polypeptide in the cell, (*e.g.,* by contacting a cell with an agent that, *e.g.*, interfers with the binding of a marker(s) to a molecule with which it interacts, changes the binding specificity of a marker(s), or post-translationally modifies a marker(s) or the expression of a marker(s), (*e.g.*, by modulating transcription of the marker gene or translation of the marker mRNA). Accordingly, the invention features methods for modulating one or more biological responses regulated by a marker(s) by contacting the cells with a modulator of the expression and/or activity the marker(s) such that the biological response is modulated.

[0333] Representative modulators are described below and include, but are not limited to, proteins, nucleic acid molecules, antibodies, nucleic acids (*e.g.*, antisense molecules, such as ribozymes and RNA interfering agents), immunoconjugates (*e.g.*, an antibody conjugated to a therapeutic agent), small molecules, fusion proteins, adnectins, aptamers, anticalins, lipocalins, and marker-derived peptidic compounds.

[0334] As used herein, the term "contacting" (*e.g.*, contacting a cell with a modulator) is intended to include incubating the modulator and the cell together *in vitro* (*e.g.,* adding the modulator to cells in culture) or administering the modulator to a subject such that the modulator and cells of the subject are contacted *in vivo.* The term "contacting" is not intended to include exposure of cells to an agent that may occur naturally in a subject *(i.e.,* exposure that may occur as a result of a natural physiological process).

[0335] In one embodiment, the modulatory methods of the invention are performed *in vitro.* In another embodiment, the modulatory methods of the invention are performed *in vivo, e.g.,* in a subject, *e.g.,* having a zoonis, *e.g.,* Brucellosis, Q-Fever, or Lyme Disease, that would benefit from modulation of the expression and/or activity of a marker of the invention.

[0336] Accordingly, the present invention also provides methods for reducing or inhibiting the development of complications associated with the disease in a subject

The methods of "inhibiting", "slowing", and/or "treating" include administration of a marker modulator to a subject in order to cure or to prolong the health or survival of a subject beyond that expected in the absence of such treatment.

[0337] The terms "patient" or "subject" as used herein is intended to include human and veterinary patients. In a particular embodiment, the subject is a human. The term "non-human animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, mice, rabbits, sheep, dog, cow, chickens, amphibians, and reptiles.

[0338] The methods of the invention also contemplate the use of marker(s) modulators in combination with other therapies, including surgery and/or life-style changes. Thus, in addition to the use of marker(s) modulators, the methods of the invention may also include administering to the subject one or more "standard" therapies. For example, the modulators can be administered in combination with (*i.e.,* together with or linked to (*i.e.,* an immunoconjugate)) cytotoxins, antibiotics, *e.g.*, streptomycin, gentamicin, doxycycline, cotrimoxazole, azithromycin, ciprofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, minocycline, co-trimoxazole, and members of the tetracycline and quinolone classes of compounds, or combinations thereof.

[0339] Marker(s) modulators and the co-therapeutic agent or co-therapy can be administered in the same formulation or separately. In the case of separate administration, the marker(s) modulators can be administered before, after or concurrently with the co-therapeutic or co-therapy. One agent may precede or follow administration of the other agent by intervals ranging from minutes to weeks. In embodiments where two or more different kinds of therapeutic agents are applied separately to a subject, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that these different kinds of agents would still be able to exert an advantageously combined effect on the target tissues or cells.

[0340] In one embodiment, the marker(s) modulators (*e.g.*, an anti- marker(s) antibody) may be linked to a second binding molecule, such as an antibody (*i.e.,* thereby forming a bispecific molecule) or other binding agent that, for example, binds to a different target or a different epitope on the marker(s).

[0341] The term "effective amount" as used herein, refers to that amount of marker(s) modulators, which is sufficient to inhibit the progression of fibrosis in a subject when administered to a subject. An effective amount will vary depending upon the subject and the severity of the disease and age of the subject, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. Marker(s) modulators dosages for administration can range from, for example, about 1 ng to about 10,000 mg, about 5 ng to about 9,500 mg, about 10 ng to about 9,000 mg, about 20 ng to about 8,500 mg, about 30 ng to about 7,500 mg, about 40 ng to about 7,000 mg, about 50 ng to about 6,500 mg, about 100 ng to about 6,000 mg, about 200 ng to about 5,500 mg, about 300 ng to about 5,000 mg, about 400 ng to about 4,500 mg, about 500 ng to about 4,000 mg, about 1 μg to about 3,500 mg, about 5 μg to about 3,000 mg, about 10 μg to about 2,600 mg, about 20 μg to about 2,575 mg, about 30 μg to about 2,550 mg, about 40 μg to about 2,500 mg, about 50 μg to about 2,475 mg, about 100 μg to about 2,450 mg, about 200 μg to about 2,425 mg, about 300 μg to about 2,000, about 400 μg to about 1,175 mg, about 500 μg to about 1,150 mg, about 0.5 mg to about 1,125 mg, about 1 mg to about 1,100 mg, about 1.25 mg to about 1,075 mg, about 1.5 mg to about 1,050 mg, about 2.0 mg to about 1,025 mg, about 2.5 mg to about 1,000 mg, about 3.0 mg to about 975 mg, about 3.5 mg to about 950 mg, about 4.0 mg to about 925 mg, about 4.5 mg to about 900 mg, about 5 mg to about 875 mg, about 10 mg to about 850 mg, about 20 mg to about 825 mg, about 30 mg to about 800 mg, about 40 mg to about 775 mg, about 50 mg to about 750 mg, about 100 mg to about 725 mg, about 200 mg to about 700 mg, about 300 mg to about 675 mg, about 400 mg to about 650 mg, about 500 mg, or about 525 mg to about 625 mg, of a marker(s) modulator. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (*i.e.,* side effects) of a marker(s) modulator are minimized and/or outweighed by the beneficial effects.

[0342] Actual dosage levels of the marker(s) modulators used in the methods of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired response, *e.g.*, inhibiting the progression of diabetes, for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular marker(s) modulator employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular modulator being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular modulator employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the modulator required. For example, the physician or veterinarian could start doses of the modulator at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a marker(s) modulator will be that amount which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous, preferably administered proximal to the site of the target. If desired, the effective daily dose of a marker(s) modulator may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a marker(s) modulator of the present invention to be administered alone, it is preferable to administer the modulator as a pharmaceutical formulation (composition).

[0343] Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. For example, the marker(s) modulators used in the methods of the present invention may be administered once or twice weekly by subcutaneous injection or once or twice monthly by subcutaneous injection.

[0344] To administer a marker(s) modulator used in the methods of the present invention by certain routes of administration, it may be necessary to include the modulator in a formulation suitable for preventing its inactivation. For example, the marker(s) modulator may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions, as well as conventional liposomes (Strejan et al. (1984) J. Neuroimmunol. 7:27).

[0345] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active marker(s) modulator, use thereof in pharmaceutical compositions is contemplated. Supplementary active compounds can also be incorporated with the marker(s) modulator.

[0346] Marker(s) modulators used in the methods of the invention typically must be sterile and stable under the conditions of manufacture and storage. The modulator can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing,

for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including an agent that delays absorption, for example, monostearate salts and gelatin.

[0347] Sterile injectable solutions can be prepared by incorporating the active modulator in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0348] Marker(s) modulators that can be used in the methods of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the modulator which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.001% to about 90 % of active ingredient, preferably from about 0.005% to about 70%, most preferably from about 0.01% to about 30%.

[0349] The phrases "parenteral administration" and "administered parenterally", as used herein, means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[0350] Examples of suitable aqueous and non-aqueous carriers which may be employed along with the marker(s) modulators utilized in the methods of the present invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0351] Marker(s) modulatos may also be administered with adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0352] When marker(s) modulators used in the methods of the present invention are administered to humans and animals, they can be given alone or as a pharmaceutical modulator containing, for example, 0.001 to 90% (more preferably, 0.005 to 70%, such as 0.01 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

[0353] Marker(s) modulators can be administered with medical devices known in the art. For example, in a preferred embodiment, a modulator can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163, 5,383,851, 5,312,335, 5,064,413, 4,941,880, 4,790,824, or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4.,486,194, which discloses a therapeutic device for administering medications through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

1. Inhibitory Agents

[0354] According to a modulatory method of the invention, the expression and/or activity of a marker(s) is inhibited in a cell or subject by contacting the cell with (or administering to a subject) an inhibitory agent. Inhibitory agents of the invention can be, for example, molecules that act to decrease or inhibit the expression and/or activity of the marker(s).

[0355] In one embodiment of the invention, the modulatory, *e.g.*, therapeutic, and diagnostic methods described herein employ an antibody that binds, *e.g.*, directly to or indirectly to, and inhibits marker(s) activity and/or down-modulates marker(s) expression.

[0356] The term "antibody" or "immunoglobulin," as used interchangeably herein, includes whole antibodies and any antigen binding fragment (*i.e.,* "antigen-binding portion") or single chains thereof. An "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[0357] The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.,* a marker). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb including VH and VL domains; (vi) a dAb fragment (Ward et al. (1989) Nature 341, 544-546), which consists of a V_H domain; (vii) a dAb which consists of a VH or a VL domain; and (viii) an isolated complementarity determining region (CDR) or (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.,* Bird et al. (1988) Science 242, 423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85, 5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antigen-binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

[0358] The term "antibody", as used herein, includes polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, and human antibodies, and those that occur naturally or are recombinantly produced according to methods well known in the art.

[0359] In one embodiment, an antibody for use in the methods of the invention is a bispecific antibody. A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.,* Songsivilai & Lachmann, (1990) Clin. Exp. Immunol. 79, 315-321; Kostelny et al. (1992) J. Immunol. 148, 1547-1553.

[0360] In another embodiment, an antibody for use in the methods of the invention is a camelid antibody as described in, for example, PCT Publication WO 94/04678, the entire contents of which are incorporated herein by reference.

[0361] A region of the camelid antibody that is the small, single variable domain identified as V_{HH} can be obtained by genetic engineering to yield a small protein having high affinity for a target, resulting in a low molecular weight, antibody-derived protein known as a "camelid nanobody". See U.S. Pat. No. 5,759,808; see also Stijlemans et al., 2004 J. Biol. Chem. 279: 1256-1261; Dumoulin et al., 2003 Nature 424: 783-788; Pleschberger et al., 2003 Bioconjugate Chem. 14: 440-448; Cortez-Retamozo et al., 2002 Int. J. Cancer 89: 456-62; and Lauwereys, et al., 1998 EMBO J. 17: 3512-3520. Engineered libraries of camelid antibodies and antibody fragments are commercially available, for example, from Ablynx, Ghent, Belgium. Accordingly, a feature of the present invention is a camelid nanobody having high affinity for a marker.

[0362] In other embodiments of the invention, an antibody for use in the methods of the invention is a diabody, a single chain diabody, or a di-diabody.

[0363] Diabodies are bivalent, bispecific molecules in which V_H and V_L domains are expressed on a single polypeptide chain, connected by a linker that is too short to allow for pairing between the two domains on the same chain. The V_H and V_L domains pair with complementary domains of another chain, thereby creating two antigen binding sites (see *e.g.,* Holliger et al., 1993 Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak et al., 1994 Structure 2:1121-1123). Diabodies can be produced by expressing two polypeptide chains with either the structure V_{HA}-V_{LB} and V_{HB}-V_{LA} (V_H-V_L configu-

ration), or V_{LA}-V_{HB} and V_{LB}-V_{HA} (V_L-V_H configuration) within the same cell. Most of them can be expressed in soluble form in bacteria.

[0364] Single chain diabodies (scDb) are produced by connecting the two diabody-forming polypeptide chains with linker of approximately 15 amino acid residues (see Holliger and Winter, 1997 Cancer Immunol. Immunother., 45(3-4):128-30; Wu et al., 1996 Immunotechnology, 2(1):21-36). scDb can be expressed in bacteria in soluble, active monomeric form (see Holliger and Winter, 1997 Cancer Immunol. Immunother., 45(34): 128-30; Wu et al., 1996 Immunotechnology, 2(1):21-36; Pluckthun and Pack, 1997 Immunotechnology, 3(2): 83-105; Ridgway et al., 1996 Protein Eng., 9(7):617-21).

[0365] A diabody can be fused to Fc to generate a "di-diabody" (see Lu et al., 2004 J. Biol. Chem., 279(4):2856-65).

[0366] Marker binding molecules that exhibit functional properties of antibodies but derive their framework and antigen binding portions from other polypeptides (e.g., polypeptides other than those encoded by antibody genes or generated by the recombination of antibody genes *in vivo*) may also be used in the methods of the present invention. The antigen binding domains (*e.g.*, marker binding domains) of these binding molecules are generated through a directed evolution process. See U.S. Pat. No. 7,115,396. Molecules that have an overall fold similar to that of a variable domain of an antibody (an "immunoglobulin-like" fold) are appropriate scaffold proteins. Scaffold proteins suitable for deriving antigen binding molecules include fibronectin or a fibronectin dimer, tenascin, N-cadherin, E-cadherin, ICAM, titin, GCSF-receptor, cytokine receptor, glycosidase inhibitor, antibiotic chromoprotein, myelin membrane adhesion molecule P0, CD8, CD4, CD2, class I MHC, T-cell antigen receptor, CD1, C2 and I-set domains of VCAM-1, I-set immunoglobulin domain of myosin-binding protein C, I-set immunoglobulin domain of myosin-binding protein H, I-set immunoglobulin domain of telokin, NCAM, twitchin, neuroglian, growth hormone receptor, erythropoietin receptor, prolactin receptor, interferon-gamma receptor, β-galactosidase/glucuronidase, β-glucuronidase, transglutaminase, T-cell antigen receptor, superoxide dismutase, tissue factor domain, cytochrome F, green fluorescent protein, GroEL, and thaumatin.

[0367] To generate non-antibody binding molecules, a library of clones is created in which sequences in regions of the scaffold protein that form antigen binding surfaces (*e.g.*, regions analogous in position and structure to CDRs of an antibody variable domain immunoglobulin fold) are randomized. Library clones are tested for specific binding to the antigen of interest (*e.g.,* a marker(s) of the invention) and for other functions (*e.g.,* inhibition of biological activity of a marker(s) of the invention). Selected clones can be used as the basis for further randomization and selection to produce derivatives of higher affinity for the antigen.

[0368] High affinity binding molecules are generated, for example, using the tenth module of fibronectin III (^{10}Fn3) as the scaffold, described in U.S. Pat. Nos. 6,818,418 and 7,115,396; Roberts and Szostak, 1997 Proc. Natl. Acad. Sci USA 94:12297; U.S. Pat. No. 6,261,804; U.S. Pat. No. 6,258,558; and Szostak et al. WO98/3170.

[0369] Non-antibody binding molecules can be produced as dimers or multimers to increase avidity for the target antigen. For example, the antigen binding domain is expressed as a fusion with a constant region (Fc) of an antibody that forms Fc-Fc dimers. See, *e.g.,* U.S. Pat. No. 7,115,396, the entire contents of which are incorporated herein by reference.

[0370] The therapeutic methods of the invention also may be practiced through the use of antibody fragments and antibody mimetics. As detailed below, a wide variety of antibody fragment and antibody mimetic technologies have now been developed and are widely known in the art. While a number of these technologies, such as domain antibodies, Nanobodies, and UniBodies make use of fragments of, or other modifications to, traditional antibody structures, there are also alternative technologies, such as Adnectins, Affibodies, DARPins, Anticalins, Avimers, and Versabodies that employ binding structures that, while they mimic traditional antibody binding, are generated from and function via distinct mechanisms. Some of these alternative structures are reviewed in Gill and Damle (2006) 17: 653-658.

[0371] Domain Antibodies (dAbs) are the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy (VH) or light (VL) chains of human antibodies. Domantis has developed a series of large and highly functional libraries of fully human VH and VL dAbs (more than ten billion different sequences in each library), and uses these libraries to select dAbs that are specific to therapeutic targets. In contrast to many conventional antibodies, domain antibodies are well expressed in bacterial, yeast, and mammalian cell systems. Further details of domain antibodies and methods of production thereof may be obtained by reference to U.S. Patent 6,291,158; 6,582,915; 6,593,081; 6,172,197; 6,696,245; U.S. Serial No. 2004/0110941; European patent application No. 1433846 and European Patents 0368684 & 0616640; WO05/035572, WO04/101790, WO04/081026, WO04/058821, WO04/003019 and WO03/002609.

[0372] Nanobodies are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3). Importantly, the cloned and isolated VHH domain is a perfectly stable polypeptide harboring the full antigen-binding capacity of the original heavy-chain antibody. Nanobodies have a high homology with the VH domains of human antibodies and can be further humanized without any loss of activity.

[0373] Nanobodies are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts, *e.g.,* E. coli (see, *e.g.,* U.S. 6,765,087), molds (for example Aspergillus or Trichoderma) and yeast (for example Saccharomyces, Kluyveromyces, Hansenula or Pichia) (see, *e.g.,* U.S. 6,838,254,). The production process is scalable

and multi-kilogram quantities of Nanobodies have been produced. Because Nanobodies exhibit a superior stability compared with conventional antibodies, they can be formulated as a long shelf-life, ready-to-use solution.

[0374] The Nanoclone method (see, *e.g.,* WO 06/079372,) is a proprietary method for generating Nanobodies against a desired target, based on automated high-throughout selection of B-cells and could be used in the context of the instant invention.

[0375] UniBodies are another antibody fragment technology, however this one is based upon the removal of the hinge region of IgG4 antibodies. The deletion of the hinge region results in a molecule that is essentially half the size of traditional IgG4 antibodies and has a univalent binding region rather than the bivalent binding region of IgG4 antibodies. It is also well known that IgG4 antibodies are inert and thus do not interact with the immune system, which may be advantageous for the treatment of diseases where an immune response is not desired, and this advantage is passed onto UniBodies. Further details of UniBodies may be obtained by reference to patent application WO2007/059782.

[0376] Adnectin molecules are engineered binding proteins derived from one or more domains of the fibronectin protein. In one embodiment, adnectin molecules are derived from the fibronectin type 21 domain by altering the native protein which is composed of multiple beta strands distributed between two beta sheets. Depending on the originating tissue, fibronectin may contain multiple type 21 domains which may be denoted, *e.g.,* ^{1}Fn3, ^{2}Fn3, ^{3}Fn3, etc. Adnectin molecules may also be derived from polymers of ^{10}Fn3 related molecules rather than a simple monomeric ^{10}Fn3 structure.

[0377] Although the native ^{10}Fn3 domain typically binds to integrin, ^{10}Fn3 proteins adapted to become adnectin molecules are altered so to bind antigens of interest, *e.g.,* a marker(s). In one embodiment, the alteration to the ^{10}Fn3 molecule comprises at least one mutation to a beta strand. In a preferred embodiment, the loop regions which connect the beta strands of the ^{10}Fn3 molecule are altered to bind to an antigen of interest, *e.g.,* a marker(s).

[0378] The alterations in the ^{10}Fn3 may be made by any method known in the art including, but not limited to, error prone PCR, site-directed mutagenesis, DNA shuffling, or other types of recombinational mutagenesis which have been referenced herein. In one example, variants of the DNA encoding the ^{10}Fn3 sequence may be directly synthesized *in vitro,* and later transcribed and translated *in vitro* or *in vivo.* Alternatively, a natural ^{10}Fn3 sequence may be isolated or cloned from the genome using standard methods (as performed, *e.g.,* in U.S. Pat. Application No. 20070082365), and then mutated using mutagenesis methods known in the art.

[0379] An aptamer is another type of antibody-mimetic which may be used in the methods of the present invention. Aptamers are typically small nucleotide polymers that bind to specific molecular targets. Aptamers may be single or double stranded nucleic acid molecules (DNA or RNA), although DNA based aptamers are most commonly double stranded. There is no defined length for an aptamer nucleic acid; however, aptamer molecules are most commonly between 15 and 40 nucleotides long.

[0380] Aptamers may be generated using a variety of techniques, but were originally developed using in vitro selection (Ellington and Szostak. (1990) Nature. 346(6287):818-22) and the SELEX method (systematic evolution of ligands by exponential enrichment) (Schneider et al. 1992. J Mol Biol. 228(3):862-9). Other methods to make and uses of aptamers have been published including Klussmann. The Aptamer Handbook: Functional Oligonucleotides and Their Applications. ISBN: 978-3-527-31059-3; Ulrich et al. 2006. Comb Chem High Throughput Screen 9(8):619-32; Cerchia and de Franciscis. 2007. Methods Mol Biol. 361:187-200; Ireson and Kelland. 2006. Mol Cancer Ther. 2006 5(12):2957-62; US Pat. Nos.: 5582981; 5840867; 5756291; 6261783; 6458559; 5792613; 6111095; and US Pat. App. Nos.: 11/482,671; 11/102,428; 11/291,610; and 10/627,543.

[0381] Aptamer molecules made from peptides instead of nucleotides may also be used in the methods of the invention. Peptide aptamers share many properties with nucleotide aptamers (*e.g.*, small size and ability to bind target molecules with high affinity) and they may be generated by selection methods that have similar principles to those used to generate nucleotide aptamers, for example Baines and Colas. 2006. Drug Discov Today. 11(7-8):334-41; and Bickle et al. 2006. Nat Protoc. 1(3):1066-91.

[0382] Affibody molecules represent a class of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG-binding domains of staphylococcal protein A. This three helix bundle domain has been used as a scaffold for the construction of combinatorial phagemid libraries, from which Affibody variants that target the desired molecules can be selected using phage display technology (Nord K, et al. Nat Biotechnol 1997;15:772-7. Ronmark J, et al., Eur J Biochem 2002;269:2647-55). Further details of Affibodies and methods of production thereof may be obtained by reference to U.S. Patent No. 5,831,012.

[0383] DARPins (Designed Ankyrin Repeat Proteins) are one example of an antibody mimetic DRP (Designed Repeat Protein) technology that has been developed to exploit the binding abilities of non-antibody polypeptides. Repeat proteins such as ankyrin or leucine-rich repeat proteins, are ubiquitous binding molecules, which occur, unlike antibodies, intra- and extracellularly. Their unique modular architecture features repeating structural units (repeats), which stack together to form elongated repeat domains displaying variable and modular target-binding surfaces. Based on this modularity, combinatorial libraries of polypeptides with highly diversified binding specificities can be generated. This strategy includes the consensus design of self-compatible repeats displaying variable surface residues and their random assembly into repeat domains.

[0384] Additional information regarding DARPins and other DRP technologies can be found in U.S. Patent Application Publication No. 2004/0132028 and International Patent Application Publication No. WO 02/20565.

[0385] Anticalins are an additional antibody mimetic technology, however in this case the binding specificity is derived from lipocalins, a family of low molecular weight proteins that are naturally and abundantly expressed in human tissues and body fluids. Lipocalins have evolved to perform a range of functions *in vivo* associated with the physiological transport and storage of chemically sensitive or insoluble compounds. Lipocalins have a robust intrinsic structure comprising a highly conserved β-barrel which supports four loops at one terminus of the protein. These loops form the entrance to a binding pocket and conformational differences in this part of the molecule account for the variation in binding specificity between individual lipocalins.

[0386] Lipocalins are cloned and their loops are subjected to engineering in order to create Anticalins. Libraries of structurally diverse Anticalins have been generated and Anticalin display allows the selection and screening of binding function, followed by the expression and production of soluble protein for further analysis in prokaryotic or eukaryotic systems. Studies have successfully demonstrated that Anticalins can be developed that are specific for virtually any human target protein can be isolated and binding affinities in the nanomolar or higher range can be obtained.

[0387] Anticalins can also be formatted as dual targeting proteins, so-called Duocalins. A Duocalin binds two separate therapeutic targets in one easily produced monomeric protein using standard manufacturing processes while retaining target specificity and affinity regardless of the structural orientation of its two binding domains.

[0388] Additional information regarding Anticalins can be found in U.S. Patent No. 7,250,297 and International Patent Application Publication No. WO 99/16873.

[0389] Another antibody mimetic technology useful in the context of the instant invention are Avimers. Avimers are evolved from a large family of human extracellular receptor domains by *in* vitro exon shuffling and phage display, generating multidomain proteins with binding and inhibitory properties. Linking multiple independent binding domains has been shown to create avidity and results in improved affinity and specificity compared with conventional single-epitope binding proteins. Other potential advantages include simple and efficient production of multitarget-specific molecules in Escherichia coli, improved thermostability and resistance to proteases. Avimers with sub-nanomolar affinities have been obtained against a variety of targets.

[0390] Additional information regarding Avimers can be found in U.S. Patent Application Publication Nos. 2006/0286603, 2006/0234299, 2006/0223114, 2006/0177831, 2006/0008844, 2005/0221384, 2005/0164301, 2005/0089932, 2005/0053973, 2005/0048512, 2004/0175756.

[0391] Versabodies are another antibody mimetic technology that could be used in the context of the instant invention. Versabodies are small proteins of 3-5 kDa with >15% cysteines, which form a high disulfide density scaffold, replacing the hydrophobic core that typical proteins have. The replacement of a large number of hydrophobic amino acids, comprising the hydrophobic core, with a small number of disulfides results in a protein that is smaller, more hydrophilic (less aggregation and non-specific binding), more resistant to proteases and heat, and has a lower density of T-cell epitopes, because the residues that contribute most to MHC presentation are hydrophobic. All four of these properties are well-known to affect immunogenicity, and together they are expected to cause a large decrease in immunogenicity.

[0392] Additional information regarding Versabodies can be found in U.S. Patent Application Publication No. 2007/0191272.

[0393] SMIPs™ (Small Modular ImmunoPharmaceuticals-Trubion Pharmaceuticals) engineered to maintain and optimize target binding, effector functions, *in vivo* half-life, and expression levels. SMIPS consist of three distinct modular domains. First they contain a binding domain which may consist of any protein which confers specificity (*e.g.*, cell surface receptors, single chain antibodies, soluble proteins, etc). Secondly, they contain a hinge domain which serves as a flexible linker between the binding domain and the effector domain, and also helps control multimerization of the SMIP drug. Finally, SMIPS contain an effector domain which may be derived from a variety of molecules including Fc domains or other specially designed proteins. The modularity of the design, which allows the simple construction of SMIPs with a variety of different binding, hinge, and effector domains, provides for rapid and customizable drug design.

[0394] More information on SMIPs, including examples of how to design them, may be found in Zhao et al. (2007) Blood 110:2569-77 and the following U.S. Pat. App. Nos. 20050238646; 20050202534; 20050202028; 20050202023; 20050202012; 20050186216; 20050180970; and 20050175614.

[0395] In another aspect, the methods of the present invention employ immunoconjugate agents that target a marker(s) and which inhibit or down-modulate the marker(s). Agents that can be targeted to a marker(s) include, but are not limited to, cytotoxic agents, antiinflammatory agents, *e.g.,* a steroidal or nonsteroidal inflammatory agent, or a cytotoxin antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0396] In another embodiment, marker(s) modulator employed in the methods of the invention are small molecules.

As used herein, the term "small molecule" is a term of the art and includes molecules that are less than about 7500, less than about 5000, less than about 1000 molecular weight or less than about 500 molecular weight, and inhibit marker(s) activity. Exemplary small molecules include, but are not limited to, small organic molecules (*e.g.,* Cane et al. 1998. Science 282:63), and natural product extract libraries. In another embodiment, the compounds are small, organic non-peptidic compounds. Like antibodies, these small molecule inhibitors indirectly or directly inhibit the activity of a marker(s).

[0397] In another embodiment, the marker(s) modulator employed in the methods of the present invention is an antisense nucleic acid molecule that is complementary to a gene encoding a marker(s) or to a portion of that gene, or a recombinant expression vector encoding the antisense nucleic acid molecule. As used herein, an "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid.

[0398] The use of antisense nucleic acids to down-modulate the expression of a particular protein in a cell is well known in the art (see *e.g.,* Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Vol. 1(1) 1986; Askari, F.K. and McDonnell, W.M. (1996) N. Eng. J. Med. 334:316-318; Bennett, M.R. and Schwartz, S.M. (1995) Circulation 92:1981-1993; Mercola, D. and Cohen, J.S. (1995) Cancer Gene Ther. 2:47-59; Rossi, J.J. (1995) Br. Med. Bull. 51:217-225; Wagner, R.W. (1994) Nature 372:333-335). An antisense nucleic acid molecule comprises a nucleotide sequence that is complementary to the coding strand of another nucleic acid molecule (*e.g.,* an mRNA sequence) and accordingly is capable of hydrogen bonding to the coding strand of the other nucleic acid molecule. Antisense sequences complementary to a sequence of an mRNA can be complementary to a sequence found in the coding region of the mRNA, the 5' or 3' untranslated region of the mRNA or a region bridging the coding region and an untranslated region (*e.g.,* at the junction of the 5' untranslated region and the coding region). Furthermore, an antisense nucleic acid can be complementary in sequence to a regulatory region of the gene encoding the mRNA, for instance a transcription initiation sequence or regulatory element. Preferably, an antisense nucleic acid is designed so as to be complementary to a region preceding or spanning the initiation codon on the coding strand or in the 3' untranslated region of an mRNA.

[0399] Antisense nucleic acids can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of marker(s) mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of marker(s) mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of marker(s) mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length.

[0400] An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.,* an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.,* RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0401] The antisense nucleic acid molecules that can be utilized in the methods of the present invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a marker(s) to thereby inhibit expression by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.,* by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also

be delivered to cells using vectors well known in the art and described in, for example, US20070111230. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[0402] In yet another embodiment, the antisense nucleic acid molecule employed by the methods of the present invention can include an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215:327-330).

[0403] In another embodiment, an antisense nucleic acid used in the methods of the present invention is a compound that mediates RNAi. RNA interfering agents include, but are not limited to, nucleic acid molecules including RNA molecules which are homologous to a marker(s) or a fragment thereof, "short interfering RNA" (siRNA), "short hairpin" or "small hairpin RNA" (shRNA), and small molecules which interfere with or inhibit expression of a target gene by RNA interference (RNAi). RNA interference is a post-transcriptional, targeted gene-silencing technique that uses double-stranded RNA (dsRNA) to degrade messenger RNA (mRNA) containing the same sequence as the dsRNA (Sharp, P.A. and Zamore, P.D. 287, 2431-2432 (2000); Zamore, P.D., et al. Cell 101, 25-33 (2000). Tuschl, T. et al. Genes Dev. 13, 3191-3197 (1999)). The process occurs when an endogenous ribonuclease cleaves the longer dsRNA into shorter, 21- or 22-nucleotide-long RNAs, termed small interfering RNAs or siRNAs. The smaller RNA segments then mediate the degradation of the target mRNA. Kits for synthesis of RNAi are commercially available from, *e.g.*, New England Biolabs and Ambion. In one embodiment one or more of the chemistries described above for use in antisense RNA can be employed.

[0404] In still another embodiment, an antisense nucleic acid is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.,* hammerhead ribozymes (described in Haselhoff and Gerlach, 1988, Nature 334:585-591) can be used to catalytically cleave marker(s) mRNA transcripts to thereby inhibit translation of the marker(s) mRNA.

[0405] Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a marker(s) (*e.g.*, the promoter and/or enhancers) to form triple helical structures that prevent transcription of the marker(s) gene. See generally, Helene, C., 1991, Anticancer Drug Des. 6(6):569-84; Helene, C. et al., 1992, Ann. N.Y. Acad. Sci. 660:27-36; and Maher, L.J., 1992, Bioassays 14(12):807-15.

[0406] In another embodiment, the marker(s) modulator used in the methods of the present invention is a fusion protein or peptidic compound derived from the marker(s) amino acid sequence. In particular, the inhibitory compound comprises a fusion protein or a portion of a marker(s) (or a mimetic thereof) that mediates interaction of the marker(s) with a target molecule such that contact of the marker(s) with this fusion protein or peptidic compound competitively inhibits the interaction of the marker(s) with the target molecule. Such fusion proteins and peptidic compounds can be made using standard techniques known in the art. For example, peptidic compounds can be made by chemical synthesis using standard peptide synthesis techniques and then introduced into cells by a variety of means known in the art for introducing peptides into cells (*e.g.*, liposome and the like).

[0407] The *in vivo* half-life of the fusion protein or peptidic compounds of the invention can be improved by making peptide modifications, such as the addition of N-linked glycosylation sites into the marker(s) or conjugating the marker(s) to poly(ethylene glycol) (PEG; pegylation), *e.g.*, via lysine-monopegylation. Such techniques have proven to be beneficial in prolonging the half-life of therapeutic protein drugs. It is expected that pegylation of marker(s) polypeptides of the invention may result in similar pharmaceutical advantages.

[0408] In addition, pegylation can be achieved in any part of a polypeptide of the invention by the introduction of a nonnatural amino acid. Certain nonnatural amino acids can be introduced by the technology described in Deiters et al., J Am Chem Soc 125:11782-11783, 2003; Wang and Schultz, Science 301:964-967, 2003; Wang et al., Science 292:498-500, 2001; Zhang et al., Science 303:371-373, 2004 or in US Patent No. 7,083,970. Briefly, some of these expression systems involve site-directed mutagenesis to introduce a nonsense codon, such as an amber TAG, into the open reading frame encoding a polypeptide of the invention. Such expression vectors are then introduced into a host that can utilize a tRNA specific for the introduced nonsense codon and charged with the nonnatural amino acid of choice. Particular nonnatural amino acids that are beneficial for purpose of conjugating moieties to the polypeptides of the invention include those with acetylene and azido side chains. Marker(s) polypeptides containing these novel amino acids can then be pegylated at these chosen sites in the protein.

2. *Stimulatory Agents*

[0409] According to a modulatory method of the invention, the expression and/or activity of a marker(s) is stimulated in a cell or subject by contacting the cell with (or administering to a subject) a stimulatory agent. Stimulatory agents of the invention can be, for example, molecules that act to stimulate or increase the expression and/or activity of the

marker(s).

[0410] Examples of such stimulatory agents include active marker(s) polypeptide and nucleic acid molecules encoding the marker(s) that are introduced into the cell to increase expression and/or activity of the marker in the cell. A preferred stimulatory agent is a nucleic acid molecule encoding a marker(s) polypeptide, wherein the nucleic acid molecule is introduced into the cell in a form suitable for expression of the active marker(s) polypeptide in the cell. To express a marker(s) polypeptide in a cell, typically a marker(s)-encoding cDNA (full length or partial cDNA sequence) is first introduced into a recombinant expression vector using standard molecular biology techniques, and the vector may be transfected into cells using standard molecular biology techniques. A cDNA can be obtained, for example, by amplification using the polymerase chain reaction (PCR), using primers based on the marker(s) nucleotide sequence or by screening an appropriate cDNA library.

[0411] The nucleic acids for use in the methods of the invention can also be prepared, *e.g.,* by standard recombinant DNA techniques. A nucleic acid of the invention can also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which has been automated in commercially available DNA synthesizers (See *e.g.,* Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071.

[0412] In one embodiment, a nucleic acid molecule encoding a marker(s) may be present in an inducible construct. In another embodiment, a nucleic acid molecule encoding marker(s) may be present in a construct which leads to constitutive expression. In one embodiment, a nucleic acid molecule encoding marker(s) may be delivered to cells, or to subjects, in the absence of a vector.

[0413] A nucleic acid molecule encoding marker(s) may be delivered to cells or to subjects using a viral vector, preferably one whose use for gene therapy is well known in the art. Techniques for the formation of vectors or virions are generally described in "Working Toward Human Gene Therapy," Chapter 28 in Recombinant DNA, 2nd Ed., Watson, J. D. et al., eds., New York: Scientific American Books, pp. 567-581 (1992). An overview of suitable viral vectors or virions is provided in Wilson, J. M., Clin. Exp. Immunol. 107(Suppl. 1):31-32 (1997), as well as Nakanishi, M., Crit. Rev. Therapeu. Drug Carrier Systems 12:263-310 (1995); Robbins, P. D., et al., Trends Biotechnol. 16:35-40 (1998); Zhang, J., et al., Cancer Metastasis Rev. 15:385-401(1996); and Kramm, C. M., et al., Brain Pathology 5:345-381 (1995). Such vectors may be derived from viruses that contain RNA (Vile, R. G., et al., Br. Med Bull. 51:12-30 (1995)) or DNA (Ali M., et al., Gene Ther. 1:367-384 (1994)).

[0414] Examples of viral vector systems utilized in the gene therapy art and, thus, suitable for use in the present invention, include the following: retroviruses (Vile, R. G., supra; U.S. Pat. Nos. 5,741,486 and 5,763,242); adenoviruses (Brody, S. L., et al., Ann. N.Y. Acad. Sci. 716: 90-101 (1994); Heise, C. et al., Nat. Med. 3:639-645 (1997)); adenoviral/retroviral chimeras (Bilbao, G., et al., FASEB J. 11:624-634 (1997); Feng, M., et al., Nat. Biotechnol. 15:866-870 (1997)); adeno-associated viruses (Flotte, T. R. and Carter, B. J., Gene Ther. 2:357-362 (1995); U.S. Patent No. 5,756,283); herpes simplex virus I or II (Latchman, D. S., Mol. Biotechnol. 2:179-195 (1994); U.S. Patent No.5,763,217; Chase, M., et al., Nature Biotechnol. 16:444-448 (1998)); parvovirus (Shaughnessy, E., et al., Semin Oncol. 23:159-171 (1996)); reticuloendotheliosis virus (Donburg, R., Gene Therap. 2:301-310 (1995)). Extrachromosomal replicating vectors may also be used in the gene therapy methods of the present invention. Such vectors are described in, for example, Calos, M. P. (1996) Trends Genet. 12:463-466, the entire contents of which are incorporated herein by reference. Other viruses that can be used as vectors for gene delivery include poliovirus, papillomavirus, vaccinia virus, lentivirus, as well as hybrid or chimeric vectors incorporating favorable aspects of two or more viruses (Nakanishi, M. (1995) Crit. Rev. Therapeu. Drug Carrier Systems 12:263-310; Zhang, J., et al. (1996) Cancer Metastasis Rev. 15:385-401; Jacoby, D. R., et al. (1997) Gene Therapy 4:1281-1283).

[0415] The term "AAV vector" refers to a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, or AAVX7. "rAAV vector" refers to a vector that includes AAV nucleotide sequences as well as heterologous nucleotide sequences. rAAV vectors require only the 145 base terminal repeats in cis to generate virus. All other viral sequences are dispensable and may be supplied in trans (Muzyczka (1992) Curr. Topics Microbiol. Immunol. 158:97). Typically, the rAAV vector genome will only retain the inverted terminal repeat (ITR) sequences so as to maximize the size of the transgene that can be efficiently packaged by the vector. The ITRs need not be the wild-type nucleotide sequences, and may be altered, *e.g.,* by the insertion, deletion or substitution of nucleotides, as long as the sequences provide for functional rescue, replication and packaging. In particular embodiments, the AAV vector is an AAV2/5 or AAV2/8 vector. Suitable AAV vectors are described in, for example, U.S. Patent No. 7,056,502 and Yan et al. (2002) J. Virology 76(5):2043-2053.

[0416] As used herein, the term "lentivirus" refers to a group (or genus) of retroviruses that give rise to slowly developing disease. Viruses included within this group include HIV (human immunodeficiency virus; including but not limited to HIV type 1 and HIV type 2), the etiologic agent of the human acquired immunodeficiency syndrome (AIDS); visna-maedi, which causes encephalitis (visna) or pneumonia (maedi) in sheep; the caprine arthritis-encephalitis virus, which causes immune deficiency, arthritis, and encephalopathy in goats; equine infectious anemia virus (EIAV), which causes autoimmune hemolytic anemia, and encephalopathy in horses; feline immunodeficiency virus (FIV), which causes immune

deficiency in cats; bovine immune deficiency virus (BIV), which causes lymphadenopathy, lymphocytosis, and possibly central nervous system infection in cattle; and simian immunodeficiency virus (SIV), which cause immune deficiency and encephalopathy in subhuman primates. Diseases caused by these viruses are characterized by a long incubation period and protracted course. Usually, the viruses latently infect monocytes and macrophages, from which they spread to other cells. HIV, FIV, and SIV also readily infect T lymphocytes (i.e., T-cells). In one embodiment of the invention, the lentivirus is not HIV.

[0417] As used herein, the term "adenovirus" ("Ad") refers to a group of double-stranded DNA viruses with a linear genome of about 36 kb. See, *e.g.,* Berkner et al., Curr. Top. Microbiol. Immunol., 158: 39-61 (1992). In some embodiments, the adenovirus-based vector is an Ad-2 or Ad-5 based vector. See, *e.g.*, Muzyczka, Curr. Top. Microbiol. Immunol., 158: 97-123, 1992; Ali et al., 1994 Gene Therapy 1: 367-384; U.S. Pat. Nos. 4,797,368, and 5,399,346. Suitable adenovirus vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types. Additionally, introduced adenovirus DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenovirus genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Haj-Ahmand et al. J. Virol. 57, 267-273 [1986]).

[0418] In one embodiment, an adenovirus is a replication defective adenovirus. Most replication-defective adenoviral vectors currently in use have all or parts of the viral E1 and E3 genes deleted but retain as much as 80% of the adenovirus genetic material. Adenovirus vectors deleted for all viral coding regions are also described by Kochanek et al. and Chamberlain et al. (U.S. Pat. No. 5,985,846 and U.S. Pat. No. 6,083,750). Such viruses are unable to replicate as viruses in the absence of viral products provided by a second virus, referred to as a "helper" virus.

[0419] In one embodiment, an adenoviral vector is a "gutless" vector. Such vectors contain a minimal amount of adenovirus DNA and are incapable of expressing any adenovirus antigens (hence the term "gutless"). The gutless replication defective Ad vectors provide the significant advantage of accommodating large inserts of foreign DNA while completely eliminating the problem of expressing adenoviral genes that result in an immunological response to viral proteins when a gutless replication defective Ad vector is used in gene therapy. Methods for producing gutless replication defective Ad vectors have been described, for example, in U.S. Pat. No. 5,981,225 to Kochanek et al., and U.S. Pat. Nos. 6,063,622 and 6,451,596 to Chamberlain et al; Parks et al., PNAS 93:13565 (1996) and Lieber et al., J. Virol. 70:8944-8960 (1996).

[0420] In another embodiment, an adenoviral vector is a "conditionally replicative adenovirus" ("CRAds"). CRAds are genetically modified to preferentially replicate in specific cells by either (i) replacing viral promoters with tissue specific promoters or (ii) deletion of viral genes important for replication that are compensated for by the target cells only. The skilled artisan would be able to identify epithelial cell specific promoters.

[0421] Other art known adenoviral vectors may be used in the methods of the invention. Examples include Ad vectors with recombinant fiber proteins for modified tropism (as described in, *e.g.,* van Beusechem et al., 2000 Gene Ther. 7: 1940-1946), protease pre-treated viral vectors (as described in, *e.g.*, Kuriyama et al., 2000 Hum. Gene Ther. 11: 2219-2230), E2a temperature sensitive mutant Ad vectors (as described in, *e.g.,* Engelhardt et al., 1994 Hum. Gene Ther. 5: 1217-1229), and "gutless" Ad vectors (as described in, *e.g.*, Armentano et al., 1997 J. Virol. 71: 2408-2416; Chen et al., 1997 Proc. Nat. Acad. Sci. USA 94: 1645-1650; Schieder et al., 1998 Nature Genetics 18: 180-183).

[0422] The vector will include one or more promoters or enhancers, the selection of which will be known to those skilled in the art. Suitable promoters include, but are not limited to, the retroviral long terminal repeat (LTR), the SV40 promoter, the human cytomegalovirus (CMV) promoter, and other viral and eukaryotic cellular promoters known to the skilled artisan.

[0423] Guidance in the construction of gene therapy vectors and the introduction thereof into affected subjects for therapeutic purposes may be obtained in the above-referenced publications, as well as in U.S. Patent Nos. 5,631,236, 5,688,773, 5,691,177, 5,670,488, 5,529,774, 5,601,818, and PCT Publication No. WO95/06486.

[0424] Generally, methods are known in the art for viral infection of the cells of interest. The virus can be placed in contact with the cell of interest or alternatively, can be injected into a subject suffering from a retinal disorder, for example, as described in United States Provisional Patent Application No. 61/169,835 and PCT Application No. PCT/US09/053730.

[0425] Gene therapy vectors comprising a nucleic acid molecule encoding a marker(s) can be delivered to a subject or a cell by any suitable method in the art, for example, intravenous injection, local administration, *e.g.*, application of the nucleic acid in a gel, oil, or cream, (see, *e.g.,* U.S. Patent No. 5,328,470), stereotactic injection (see, *e.g.,* Chen et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91:3054), gene gun, or by electroporation (see, *e.g.*, Matsuda and Cepko (2007) Proc. Natl. Acad. Sci. U.S.A. 104:1027), using lipid-based transfection reagents, or by any other suitable transfection method.

[0426] As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized

techniques for introducing foreign nucleic acid (*e.g.,* DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection (*e.g.*, using commercially available reagents such as, for example, LIPOFECTIN® (Invitrogen Corp., San Diego, CA), LIPOFECTAMINE® (Invitrogen), FUGENE® (Roche Applied Science, Basel, Switzerland), JETPEI™ (Polyplustransfection Inc., New York, NY), EFFECTENE® (Qiagen, Valencia, CA), DREAMFECT™ (OZ Biosciences, France) and the like), or electroporation (*e.g.*, in vivo electroporation). Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

[0427] In one embodiment, a marker(s) is delivered to a subject or cells in the form of a peptide or protein. In order to produce such peptides or proteins, recombinant expression vectors of the invention can be designed for expression of one or more marker(s) proteins, and/or portion(s) thereof in prokaryotic or eukaryotic cells. For example, one or more marker proteins and/or portion(s) thereof can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

[0428] In one embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include retinal cell-type-specific promoters (*e.g.*, rhodopsin regulatory sequences, Cabp5, Cralbp, Nrl, Crx, Ndrg4, clusterin, Rax, Hes1 and the like (Matsuda and Cepko, supra)), the albumin promoter (liver-specific, Pinkert et al. (1987) Genes Dev. 1:268), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) Proc. Natl. Acad. Sci. U.S.A. 86:5473). Developmentally-regulated promoters are also encompassed, for example the α-fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537).

[0429] Application of the methods of the invention for the treatment and/or prevention of a retinal disorder can result in curing the disorder, decreasing at least one symptom associated with the disorder, either in the long term or short term or simply a transient beneficial effect to the subject. Accordingly, as used herein, the terms "treat," "treatment" and "treating" include the application or administration of agents, as described herein, to a subject who is suffering from Brucellosis, or who is susceptible to such conditions with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting such conditions or at least one symptom of such conditions. As used herein, the condition is also "treated" if recurrence of the condition is reduced, slowed, delayed or prevented.

[0430] A modulatory agent, such as a chemical compound, can be administered to a subject as a pharmaceutical composition. Such compositions typically comprise the modulatory agent and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be prepared as described above.

E. Methods of Identifying Brucellosis Biomarkers

[0431] The present invention further provides methods for identifying biomarkers useful as markers for, *e.g.*, disease (prognostics and diagnostics), therapeutic effectiveness of a drug (theranostics) and of drug toxicity. For example, as described above, the markers described herein and the markers identified using the methods for biomarker discovery are useful for, *e.g.*, determining whether a subject has Brucellosis; determining whether a subject has Brucellosis or Q-fever; determining whether a subject has Brucellosis or Lyme Disease; monitoring the effectiveness of a therapy for treating Brucellosis, reducing or slowing down the progression of Bruceelosis, and/or reducing or inhibiting the development of complications associated with Brucellosis in a subject; in screening assays to identify molecules which modulate, *e.g.*, decrease or increase, the level and/or activity of a marker(s) of the invention for *e.g.,* use as therapeutics.

[0432] Methods for identifying a Brucellosis marker are described in the working examples and include identifying proteins differentially expresed in cells that are not infected with *Brucella* and cells infected with *Brucella,* thereby generating a provisional list of markers, determining the level of a marker in a sample form a control subject, *e.g.,* a healthy subject, a subject having Q-fever, a subject having Lyme Disease, a subject having Salmonellosis, and determining the level of the marker in a test sample from a subject, *e.g.,* a subject having Brucellosis. A difference in the level of a marker in the control sample as compared to the level in the test sample, *e.g.,* a statistically significant level, identifies the marker as a Brucellosis marker.

IV. Kits of the Invention

[0433] The invention also provides kits for determining whether a subject has Brucellosis, Lyme Disease, or Q-Fever. Kits for monitoring the effectiveness of a treatment for Brucellosis, Q-Fever, or Lyme Disease, kits for determining whether a subject has Brucellosis or Q-Fever, kits for determining whether a subject has Brucellosis or Lyme Disease, lits for determining whether a subject has Q-Fever or Lyme Disease, and kits for determining whether a subject has Brucellosis or Q-Fever or Lyme Disease are also provided.

[0434] These kits include means for determining the level of one or more markers of the invention and instructions for use of the kit.

[0435] The kits of the invention may optionally comprise additional components useful for performing the methods of the invention. By way of example, the kits may comprise reagents for obtaining a biological sample from a subject, a control sample, one or more sample compartments, a diabetic therapeutic, and instructional material which describes performance of a method of the invention and tissue specific controls/standards.

[0436] The reagents for determining the level of one or more marker(s) can include, for example, buffers or other reagents for use in an assay for evaluating the level of one or more markers, *e.g.,* expression level (*e.g.,* at either the mRNA or protein level). The instructions can be, for example, printed instructions for performing the assay for evaluating the level of one or more marker(s) of the invention.

[0437] The reagents for isolating a biological sample from a subject can comprise one or more reagents that can be used to obtain a fluid or tissue from a subject, such as means for obtaining a saliva or blood.

[0438] The kits of the invention may further comprise reagents for culturing a sample obtained from a subject.

[0439] Preferably, the kits are designed for use with a human subject.

[0440] The present invention is further illustrated by the following examples which should not be construed as further limiting.

EXAMPLES

EXAMPLE I. Biomarker Identification

Materials and Methods

Infection conditions.

[0441] THP-1 cells were obtained from ATCC (#TIB-202) and routinely maintained in RPMI 1640 media containing 10% heat-inactivated fetal bovine serum. THP-1 cells were differentiated using Phorbol 12-Myristate 13-acetate (PMA) (Sigma, P-8139) at a final concentration of 10^{-7}M 24 hours before use in infection experiments. AC-1M88 (#ACC-457) and JAR (#ACC-462) cells were obtained from DSMZ and maintained in Hams-F12 + 10% FBS and RPMI-1640 + 10% FBS, respectively.

[0442] All experiments involving live *B. abortus* were conducted under Biosafety Level 3 (BSL3) conditions. Bacterial cultures were prepared as described (Lamontagne J, et al. (2009) J Proteome Res 8:1594-1609). *B. abortus* strains 2308 and 544 were used to infect human THP-1 macrophages at a multiplicity of infection (MOI) of 250, as described (Lamontagne J, et al. (2009) J Proteome Res 8:1594-1609). Bacteria were incubated with the host macrophages for 30 minutes, the extracellular bacteria were then removed, and the infection allowed to continue for 24 hours. The same bacterial culture protocols were used to prepare *B. abortus* 2308 for infection of the JAr and AC-1M88 human trophoblast cell lines. Bacteria were incubated with the host trophoblasts at an multiplicity of infection (MOI) of 250 for 24 hrs before the extracellular bacteria were removed and the infection allowed to continue for an additional 24 hrs. Lengthening the incubation period reliably allowed 85-100% of the trophoblasts per culture to be infected.

Immunofluorescence

[0443] THP-1 macrophages were seeded on microscope coverslips and infected with *B. abortus* 2308 or 544 as described above. Cells were then washed with phosphate-buffered saline and incubated with anti-LPS primary antibody for 30 minutes. This step was used to stain the extracellular bacteria. The cells were washed, fixed with cold 4% paraformaldehyde (Merck) for 20 min on ice, washed, incubated for 2 min with PBS-1% Triton X-100 (ACE Chemicals, #T935050), washed, and incubated with blocking buffer (5% Goat Serum, 5% Glycerol; 0.04% Na Azide in D-PBS) for 2 minutes. The cells were then incubated with anti-bovine-Rhodamine conjugated antibody (Jackson Immuno Research, #101-025-165) diluted 1/500) for 20 minutes, washed, incubated with blocking buffer for 30 min, washed, and incubated with the anti-LPS primary antibody for 30 min. This latter step was to allow staining of intracellular bacteria. The cells were washed, incubated with blocking buffer for 30 minutes, washed, and incubated with anti-bovine-FITC (KPL,

#02-12-06) for 20 min. The coverslips were washed with PBS, mounted using Prolong anti-fade kit (Molecular Probes, #P7481), and analyzed by fluorescence microscopy. Counts of green and red intracellular fluorescent bacteria were performed in at least 100 infected cells and expressed as a mean of green-red/red fluorescent bacteria per cell. The same protocol was used to determine *B. abortus* infectivity in the trophoblast cell lines, except that 2-well chamber slides (Fisher Scientific, #FSSP9772261) were used instead of the microscope coverslips.

Light membrane sample preparation

[0444] Six to eight independent replicates of infected and non-infected cultures for each cell line were independently prepared and processed. Secretory vesicle isolation was done in batches using the cell homogenization and density centrifugation methods modified from a previous study (Sicar K, et al, (2006) Clin Cancer Res 12:4178-4184). Briefly, cells were harvested by scraping, centrifuged and resuspended in homogenization buffer. They were homogenized using 15 strokes with a stainless steel dounce homogenizer (Wheaton Science, Millville NJ). The homogenate was adjusted to 1.4M sucrose. A 14ml SW40Ti ultra-clear centrifuge tube (Beckman Coulter #344060) was layered with homogenate followed by 4ml of 1.2M sucrose and topped with 0.8M sucrose. The samples were centrifuged for 2hrs at 155,000 xg at 40C and vesicles were harvested from the 0.8-1.2M interface. The vesicles were washed in 0.5M KCl followed by incubation in ammonium carbonate pH11. Vesicle content was separated from the vesicle membranes by centrifugation at 112,000 g. Protein yields were measured using the BCA Protein Assay (Pierce #23227). Secretory vesicle content yield from infected THP-1 macrophages was 0.85 μg per 10 million cells, and from uninfected macrophages it was 1.9 μg per 10 million cells. Infected AC-1M88 cells yielded 12.9 μg per 10 million cells, whereas uninfected cells yielded 7.8 μg per 10 million cells. Infected JAr cells yielded 20μg per 10 million cells whereas uninfected JAr cells yielded and 17.8μg per million cells. Western blot characterization of the starting cell line homogenates and secretory protein final products were done using antibodies against Na+ K+ ATPase (Upstate, #05-369), BIP (BD #610979), Nucleoporin p62 (BD #610498, PAN Actin (Neomarkers, #MS-1295P), GS-28 (BD #G83820), Tom20 (BD #612278), Beta-2-microglobulin (Abcam #Ab6608), and Brucella LPS (Hyperomics Farma #Ba05-0001).

Tandem mass spectrometry analysis

[0445] All samples were digested with trypsin. The resulting peptides were then separated by SCX into three fractions. Each of the three fractions per sample was analyzed by reversed phase liquid chromatography, coupled by electrospray to a Waters QTOF mass spectrometer (LC-MS). Components were detected and matched across all samples and compared for relative peak intensity. Peak intensity was normalized to account for small differences in protein concentration between samples. ANOVA was then applied to identify peptides that were differentially expressed between the groups of interest. High stringency thresholds were used to ensure the statistical significance of the identified peptides. All intensity values were log (base e) transformed with values < 0 replaced by 0. A subset of the samples was used to create an average sample (*i.e.* the Reference sample) against which all samples were then normalized. The normalization factors were chosen so that the median of log ratios between each sample and the Reference sample over all the peptides was adjusted to zero. For the macrophage analysis, a one-way ANOVA (infection status) was used to find the differentially expressed peptides. For the trophoblast analysis, a two-factor ANOVA (cell line, infection status, and the interaction between the two) was used. FDR (false detection rate) and q-value were calculated, based on the p-values obtained from the ANOVA, using Storey's method to make multiple testing adjustments (implemented in MATLAB). 'Post hoc' contrast analyses were conducted using Tukey's hsd method to calculate p-values associated with each pair wise comparison (Hochberg Y, Tamhane AC (1987) Multiple Comparison Procedures. Canada: Wiley & Sons). For the macrophage analysis, peptides meeting q-value $\leq$ 0.05, Tukey's hsd p-value $\leq$ 0.05, fold-change $\geq$ 2.0; peptide intensity $\geq$ 70 in at least 6 samples were selected. For the trophoblast analysis, peptides meeting q-value (either for the factor 'infection status' or for the interaction) < 0.05, Tukey's hsd p-value < 0.05, fold-change $\geq$ 2.0 were selected. Protein identification was accomplished by the analysis of replicate samples by tandem mass spectrometry (LC-MS/MS). Differentially expressed peptides were targeted for sequencing, and the resulting fragmentation patterns were matched to the corresponding peptide sequences found in a custom protein database using Mascot (Matrix Science) software. For the macrophages, the database was composed of the IPI (International Protein Index) human protein sequences and the NCBI protein sequences from *Brucella* species which infect humans (NCBI taxonomy ID 235, 29459, 29461, 36855, 120576 and 120577). Several strains of *Brucella* were included in the database, because the strain 544 had not yet been sequenced. For the trophoblasts, since only the sequenced strain 2308 was used the database was composed of the IPI (International Protein Index) human protein sequences and the NCBI protein sequences from Brucella melitensis biovar abortus 2308 species only (NCBI taxonomy ID 359391).

Biomarker candidate Selection

[0446] Biomarker candidates were drawn from the proteins upregulated in the infected host secretomes. They were further evaluated using the Meng importance score (Lamontagne J, et al. (2009) J Proteome Res 8:1594-1609), which ranked each peptide by their ability to separate the infected from non-infected samples, and a detailed literature review. The literature review focused on the known roles of each protein, association with brucellosis with other infectious diseases, or association with other relevant conditions which has been associated with aberrant trophoblast function, such as spontaneous abortion. The combination of the Meng scores and the literature annotation was used to produce a ranking of the biomarker candidates.

Enxyme-linked Immunosorbent Assay (ELISA)

[0447] Twenty-three candidates for which commercially ELISA kits were available, were evaluated (from manufacturer Uscn Life Science Inc.: IL-25 (#E91694Hu), CALU (#E91854Hu), SEPT6 (#E95866Hu), S100A11 (#E90568Hu), MYH10 (#E93423Hu), MAT2A (#E96469Hu), S100P (#E92054Hu), GPI (#E90725Hu), RPL23A (#E83643Hu), ANXA2 (#E91944Hu), RSAD2 (#E81762Hu), LYN (#E96523Hu), EVL (#E84820Hu), HNRNPU (#E95128Hu), ARHGDIA (#E94329Hu), SFRS2 (#E95102Hu), PFDN1 (#E92722Hu), and GSTP1 (#E91090Hu); from other manufacturers: ANXA5 (US Biological, #A2296-15 or Uscn Life Science Inc., #E90259Hu), PKM2 (ScheBo Biotech, #08), IL1B (Abcam, #ab46052 and Thermo Scientific, #EH2IL1B), and HSPA8 (StressMarq, #SKT-106). All ELISAs were performed according to manufacturer's instructions. An initial small scale analysis used 28 serum samples from subjects with acute, non-complicated brucellosis along with 28 age and gender matched healthy controls and 24 age and gender matched samples from subjects infected with Lyme disease (ProMedDx, Norton, MA, USA). Four biomarker candidates (ANXA5, MAT2A, RPL23A, and LYN) were further evaluated using 278 serum samples as described in Table 1.

Multiple reaction monitoring mass spectrometry

[0448] One MRM assay for the macrophage model of infection and one for the trophoblast model of infection was developed. The macrophage MRM assay contained 70 proteins upregulated after infection, represented by 149 peptides and the trophoblast MRM assay contained 123 proteins upregulated after infection represented by 254 peptides. Peptides were synthesized by JPT Peptide Technologies (Berlin, Germany). The synthesized peptides were resolubilized in 72/25 water/DMSO, pooled and diluted with water + 0.2 % formic acid to a final concentration of 2 nmol/mL. 5 uL of this solution was analyzed on a QTRAP 4000 mass spectrometer (ABSciex, Canada) using a 320 um x 150 mm, 5um particle size, Thermo Biobasic C18 column. A linear gradient of 10-40% acetonitrile (0.2% formic acid) in 30 minutes was used for peptide separation. MS/MS spectra of the synthetic peptides were acquired using selected reaction monitoring (SRM)-triggered MS/MS allowing the identification of peptide and peptide fragments (transitions). The two most intense fragment ions (b or y fragment ions only) in the MS/MS spectrum and its elution time were determined for each acquired peptide. The collision energy was then optimized for each of the chosen transitions. The CE values evaluated were the empirical calculated CE value and the empirical CE value -3 and +3. Study samples were then subjected to immunodepletion of high and medium abundance proteins using a Human IgY-14/SuperMix depletion column (Sigma, USA). Depleted samples were digested with trypsin overnight (Trypsin to protein ratio 1:10) and desalted by solid phase extraction using a 3M Empore C18 desalting plate. Samples were after analyzed by the MRM assay.

Expression analysis on MRM results

[0449] Expression analysis on MRM results was performed using essentially the same principles as for the tandem MS analysis described above, with the following adaptations for MRM analysis. All the statistical analyses were performed using R version 2.12.0, platform i386-pc-mingw32/i386 (32-bit). The calculation of q-values was done using function "qvalue" from Storey's package "qvalue" version 1.24.0 (Storey, John D. (2002). Journal of the Royal Statistical Society, Series B (Methodological) 64(3):479-498; Storey, John D. (2003). Annals of Statistics 31(6):2013-2035; R Development Core Team (2010). R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0). A limit of quantification (LOQ) (defined as an intensity value below which the measure is deemed unreliable) was determined empirically according to the instrument that was used for the study (QTrap), and was set to 10000, pre-normalization. The detection rate (DR), defined for each group that needed to be compared, was defined as the proportion of samples with a raw *(i.e.* pre-normalized) intensity value at least equal to the LOQ. Prior expression analysis, an outlier and pattern detection analysis was performed using a standard Principal Component Analysis (PCA) that was applied to the transformed intensities in order to visually assess any pattern in the data that are likely to be unrelated to sample condition. (The PCA was performed as follow. If L is the matrix of transformed intensities with samples as rows and transitions as intensities, then we define L* as the matrix of intensities doubly centered. Double centering means that each cell of the

matrix has both its column and row mean subtracted from it, to which we also add back the grand mean - i.e. the mean of all intensity values. This is done to remove principal component dependencies to systematic shifts in intensity levels (*i.e.* transitions that are over all samples less or more intense than other transitions, or samples that are, over all transitions, more or less intense than other samples). The SVD L* = UDVT was performed, with U containing the components for samples. This analysis identified a clustering of samples, based on their region of origin (data not shown). Therefore, samples were always compared to their corresponding regional controls. Differential intensity ratios (DI) were then calculated for each transition, for two-group comparisons (disease vs corresponding regional controls), as the ratio of the median normalized intensities of each group. Prior to calculating the DI, all intensity values that were below the LOQ quantity in the raw (i.e. prior to normalization) data were replaced by the half-LOQ value. ANOVA or t-test with gender as covariate were used.

Normalization and box plots generation

[0450] Samples were normalized, using the regional non-infected controls as reference. The actual normalization was based on two regression models predicting intensity level:

$$M1: I_i = \mu_1 + \gamma S_i + \varepsilon_i, \hat{I}_{i1} = \mu_1 + \gamma S_i$$

$$M2: I_i = \mu_2 + \varepsilon_i, \hat{I}_{i2} = \mu_2$$

[0451] In each model, I and S are respectively intensity value (or ELISA value) and Sample Source (i.e. France, Spain, USA). The parameters were estimated using the control samples only, and the following normalization was applied on all samples:
$I_{it} = I_i - (\hat{I}_{i1} - \hat{I}_{i2}) = I_i - \hat{I}_{i1} + \hat{I}_{i2}$, applied to each transition/ELISA. This was done independently for MRM and ELISA. For each protein, normalized intensities for each sample, grouped by condition, were then plotted as box plots, using XYZ software.

Panel Definition

[0452] Area under the ROC curve (AUC) was computed from bootstrap. Boostrap samples (n) were selected with replacement *i.e.* take a sample at random, then a second - with the first selected sample being possibly selected again, and so on. Some samples, called out-of-bag, were left out by design. The panel was built on the bootstrap samples and evaluated on the out-of-bag samples by calculating AUC. The process was repeated 100 times. The reported AUC was the average of the 100 AUCs. Each protein was represented by a single MRM transition. Transitions with a DR lower than 80% and transitions that did not trend according to the protein DI were filtered out. The transition selected for a protein was the one with the highest DR. Logistic Regression models were built with the secretome proteins as explanatory variables. All combinations of 1 to 6 proteins were systematically fitted into such logistic models. These combinations were either used as is to build the models, or with the addition of either or both of the proteins measured by ELISA that were found to have appropriate performance. The proteins were ranked by their propensity to be a good team player. For k from 1 to 6, combinations were ranked by their AUC, and for each protein, the mean rank of the combinations they appear in, for a given k, was calculated. Within each k, the protein rank was calculated as the rank of the average rank. The final rank was taken as the weighted average over k of the ranks. The protein ranking was done for classifying brucellosis vs. controls, brucellosis vs. Lyme and brucellosis vs. Q-fever.

Results

Protein biomarker discovery

[0453] The infection models used were selected to reflect *Brucella* cell tropism In particular, the well characterized human THP-1 macrophage cell line was used for the macrophage infection experiments (Auwerx J (1991) Experientia 47: 22-31), and selected trophoblast cell lines that have been used as models of first trimester pregnancy (Anderson TD, et al. (1986) Vet Pathol 23: 227-239; Anderson TD, et al. (1986) Vet Pathol 23: 219-226), a time period where trophoblasts can be prone to *Brucella* infection were also used. Two different trophoblast cell lines, JAr and ACM188 were used. The choriocarcinoma cell line (JAr) was derived from a human trophoblastic tumor of the placenta (Hochberg A, et al. (1992) Cancer Research 52: 3713-3717) whereas the trophoblastic hybridoma cell line AC-1M88 was a fusion of primary extravillous trophoblast from term placenta with a choriocarcinoma cell line (Gellersen B, et al. (2010) Hum

Reprod 25: 862-873).

[0454] THP-1 macrophages were infected with either *B. abortus* 2308 or 544. Seven independent replicates of *B. abortus* 2308-infected as well as 8 independent replicates of *B. abortus* 544-infected cultures were produced, matched by an equal number of non-infected cultures, respectively. Twenty four hours after infection approximately 70% of the macrophages had become infected, typically with 1-3 bacteria per cell (Figure 1, panel A). Infecting the THP-1 macrophages with two virulent *B. abortus* strains was done to determine if any of the protein expression changes observed were strain-specific. As will be illustrated in more detail below, 90% of the significantly differentially expressed proteins identified were common to both infections. Therefore, only *B. abortus* 2308 was used to infect the trophoblast cell lines. The macrophage cultures were homogenized and light density membrane vesicles were prepared as described in the methods section. The preparations appeared to be enriched for secreted proteins by western blot (Figure 1, panel B).

[0455] Eight independent replicate cultures of infected and non-infected cells of each of the 2 trophoblast cell lines were produced and evaluated as described above (Figure 1). The two trophoblast cell lines displayed different growth characteristics and infection patterns. The AC-1M88 cell line formed monolayers, and the cultures became infected with a pattern consistent with in vivo trophoblast tissue infections (Carvalho Neta AV, et al. (2008) Infect Immun 76: 1897-1907). The trophoblasts on the outer part of the monolayer contained typically 50-100 bacteria per cell, whereas the trophoblasts in the inner part of the monolayer contained much fewer bacteria per cell, on the order of 8-10. In contrast, the JAr cell line did not have contact inhibition, and the cells of those cultures became uniformly infected with 8-10 bacteria per cell. Thus while the AC-1M88 cell line became infected in a manner consistent with *in vivo* infections, its cultures had cells with both heavy and comparably lighter bacterial burdens. The JAr cell line was far more uniformly infected, although too uniform compared to *in vivo* infections. Analysis of the results from both trophoblast cell lines thus allows insights that would not be possible from one cell line alone.

[0456] Expression analysis of the mass spectrometry data acquired identified 3917 components differentially expressed between infected and non-infected macrophages. Targeted sequencing of the differentially expressed peptides resulted in the identification of 184 candidate protein biomarkers (Table 2). There was a 90% overlap of the differentially expressed proteins identified after THP-1 macrophage infection with either bacterial strain. Moreover the proteins in common had the same expression profiles in both infection conditions. The two macrophage datasets have therefore been merged and will not consider them separately. Of the 184 differentially expressed proteins identified in the secretory vesicle contents of infected cells, 77 (41%) were over-expressed by infected cells and 107 (59%) were under-expressed by infected cells compared to non-infected cells.

[0457] Expression analysis of the mass spectrometry data acquired identified 812 components differentially expressed between the non-infected and the infected AC-1M88 cultures, and 2295 components differentially expressed between the non-infected and the infected JAr cultures. Targeted sequencing of the differentially expressed peptides resulted in the identification of 143 (Table 2) candidate biomarkers proteins from the AC-1M88 cell infections, and 287 from the JAr cell infections. Only 25% of the differentially expressed proteins identified were common to both cell lines. Since the two trophoblast datasets are mostly dissimilar, they were not merged but rather considered independently. For the secretory vesicle content dataset derived from the JAr trophoblasts, 66% (189 proteins) were upregulated in the infected cells, and 42% (61 proteins) were upregulated in the AC-1M88 cells.

[0458] The differentially expressed proteins identified in each infection model modestly overlapped with each other.

[0459] The differentially expressed proteins identified and the DI (Differential Intensity) values of the proteins are shown in Table 2. If the DI value is above 1, the level of the protein is upregulated. If the DI value is less than 1, the level of the marker is downregulated.

Table 2. Differetially Expressed Proteins

		Trophoblasts	
Protein	**Macrophage (combined)**	**Jar**	**AC-1M88**
MCCC2	0.16		
GLS	0.28		
GATM	0.29		
SHMT2	0.30		
IVD	0.33		
ALDH4A1	0.38		
GLUD1	0.39		
HSD17B10	0.43	2.51	
DLD	0.45		
GOT2	0.46	2.22	
LRPPRC	0.26		

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
PHB2	0.30	0.47	0.50
POLDIP2	0.34		
PNPT1	0.50		
HIST1H1 D	2.52		
PARP1	2.59		
HIST1H1B	2.68	4.48	2.47
HIST2H2BF	2.99		
HIST1H4	3.77		
IDH2	0.18		
MTHFD1L	0.22		
NNT	0.23		
SUCLG2	0.27		
ALDH2	0.27		
DLAT	0.28		0.41
IDH3A	0.28		
ACO2	0.29		
PDHB	0.31		
CS	0.35		
FH	0.44	2.17	
AKR7A2	0.46	7.93	
ME2	0.47		
DLST; DLSTP	0.47		
MTHFD2	0.51		
MDH2	1.32		0.08
H6PD	2.04		
PLD4	0.04		
OXCT1	0.16		
HADHA	0.22		
ACAT1	0.29		
ECHS1	0.31		
CPT1A	0.33		
CPT2	0.33		
ECH1	0.33		
HADHB	0.34		
PCCA	0.37		
HADH	0.42		0.35
ACAA2	0.43		
DCI	0.43		
HSD17B4	2.00	2.09	
AARS2	0.09		
TUFM	0.26		
DARS2	0.29		
GFM1	0.29		
HARS	0.31		
SARS2	0.32		
RPN1	0.42		
GATC	0.44		
ICT1	0.51		
P4HB	1.92		

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
RPL14	1.99		
IARS2	2.07		
RPLP2	2.07		3.04
IL4I1	0.17		
LONP1	0.23		
PMPCB	0.27		
PMPCA	0.29		
CPVL	0.38	2.40	
CLPP	0.38		
PPT1	0.46		
GLB1	0.48		0.44
ANPEP	1.94		
ASAH1	2.01		
MMP9	3.19		
SGSH	4.00		
CLPX	0.22		
TRAP1	0.29		
HSPD1	0.31		0.05
DNAJA3	0.37		
HSP90B1	0.38	1.56	0.48
HSPA9	0.39		4.00
ERP29	0.47		
SQRDL	0.50		
PLOD3	1.24		
HSPA5	1.90	2.10	1.09
CNPY2	1.94		
PPIB	1.94		
UGGT1	1.96		
CALU	2.12		
ERO1L	2.68		
PRDX1	2.69		
CLEC11A	0.32		
OPA1	0.36		
SPRED1	1.90		
S100A6	1.90		
PIP4K2A	2.02		
TBL2	2.03		
PIP5K1A	2.19		
CAMK2D	2.20		
ITPR1	2.24		
LYN	2.33		
ESYT2	2.80		
ITGB2	0.39		
PECAM1	1.82		
ARPC3	2.01		
EMR2	2.03		
YBX1	2.09		2.01
ENG	2.09		
AHNAK	2.11	0.52	0.22

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
LCP1	2.11	2.15	
ICAM1	2.11		
ITGAM	2.36		
EVL	2.59		
ITGA5	2.78		
STOML2	0.24		
PHB	0.32	0.42	0.60
IMMT	0.36	0.46	
C5orf33	0.37		
TUBA1A	0.48		
TUBB2C	0.62	2.21	
TUBB	0.69	2.30	
LASP1	1.74	0.49	
LRCH1	1.94		
SEPT2	1.97	0.43	
CAPZB	2.06		
CORO1B	2.07		
TLN1	2.15	0.48	0.61
PLEKHO2	2.21		
ANXA2	2.25	0.39	0.17
CORO1C	2.29	0.48	
SEPT7	2.34		
CLIC1	2.40		
SEPT6	2.43		
S100A11	2.54	1.20	0.85
SEPT9	2.76		2.09
EPB41L3	2.90	0.47	
GBAS	0.40		
GLG1	0.44		
RAB7A	0.50		
RAB6A	2.06		
MYO6	2.13		
ANXA6	2.25		0.42
EHD1	2.39		
CORO1A	2.46		
NAPA	2.51	2.68	
MYO1E	2.60		
STX11	2.69		
C190rf10	1.98	2.50	
CXCL10	2.10		
PRKCSH	2.13		
IL1B	2.89		
F5	3.21		
OAS2	4.60		
ANXA5	6.19	8.82	
RSAD2	16.29		
PPA2	0.47		
SOD2	2.56		
MRPL37	0.23		

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
MRPS28	0.26		
MRPS22	0.29		
MRPS27	0.30		
MRPS7	0.31		
MRPL46	0.36		
ACADM	0.20		
ACADVL	0.42	2.10	
ACADS	0.45		
UQCRC2	0.20	0.31	
ATP5A1	0.31		
UQCRC1	0.32	0.47	
ETFA	0.32		
NDUFS7	0.33		
ETFB	0.34		
NDUFV1	0.37		
TIMM13	0.43		
ATP5O	0.44		
NDUFS1	0.44	0.42	0.26
ATP5B	0.44	0.37	0.20
NDUFS3	0.46		
COX5A	0.46		0.35
NDUFA5	0.50		
POR	1.46		
TIMM44	0.25		
VDAC1	0.34	0.34	
C14orf159	0.30		
PHGDH		2.15	
MAT2A		4.74	
GOT1	8.74		
DNASE2	2.18		
ATIC	2.19		
TRA2B	2.39		
HNRNPK	2.42	3.79	
HNRNPD	2.54		
SF3B3	2.79		
HNRNPA1	3.04		
HNRNPC	3.20	1.43	
HNRNPU	3.62	2.24	
HNRNPR	3.66		
PAICS	3.76		
PNP	3.91		
SFPQ	3.97		
HNRNPA2B1	4.31		
SFRS2B	4.75	4.46	
STRAP	5.32		
SNRPD3	6.94		
GPD2	0.38	0.37	
TKT	2.13		
GNS	2.15	1.69	

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
TALDO1	2.16		
NAGA	2.28		
ENO3	2.46	1.46	
PKM2	2.49		
PGK1	2.64		
ENO1	2.67	1.40	
GAA	2.91		
DDT	2.94		
GLT25D1	3.07		
GGH	3.13	0.52	
HEXA	3.68		
GPI	3.73		
MDH1	4.05		
LDHB	4.29		
AKR1B1	4.71		
PGAM1	4.78		
TPI1	6.15	2.73	
APOE	0.37		
ACSL4	0.50	0.53	
GBA	2.74	0.30	
PRDX6	2.80		
PAFAH1B2	2.93		
ISYNA1	3.23		
ACOX1	3.88		
PDIA3	0.12		
PDIA4	0.28	0.24	
MAN2A1	0.59	0.36	
C1GALT1	0.76	0.46	
RPL8	1.82	2.64	
PDIA5	1.94	1.71	
RPSA	1.97	1.97	
FKBP4	2.05		
EEF1A1	2.12		
AIMP1	2.14		
EEF1B2	2.35		
STIP1	2.35		
EEF2	2.45		
PA2G4	2.46		
EEF1D	2.51	1.91	
RPS23	2.54		
ELAVL1	2.55		
EPRS	3.37		
EIF5A2	5.30		
B4GALT1	5.39	0.08	
CTSL2	0.21	0.37	
CTSL1	0.22		
CTSC	0.49		
CTSB	0.72	0.64	
PSMA7	2.01		

(continued)

Protein	Macrophage (combined)	Trophoblasts Jar	Trophoblasts AC-1M88
USP14	2.06		
NPEPPS	2.31		
PSMC2	2.38		
PSMB5	2.42		
PRCP	2.57		
PSMD11	2.76		
SIAE	2.80		
PSMC6	3.01		
PSMC3	3.18		
CNDP2	3.21		
SUMO3	3.31		
PSMD4	3.34		
PSMD14	3.72		
PSMD10	6.03	0.49	
PSMD2	13.69		
PSMD1	14.29		
HBA1; HBA2	0.38	0.39	
CACYBP	1.92	2.28	
MESDC2	2.03		
HSPA1A; HSPA1B	2.03	1.54	
CCT8	2.11	2.35	
NPM1	2.22	2.42	
PRDX2	2.25		
HSPA8	2.25	1.26	
CDC37	2.27		
PFDN2	2.35		
HSP90AB1	2.44	1.29	
ST13	2.48		
PARK7	2.49		
HSP90AA1	2.52	1.26	
PPIA	2.53		
NDRG1	2.54		
CCT4	2.68		
PFDN1	3.09		
GSTP1	3.49		
ILK	0.29		
PTPN11	0.36	0.54	
AKAP2	0.37		
MPP6	0.38		
IQGAP1	0.44	0.89	
CTNNA1	0.47	0.18	
CTNNB1	0.47	0.76	
MPP1	0.48	0.90	
RANGAP1	3.65		
ARHGDIA	4.36	3.96	
RANBP1	4.50		
THBS1	0.20		
CTNND1	0.24	0.69	

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
ITGA6	0.25		
FN1	0.29	0.47	
FBLN1	0.29	0.41	
FAT2	0.36	0.50	
PTPRJ	0.36		
PARVA	0.38		
ZYX	0.39		
LIMS1	0.41		
PTPRF	0.42		
MPDZ	0.46		
DAG1	0.47		
CDH3	0.48		
MLLT4	0.54	0.44	
DSP	1.10	2.25	
MMP2	0.18	0.14	
CSRP2	0.22		
NEBL	0.25		
DMD	0.29		
BAIAP2	0.30		
FLNB	0.31	0.28	
FMNL2	0.31		
NUCB1	0.32	0.62	
SEPT10	0.34		
CNN2	0.38		
PALLD	0.40		
CALD1	0.40		
NUCB2	0.40		
CNN3	0.41		
CSRP1	0.42		
SLC9A3R1	0.42		
EPB41L2	0.42		
EPB41	0.43		
MYO1B	0.44		
FLNA	0.44	1.34	
EZR	0.45	0.64	
RDX	0.46	0.57	
MYO1C	0.46		
ACTB	0.47	0.17	
CNP	0.49	0.48	
ATP6V1A	1.82	1.86	
ATP6V1F	2.01		
PLEC	2.06	1.66	
CAPZA2	2.08		
TUBA1	2.14		
MYH10	2.18		
KRT8	2.20		
ISOC1	2.23		
MYH9	2.26		
SPTAN1	2.30		

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
SPTBN1	2.32		
ALPP	2.34		
KRT7	2.34		
ATP6V1D	2.40		
MYL12A	2.40	1.25	
ATP6V1B2	2.49		
ATP6V1E1	2.51		
KRT9	2.54		
TMOD3	2.62		
TDRD6	2.74		
ATP6V1G1	2.78		
PEBP1	2.93		
STMN1	3.48	1.55	
ROD1	3.62		
CAPG	4.28		
S100P	7.42		
GOLGA4	0.25	0.50	
GOLGA2	0.25		
TMED10	0.26	0.53	
GOLGA1	0.31	0.51	
SDF4	0.36	0.55	
OCIAD1	0.39		
TRIP11	0.43	0.65	
SCFD1	0.49	0.47	
LRP1	0.50		
GOLGB1	0.54	0.52	
VPS4A	2.07		
VCP	2.21		
DYNLL1	2.58		
COPA	2.58		
MUTED	2.62		
SORT1	2.75		
PDCD61P	2.81		
COPB2	4.00		
B2M	0.39		
PIP4K2C	0.42		
YES1	0.43		
CBX1	2.10		
NCL	2.17		
NUDC	2.28		
RAD23B	2.53		
NAP1L1	2.65		
PRPF19	2.76	6.49	
NME1-NME2	2.80		
LMNA	2.81		
HMGB3	2.92		
LMNB2	3.35		
LMNB1	3.43		
RBBP4	3.56		

(continued)

		Trophoblasts	
Protein	Macrophage (combined)	Jar	AC-1M88
HIST1H1	4.27	2.11	
RUVBL2	4.34		
RUVBL1	4.46	2.12	
IMPDH2	5.27		
HIST2H2B	5.63	1.82	
H2AFY	8.29		
HIST3H3	9.80	2.38	
HIST4	10.63	2.52	
HIST3H2A	10.92		
ATP5J2	0.16		
ATP5I	0.29		
ATP5F1	0.34		
ATP5H	0.44		
MRPL39	2.06		
VDAC2	0.21	0.20	
SLC25A6	0.29	0.38	
SLC25A5	0.29	0.37	
CLIC4	0.40		
SFXN1	0.52	0.36	
SLC25A13	0.60	0.30	
PXMP2	2.17		
RAN	2.20		
SEC61B	2.49	2.89	
GDI2	2.80		
NUTF2	2.90		
RABIF	3.08		
CHMP4B	4.29		
CHMP1A	4.30		
TXNRD1	4.40		
CHMP5	4.44		
DBI	4.66		
CHMP2A	4.82	0.46	
CHMP1B	5.80		
CHMP4A	18.56		
SPG11	2.79		
TTC35	3.64		
CXorf26	6.47		
SLC3A2		2.32	
SFRS3		2.03	
GAPDH		0.18	
GLA		0.45	
HEXB		0.48	
GM2A		0.45	
MGAT3		0.28	
PDIA6		0.30	
DDOST		0.35	
RPL24		2.07	
RPL7A		2.10	
RPL26		2.15	

(continued)

		Trophoblasts	
Protein	**Macrophage (combined)**	**Jar**	**AC-1M88**
RPL23A	2.36		
RPS8	2.38		
RPL3	2.70		
RPL29	2.84		
RPL31	3.53		
PSMA5	0.49		
PRSS8	2.07		
HMOX1	0.33		
HSPE1	2.19		
PRDX3	3.83		
C11orf59	0.46		
RAB2A	0.50		
PPP1R12A	2.15		
DLG1	2.23		
L1CAM	0.41		
BCAM	2.01		
LAMP2	0.09		
MYL6B	0.28		
TAGLN2	2.05		
RAI14	2.08		
SEPT11	2.11		
LIMA1	2.14		
GOLGA7	0.24		
GCC2	0.26		
GOSR1	0.45		
UXS1	0.43		
XRCC6	2.26		
ATP5L	0.18		
NDUFB10	0.37		
COX5B	0.43		
NDUFA2	2.45		
SLC25A11	0.12		
PGRMC1	0.37		
CYB5A	0.38		

Protein biomarker selection and MRM assay development

[0460] The proteomics analysis was focused on host compartments associated with secretion in order to enrich for proteins that were likely to be eventually found in the circulation. Both upregulated and down regulated proteins were identified following infection, and that combined dataset was useful for deriving insights into the host-pathogen interactions. However, for selection of biomarker candidates of infection, the secretory proteins that appear to become upregulated in the host cell secretomes after infection were focused on.

[0461] A multiplex MRM assay was developed for 169 proteins that were upregulated in infected macrophages and trophoblasts, represented by 331 peptides. The proteins were selected from all the biological processes identified to be affected by the infection. A relatively small number of the upregulated candidate biomarker proteins identified were excluded from the multiplex MRM assay. That number included those candidates for which protein-specific peptides meeting the selection criteria described in the Materials and Methods could not be identified, as well as those where the selected peptides failed to produce an appropriate signal during MRM method development.

Biomarker verification by ELISA and MRM

[0462] The majority of the candidate biomarkers in the MRM assay did not have known concentrations in plasma or serum. Although sera depleted of multiple high abundance proteins was used, it remained a distinct possibility that many of the biomarker candidates included in the multiplex MRM assay would be beneath its limit of detection. For a few of the secreted proteins that became upregulated after infection, such as IL-1b, serum concentrations were expected to be beneath the level of detection of the MRM assay. However, for the vast majority of the other biomarker candidates there was insufficient information to make that assessment. Twenty-two commercially available ELISA kits against a selection of biomarker candidates were obtained, 20 of which were also included in the MRM assay. A two-stage evaluation was used. Initially, each ELISA kit was used to measure the candidate biomarker levels in sera of 28 brucellosis patients, an equal number of age and gender matched controls, and 24 samples from subjects with Lyme disease. The biomarker candidates that were able to discriminate the brucellosis samples from the combination of controls and other infectious disease samples were selected for more extensive evaluation. Four candidate biomarkers were thus selected, LYN, MAT2A, ANXA5, and RPL32A.

[0463] A total of 254 serum samples were used for the second stage ELISA-based evaluation. These samples represented subjects with acute brucellosis, healthy controls, as well as subjects with Lyme disease, Q-fever, and salmonellosis (Table 1). Brucellosis presents clinically as recurring fever typically with joint and abdominal pain. Lyme disease has a similar clinical presentation and epidemiology as brucellosis, but a dissimilar infection mechanism. Q-fever also has a similar presentation and epidemiology as brucellosis, and a similar infection mechanism. Salmonellosis, in contrast, has a dissimilar clinical presentation and epidemiology as brucellosis but also a similar infection mechanism. These three additional diseases, therefore, were useful in assessing the specificity of the candidate biomarkers as well as their physiological relevance. All the serum samples were age and gender matched to the brucellosis cohort (Table 3). The disease samples were all archival and obtained from different regions. To control for regional differences in immune profiles and site to site sample handling, healthy control samples from the same regions, matching site and geography were also obtained.

Table 3. Clinical samples used in biomarker candidate verification.

Condition	Country	Number	Male/Female ratio (%)	Age (mean +/- standard deviation)
Healthy	Spain	49*	73/27	27.02 +/- 12.25
Brucellosis	Spain	28	82/18	25.11 +/- 12.60
Healthy	USA	28	86/14	28.29 +/- 18.09
Lyme disease	USA	44	77/23	31.41 +/- 16.47
Healthy	France	19	68/32	33.63 +/- 3.24
Q-fever	France	55	60/40	32.55 +/-15.93
Salmonellosis	France	55	53/47	30.11 +/- 15.37
* For the ELISAs 25 samples were used (Male/Female ratio (%): 68/32; Age (mean +/- standard deviation): 26.72 +/-12.41)				

[0464] Two hundred seventy eight serum samples were used for the multiplex MRM assay. These included all the samples used for the ELISA assays described above, and included an additional 25 healthy control samples from Spain which had limiting volumes. The mass spectrometry data acquired was normalized using the appropriate regional control samples per cohort and the differential expression ratios were calculated for each candidate biomarker detected. Twenty of the biomarker candidates were reliably detected by the MRM assay. The biomarker candidates assessed by ELISA were not detected by the MRM assay. The ability of each biomarker candidate to classify the experimental and control sample groups was assessed individually (Figures 2-12) as well as in combination (Tables 3A-3D).

[0465] The candidate biomarkers that formed the best performing panels for brucellosis comparisons to Q fever, Lyme disease and to healthy controls have good individual performance but were also able to complement each other. Classification performance improved when these biomarkers were used in combination compared to when they were used individually.

Table 3A.

Bru vs q-fever

	AUC
B4GALT1	0.876
CALU	0.883
HIST3H3	0.897
HIST4	0.999
ICAM1	0.874
LDHB	0.964
LYN	0.504
MAT2A	0.954
MDH1	0.972
MMP9	0.776
TPI1	0.875

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
LDHB	MDH1	TPI1	MAT2A				0.980
B4GALT1	LDHB	MDH1	MAT2A				0.980
B4GALT1	LDHB	MAT2A					0.977
LDHB	MDH1	MAT2A					0.975
CALU	MAT2A						0.975
LDHB	MDH1						0.974
B4GALT1	LDHB	MMP9	MAT2A				0.973
CALU	LDHB	MDH1	MAT2A				0.973
LDHB	MDH1	MMP9	MAT2A				0.972
ICAM1	LDHB	MDH1					0.972
LDHB	MDH1	TPI1					0.971
B4GALT1	LDHB	TPI1	MAT2A				0.971
CALU	MDH1						0.970
B4GALT1	LDHB	MDH1					0.970
CALU	LDHB	MDH1					0.970
ICAM1	MDH1						0.970
B4GALT1	ICAM1	LDHB	MAT2A				0.970
B4GALT1	CALU	MAT2A					0.969
B4GALT1	CALU	LDHB	MAT2A				0.969
LDHB	MDH1	MMP9					0.968
CALU	LDHB	MAT2A					0.968
B4GALT1	CALU	LDHB	MDH1				0.968
LDHB	MAT2A						0.968
B4GALT1	LDHB	MDH1	TPI1				0.968
MDH1	MAT2A						0.968
B4GALT1	MDH1						0.967
CALU	LDHB	MMP9	MAT2A				0.967
B4GALT1	ICAM1	LDHB	MDH1				0.967
ICAM1	MAT2A						0.967
CALU	MDH1	MAT2A					0.967
ICAM1	MDH1	MAT2A					0.967
ICAM1	LDHB	MDH1	MAT2A				0.967
CALU	LDHB	TPI1	MAT2A				0.967
CALU	ICAM1	MDH1	MAT2A				0.966
CALU	LDHB	MDH1	TPI1				0.966

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	MDH1	MAT2A					0.966
LDHB	MMP9	MAT2A					0.966
B4GALT1	LDHB	MDH1	MMP9				0.966
CALU	MMP9	MAT2A					0.966
B4GALT1	ICAM1	MDH1	MAT2A				0.966
CALU	ICAM1	LDHB	MDH1				0.965
ICAM1	LDHB	MDH1	TPI1				0.965
LDHB	TPI1	MAT2A					0.965
CALU	MDH1	MMP9	MAT2A				0.965
CALU	ICAM1	MDH1					0.965
ICAM1	LDHB	MDH1	MMP9				0.964
B4GALT1	CALU	MDH1	MAT2A				0.964
ICAM1	MMP9	MAT2A					0.964
ICAM1	LDHB	MAT2A					0.964
B4GALT1	MDH1	TPI1	MAT2A				0.964
CALU	LDHB	MDH1	MMP9				0.964
B4GALT1	MDH1	MMP9	MAT2A				0.963
CALU	ICAM1	LDHB	MAT2A				0.963
ICAM1	MDH1	MMP9	MAT2A				0.963
ICAM1	MDH1	TPI1	MAT2A				0.963
LDHB	TPI1						0.962
LDHB	MMP9						0.962
B4GALT1	ICAM1	MDH1					0.962
B4GALT1	CALU	MDH1					0.962
B4GALT1	CALU	MMP9	MAT2A				0.962
ICAM1	TPI1	MAT2A					0.961
B4GALT1	CALU	ICAM1	MAT2A				0.961
CALU	LDHB						0.961
LDHB	MDH1	MMP9	TPI1				0.961
CALU	TPI1	MAT2A					0.961
B4GALT1	LDHB						0.960
MDH1	MMP9	MAT2A					0.960
MDH1	TPI1	MAT2A					0.960
B4GALT1	ICAM1	MAT2A					0.960
MDH1	MMP9						0.960
ICAM1	LDHB	MMP9	MAT2A				0.960
LDHB	MDH1	LYN					0.960
LDHB	MMP9	TPI1					0.960
B4GALT1	LDHB	TPI1					0.959
ICAM1	LDHB						0.959
MDH1	TPI1						0.959
CALU	MDH1	TPI1	MAT2A				0.959
CALU	MDH1	MMP9					0.959
ICAM1	MDH1	MMP9					0.958
ICAM1	MDH1	TPI1					0.958
LDHB	MDH1	TPI1	LYN				0.958
ICAM1	LDHB	MMP9					0.958
CALU	ICAM1	LDHB					0.958
CALU	LDHB	TPI1					0.958
CALU	ICAM1	MAT2A					0.957

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
ICAM1	LDHB	MDH1	LYN				0.957
B4GALT1	LDHB	LYN	MAT2A				0.957
ICAM1	LDHB	TPI1					0.957
B4GALT1	LDHB	MMP9					0.956
TPI1	MAT2A						0.956
B4GALT1	ICAM1	MMP9	MAT2A				0.956
B4GALT1	MDH1	TPI1					0.956
CALU	LDHB	MMP9					0.956
LDHB	MDH1	LYN	MAT2A				0.955
B4GALT1	ICAM1	LDHB					0.955
B4GALT1	MDH1	MMP9					0.955
ICAM1	LDHB	TPI1	MAT2A				0.955
MMP9	TPI1	MAT2A					0.954
ICAM1	MMP9	TPI1	MAT2A				0.954
B4GALT1	CALU	TPI1	MAT2A				0.954
CALU	MDH1	TPI1					0.954
B4GALT1	CALU	ICAM1	MDH1				0.954
B4GALT1	LDHB	MMP9	TPI1				0.954
LDHB	MDH1	MMP9	LYN				0.954
MMP9	MAT2A						0.954
B4GALT1	CALU	LDHB					0.954
CALU	ICAM1	LDHB	TPI1				0.953
ICAM1	LDHB	MMP9	TPI1				0.953
B4GALT1	ICAM1	TPI1	MAT2A				0.953
B4GALT1	ICAM1	LDHB	TPI1				0.952
MDH1	LYN						0.952
LDHB	MMP9	TPI1	MAT2A				0.952
B4GALT1	ICAM1	LDHB	MMP9				0.952
CALU	ICAM1	LDHB	MMP9				0.951
MDH1	MMP9	TPI1	MAT2A				0.951
CALU	LDHB	MMP9	TPI1				0.951
CALU	ICAM1	MDH1	MMP9				0.951
CALU	ICAM1	MMP9	MAT2A				0.951
LDHB	MMP9	LYN	MAT2A				0.951
B4GALT1	CALU	LDHB	TPI1				0.951
CALU	ICAM1	TPI1	MAT2A				0.951
MDH1	MMP9	TPI1					0.950
B4GALT1	CALU	MDH1	MMP9				0.950
B4GALT1	ICAM1	MDH1	TPI1				0.950
B4GALT1	CALU	LDHB	MMP9				0.950
B4GALT1	ICAM1	MDH1	MMP9				0.950
ICAM1	LDHB	LYN	MAT2A				0.950
B4GALT1	CALU	ICAM1	LDHB				0.950
LDHB	TPI1	LYN	MAT2A				0.949
B4GALT1	TPI1	MAT2A					0.949
ICAM1	MDH1	LYN					0.949
B4GALT1	MMP9	MAT2A					0.948
CALU	MMP9	TPI1	MAT2A				0.948
CALU	LDHB	MDH1	LYN				0.948
CALU	MDH1	LYN					0.948

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
LDHB	LYN	MAT2A					0.948
B4GALT1	MAT2A						0.948
CALU	ICAM1	MDH1	TPI1				0.947
ICAM1	MDH1	MMP9	TPI1				0.947
B4GALT1	MMP9	TPI1	MAT2A				0.947
B4GALT1	LDHB	MDH1	LYN				0.946
LDHB	LYN						0.946
LDHB	TPI1	LYN					0.945
CALU	MDH1	MMP9	LYN				0.945
B4GALT1	MDH1	MMP9	TPI1				0.945
CALU	LYN	MAT2A					0.945
CALU	MDH1	LYN	MAT2A				0.945
CALU	LDHB	LYN	MAT2A				0.944
ICAM1	LYN	MAT2A					0.944
B4GALT1	CALU	MDH1	TPI1				0.943
MDH1	LYN	MAT2A					0.943
CALU	MDH1	MMP9	TPI1				0.942
B4GALT1	MDH1	LYN					0.942
ICAM1	MDH1	LYN	MAT2A				0.942
MDH1	MMP9	LYN					0.942
CALU	ICAM1	MDH1	LYN				0.941
MDH1	TPI1	LYN					0.941
LDHB	MMP9	LYN					0.941
B4GALT1	MDH1	LYN	MAT2A				0.940
ICAM1	MMP9	LYN	MAT2A				0.940
CALU	MMP9	LYN	MAT2A				0.939
ICAM1	TPI1	LYN	MAT2A				0.938
MDH1	TPI1	LYN	MAT2A				0.938
ICAM1	MDH1	MMP9	LYN				0.938
ICAM1	LDHB	LYN					0.938
B4GALT1	LDHB	LYN					0.938
CALU	LDHB	TPI1	LYN				0.937
B4GALT1	CALU	LYN	MAT2A				0.937
LDHB	MMP9	TPI1	LYN				0.937
ICAM1	MDH1	TPI1	LYN				0.937
CALU	ICAM1	LYN	MAT2A				0.937
CALU	LDHB	LYN					0.936
LYN	MAT2A						0.935
B4GALT1	LDHB	TPI1	LYN				0.934
ICAM1	LDHB	MMP9	LYN				0.934
CALU	TPI1	LYN	MAT2A				0.934
MDH1	MMP9	TPI1	LYN				0.934
CALU	MDH1	TPI1	LYN				0.933
B4GALT1	LDHB	MMP9	LYN				0.933
B4GALT1	ICAM1	MDH1	LYN				0.932
B4GALT1	MDH1	MMP9	LYN				0.932
TPI1	LYN	MAT2A					0.932
MDH1	MMP9	LYN	MAT2A				0.932
B4GALT1	ICAM1	LYN	MAT2A				0.931
B4GALT1	ICAM1	LDHB	LYN				0.931

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
MMP9	TPI1	LYN	MAT2A				0.931
CALU	LDHB	MMP9	LYN				0.931
B4GALT1	CALU	MDH1	LYN				0.930
MMP9	LYN	MAT2A					0.930
CALU	ICAM1	LDHB	LYN				0.929
B4GALT1	MDH1	TPI1	LYN				0.928
ICAM1	LDHB	TPI1	LYN				0.928
B4GALT1	CALU	LDHB	LYN				0.926
B4GALT1	LYN	MAT2A					0.924
B4GALT1	TPI1	LYN	MAT2A				0.923
B4GALT1	MMP9	LYN	MAT2A				0.921
B4GALT1	CALU						0.900
B4GALT1	CALU	TPI1					0.899
CALU	TPI1						0.898
B4GALT1	CALU	MMP9					0.896
B4GALT1	CALU	MMP9	TPI1				0.895
B4GALT1	ICAM1	TPI1					0.893
CALU	MMP9	TPI1					0.893
B4GALT1	TPI1						0.893
ICAM1	TPI1						0.892
CALU	ICAM1	TPI1					0.890
B4GALT1	CALU	ICAM1	TPI1				0.889
B4GALT1	CALU	ICAM1					0.888
B4GALT1	ICAM1						0.886
CALU	ICAM1	MMP9	TPI1				0.886
B4GALT1	CALU	ICAM1	MMP9				0.885
CALU	MMP9						0.879
ICAM1	MMP9	TPI1					0.879
B4GALT1	MMP9	TPI1					0.878
B4GALT1	ICAM1	MMP9	TPI1				0.878
CALU	ICAM1						0.877
CALU	ICAM1	MMP9					0.876
B4GALT1	ICAM1	MMP9					0.872
B4GALT1	TPI1	LYN					0.866
B4GALT1	CALU	LYN					0.864
B4GALT1	CALU	TPI1	LYN				0.863
MMP9	TPI1						0.863
B4GALT1	CALU	MMP9	LYN				0.862
ICAM1	MMP9						0.860
CALU	TPI1	LYN					0.858
B4GALT1	MMP9						0.858
B4GALT1	ICAM1	TPI1	LYN				0.853
B4GALT1	ICAM1	LYN					0.852
ICAM1	TPI1	LYN					0.852
CALU	MMP9	TPI1	LYN				0.851
B4GALT1	LYN						0.851
B4GALT1	MMP9	TPI1	LYN				0.850
B4GALT1	CALU	ICAM1	LYN				0.849
CALU	ICAM1	TPI1	LYN				0.846
ICAM1	MMP9	TPI1	LYN				0.844

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	ICAM1	MMP9	LYN				0.844
B4GALT1	MMP9	LYN					0.840
TPI1	LYN						0.839
CALU	LYN						0.838
CALU	MMP9	LYN					0.837
CALU	ICAM1	MMP9	LYN				0.835
ICAM1	LYN						0.832
CALU	ICAM1	LYN					0.832
ICAM1	MMP9	LYN					0.826
MMP9	TPI1	LYN					0.825
MMP9	LYN						0.746

Table 3B.

Bru vs lyme

	AUC
B4GALT1	0.842
CALU	0.543
HIST3H3	0.753
HIST4	0.995
ICAM1	0.896
LDHB	0.885
LYN	0.841
MAT2A	0.965
MDH1	0.946
MMP9	0.902
TPI1	0.652

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	HIST3H3	LYN					0.932
B4GALT1	CALU	ICAM1	LDHB	TPI1	MAT2A		0.931
B4GALT1	ICAM1	LDHB	TPI1				0.896
CALU	HIST3H3	HIST4	MMP9	LYN			1.000
CALU	HIST4	ICAM1	LDHB	MMP9	LYN		1.000
CALU	HIST4	MMP9	TPI1	LYN			1.000
CALU	HIST4	ICAM1	MDH1	MMP9	TPI1		1.000
CALU	HIST3H3	HIST4	ICAM1				1.000
CALU	HIST3H3	HIST4	ICAM1	MMP9			1.000
CALU	HIST3H3	HIST4	TPI1	LYN			0.999
CALU	HIST4	ICAM1	LDHB	MMP9			0.999
CALU	HIST3H3	HIST4	LDHB	MDH1	MMP9		0.999
CALU	HIST3H3	HIST4	ICAM1	TPI1			0.999
CALU	HIST4	MDH1	MMP9	TPI1			0.999
CALU	HIST3H3	HIST4	LDHB	MMP9	TPI1		0.999
CALU	HIST3H3	HIST4	ICAM1	LDHB			0.999
CALU	HIST3H3	HIST4	MMP9	TPI1			0.999
CALU	HIST3H3	HIST4	MDH1	MMP9	TPI1		0.999
CALU	HIST3H3	HIST4	MMP9				0.999
CALU	HIST3H3	HIST4	MDH1	TPI1	LYN		0.999
CALU	HIST3H3	ICAM1	MDH1	MMP9	LYN		0.999

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST3H3	HIST4	LDHB	TPI1			0.998
CALU	HIST3H3	HIST4	ICAM1	MDH1			0.998
CALU	HIST4	ICAM1					0.998
CALU	HIST3H3	HIST4	LDHB	MDH1	TPI1		0.998
CALU	HIST3H3	HIST4	ICAM1	MDH1	TPI1		0.998
CALU	ICAM1	MMP9	LYN				0.998
CALU	HIST4	ICAM1	TPI1	LYN			0.998
CALU	HIST4	ICAM1	TPI1				0.998
CALU	HIST3H3	HIST4	ICAM1	LDHB	LYN		0.998
CALU	HIST4	ICAM1	MMP9	TPI1	MAT2A		0.997
CALU	HIST4	LDHB	MMP9	TPI1			0.997
CALU	HIST3H3	HIST4	MDH1	LYN			0.997
CALU	HIST4	LDHB	MMP9				0.997
CALU	HIST4						0.997
CALU	HIST4	MDH1	TPI1				0.997
CALU	ICAM1	MDH1	MMP9	LYN			0.997
CALU	HIST3H3	HIST4	LDHB	MDH1	LYN		0.997
CALU	HIST4	LDHB	MMP9	TPI1	MAT2A		0.997
CALU	HIST4	MDH1	TPI1	LYN			0.997
CALU	HIST4	MMP9	TPI1				0.996
CALU	HIST4	ICAM1	MDH1	TPI1	LYN		0.996
CALU	HIST4	MDH1					0.996
CALU	HIST4	LDHB					0.996
CALU	HIST4	ICAM1	LDHB	LYN			0.995
CALU	LDHB	MDH1	MMP9	LYN			0.995
CALU	HIST4	LDHB	MDH1	TPI1			0.995
CALU	HIST4	LDHB	MDH1				0.995
CALU	ICAM1	MMP9	TPI1	LYN			0.995
CALU	MDH1	MMP9	LYN				0.995
CALU	HIST4	ICAM1	LDHB	MDH1			0.995
CALU	HIST4	ICAM1	LDHB	MDH1	LYN		0.993
CALU	HIST4	LDHB	MDH1	LYN			0.993
CALU	ICAM1	LDHB	MDH1	MMP9	LYN		0.993
CALU	HIST3H3	MDH1	MMP9	LYN			0.993
CALU	HIST4	ICAM1	MMP9	LYN	MAT2A		0.992
CALU	HIST4	MDH1	MMP9	MAT2A			0.991
CALU	LDHB	MDH1	MMP9	TPI1	LYN		0.991
CALU	HIST4	MMP9	TPI1	MAT2A			0.989
CALU	HIST4	ICAM1	LDHB	MMP9	MAT2A		0.988
CALU	ICAM1	LDHB	MMP9	MAT2A			0.987
CALU	HIST3H3	HIST4	ICAM1	MMP9	MAT2A		0.987
CALU	LDHB	MMP9	MAT2A				0.986
CALU	HIST3H3	ICAM1	LDHB	MMP9	MAT2A		0.986
CALU	ICAM1	MMP9	LYN	MAT2A			0.984
CALU	ICAM1	MMP9	TPI1	LYN	MAT2A		0.983
CALU	HIST3H3	HIST4	MMP9	MAT2A			0.983
CALU	HIST3H3	HIST4	LDHB	MMP9	MAT2A		0.982
CALU	HIST3H3	ICAM1	LDHB	MMP9			0.982
CALU	HIST3H3	HIST4	LDHB	TPI1	MAT2A		0.982
CALU	ICAM1	LDHB	MDH1	MMP9	MAT2A		0.982

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	MDH1	MMP9	MAT2A				0.981
CALU	LDHB	MMP9	LYN				0.980
CALU	HIST3H3	HIST4	TPI1	LYN	MAT2A		0.979
CALU	LDHB	MMP9	TPI1	MAT2A			0.979
CALU	HIST4	ICAM1	LDHB	TPI1	MAT2A		0.978
CALU	ICAM1	MDH1	MMP9	TPI1			0.978
CALU	ICAM1	LDHB	MMP9	LYN	MAT2A		0.978
CALU	HIST3H3	HIST4	LDHB	MAT2A			0.977
CALU	ICAM1	MDH1	MMP9	LYN	MAT2A		0.977
CALU	MDH1	MMP9	TPI1	LYN	MAT2A		0.976
CALU	HIST4	ICAM1	MDH1	TPI1	MAT2A		0.976
CALU	HIST3H3	MMP9	TPI1	LYN	MAT2A		0.976
CALU	MMP9	LYN	MAT2A				0.975
CALU	HIST3H3	HIST4	MAT2A				0.975
CALU	HIST4	MDH1	TPI1	LYN	MAT2A		0.974
CALU	ICAM1	MMP9	MAT2A				0.974
CALU	LDHB	MMP9	LYN	MAT2A			0.974
CALU	MDH1	MMP9	LYN	MAT2A			0.973
CALU	MMP9	LYN					0.973
CALU	MDH1	MMP9					0.973
CALU	HIST3H3	MMP9	LYN	MAT2A			0.972
CALU	HIST4	LDHB	LYN	MAT2A			0.972
CALU	ICAM1	LDHB	MDH1				0.972
CALU	HIST3H3	MDH1	MMP9	LYN	MAT2A		0.972
CALU	HIST4	MDH1	LYN	MAT2A			0.971
CALU	HIST4	ICAM1	MDH1	MAT2A			0.970
CALU	ICAM1	LDHB	MMP9	TPI1			0.969
CALU	HIST4	ICAM1	MAT2A				0.969
CALU	HIST4	ICAM1	LDHB	MAT2A			0.968
CALU	HIST4	ICAM1	MDH1	LYN	MAT2A		0.968
CALU	HIST4	ICAM1	LYN	MAT2A			0.966
CALU	LDHB	MDH1	MMP9				0.966
CALU	HIST4	ICAM1	LDHB	MDH1	MAT2A		0.965
CALU	MMP9	TPI1	MAT2A				0.962
CALU	HIST3H3	MDH1	MMP9	TPI1			0.962
CALU	HIST3H3	LDHB	MDH1				0.955
CALU	ICAM1	LDHB	MDH1	TPI1	LYN		0.955
CALU	LDHB	MDH1	TPI1	LYN			0.954
CALU	HIST3H3	ICAM1	TPI1				0.953
CALU	HIST3H3	ICAM1	LDHB				0.950
CALU	HIST3H3	ICAM1	LYN				0.949
CALU	ICAM1	LYN					0.949
CALU	ICAM1	TPI1	MAT2A				0.948
CALU	ICAM1	MDH1	TPI1	MAT2A			0.944
CALU	TPI1	MAT2A					0.944
CALU	ICAM1	LDHB	MAT2A				0.943
CALU	ICAM1	LDHB	LYN				0.943
CALU	HIST3H3	MDH1	MAT2A				0.941
CALU	ICAM1	LDHB	TPI1	MAT2A			0.941
CALU	ICAM1	LDHB	MDH1	TPI1	MAT2A		0.941

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST3H3	ICAM1	MDH1	TPI1	MAT2A		0.938
CALU	ICAM1						0.938
CALU	HIST3H3	MAT2A					0.938
CALU	LDHB	MDH1	TPI1	MAT2A			0.937
CALU	LDHB	MDH1	TPI1	LYN	MAT2A		0.937
CALU	ICAM1	LDHB	TPI1	LYN			0.937
CALU	HIST3H3	TPI1	MAT2A				0.935
CALU	HIST3H3	LDHB	MDH1	MAT2A			0.933
CALU	ICAM1	MDH1	LYN	MAT2A			0.933
CALU	HIST3H3	MDH1	LYN	MAT2A			0.931
CALU	HIST3H3	MDH1	TPI1	MAT2A			0.929
CALU	HIST3H3	MDH1	TPI1	LYN			0.928
CALU	LDHB	MAT2A					0.928
CALU	HIST3H3	LDHB	MDH1	LYN	MAT2A		0.922
CALU	HIST3H3	LDHB	MDH1	TPI1	LYN		0.922
CALU	LDHB	TPI1	MAT2A				0.922
CALU	LDHB						0.903
CALU	LDHB	TPI1	LYN				0.898
CALU	LDHB	TPI1					0.891
CALU	HIST3H3	LDHB	TPI1	LYN			0.891
HIST3H3	HIST4	MMP9	TPI1	LYN			1.000
HIST3H3	HIST4	LDHB	MMP9	LYN			1.000
HIST3H3	HIST4	ICAM1	MDH1	MMP9	LYN		1.000
HIST3H3	HIST4	ICAM1	LDHB	MMP9	TPI1		1.000
HIST3H3	HIST4	ICAM1	MDH1	MMP9	TPI1		1.000
HIST3H3	HIST4	LYN					1.000
HIST3H3	HIST4	LDHB	MMP9	TPI1			1.000
HIST3H3	HIST4	ICAM1	LDHB	TPI1	LYN		0.999
HIST3H3	HIST4	LDHB	TPI1				0.998
HIST3H3	HIST4	ICAM1	TPI1				0.998
HIST3H3	HIST4	LDHB	MDH1	TPI1			0.998
HIST3H3	HIST4	ICAM1	LDHB				0.998
HIST3H3	ICAM1	MDH1	MMP9	LYN			0.997
HIST3H3	HIST4	ICAM1	MDH1	LYN			0.997
HIST3H3	ICAM1	MMP9	TPI1				0.994
HIST3H3	HIST4	ICAM1	MMP9	LYN	MAT2A		0.991
HIST3H3	LDHB	MDH1	MMP9	LYN			0.991
HIST3H3	ICAM1	MMP9	MAT2A				0.990
HIST3H3	ICAM1	MDH1	MMP9	TPI1			0.990
HIST3H3	ICAM1	LDHB	MMP9	TPI1			0.988
HIST3H3	HIST4	LDHB	MDH1	MMP9	MAT2A		0.986
HIST3H3	HIST4	ICAM1	LDHB	TPI1	MAT2A		0.985
HIST3H3	HIST4	ICAM1	MDH1	MMP9	MAT2A		0.985
HIST3H3	ICAM1	LDHB	MDH1	MMP9			0.985
HIST3H3	HIST4	LDHB	MMP9	LYN	MAT2A		0.984
HIST3H3	ICAM1	LDHB	MDH1	MMP9	TPI1		0.980
HIST3H3	MDH1	MMP9					0.980
HIST3H3	ICAM1	MDH1	MMP9	MAT2A			0.979
HIST3H3	HIST4	LDHB	MAT2A				0.978
HIST3H3	HIST4	ICAM1	LDHB	MAT2A			0.977

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
HIST3H3	HIST4	ICAM1	LDHB	MDH1	MAT2A		0.976
HIST3H3	MMP9	TPI1	LYN	MAT2A			0.970
HIST3H3	LDHB	MDH1	MMP9	TPI1	MAT2A		0.967
HIST3H3	MDH1	MMP9	TPI1	MAT2A			0.967
HIST3H3	LDHB	MMP9	TPI1	LYN	MAT2A		0.965
HIST3H3	MMP9	TPI1	MAT2A				0.964
HIST3H3	MAT2A						0.944
HIST3H3	LDHB	MDH1					0.939
HIST3H3	ICAM1	LDHB	MDH1	MAT2A			0.939
HIST3H3	MDH1	TPI1	LYN	MAT2A			0.936
HIST3H3	ICAM1	MDH1	LYN	MAT2A			0.936
HIST3H3	ICAM1	LDHB	LYN	MAT2A			0.934
HIST3H3	LDHB	MDH1	TPI1				0.932
HIST3H3	ICAM1	LDHB	MDH1				0.931
HIST3H3	ICAM1	MDH1					0.931
HIST3H3	TPI1	LYN	MAT2A				0.929
HIST3H3	LDHB	MDH1	LYN				0.919
HIST3H3	LDHB	TPI1	MAT2A				0.918
HIST3H3	ICAM1	TPI1	LYN				0.888
HIST3H3	LDHB	TPI1	LYN				0.853
HIST3H3	TPI1	LYN					0.778
HIST4	ICAM1	MDH1	MMP9				1.000
HIST4	MMP9						0.999
HIST4	LDHB	MMP9	TPI1				0.999
HIST4	LDHB	MMP9	TPI1	MAT2A			0.995
HIST4	ICAM1	LDHB	MDH1	LYN	MAT2A		0.971
HIST4	ICAM1	LDHB	MAT2A				0.971
HIST4	ICAM1	MDH1	MAT2A				0.970
ICAM1	LDHB	MMP9	LYN				0.998
ICAM1	MMP9	TPI1	LYN				0.998
ICAM1	MDH1	MMP9	TPI1	LYN			0.996
ICAM1	LDHB	MMP9	TPI1	LYN			0.994
ICAM1	MMP9	LYN	MAT2A				0.985
ICAM1	MMP9	TPI1	LYN	MAT2A			0.984
ICAM1	LDHB	MDH1	MMP9	TPI1			0.981
ICAM1	LDHB	MMP9	MAT2A				0.977
ICAM1	MMP9	MAT2A					0.977
ICAM1	LDHB	MDH1	MMP9	LYN	MAT2A		0.975
ICAM1	MDH1	MMP9	LYN	MAT2A			0.975
ICAM1	LDHB	MMP9	LYN	MAT2A			0.974
ICAM1	MDH1	TPI1	LYN	MAT2A			0.944
ICAM1	MDH1	LYN	MAT2A				0.943
ICAM1	MDH1						0.942
ICAM1	LDHB	LYN	MAT2A				0.942
ICAM1	MDH1	TPI1					0.941
ICAM1	LDHB	TPI1	LYN	MAT2A			0.938
ICAM1	LDHB	MDH1	LYN				0.925
ICAM1	LDHB	MDH1	TPI1	LYN			0.922
ICAM1	LDHB	LYN					0.917
ICAM1	LYN						0.917

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
ICAM1	LDHB	TPI1	LYN				0.915
ICAM1	TPI1	LYN					0.912
ICAM1	TPI1						0.886
LDHB	MDH1	MMP9	LYN				0.994
LDHB	MDH1	MMP9	TPI1	LYN			0.992
LDHB	MMP9	MAT2A					0.969
LDHB	MDH1	MMP9	TPI1	MAT2A			0.969
LDHB	MDH1	MMP9	TPI1				0.968
LDHB	MMP9						0.962
LDHB	MMP9	LYN	MAT2A				0.957
LDHB	MMP9	TPI1					0.955
LDHB	MMP9	TPI1	LYN	MAT2A			0.953
LDHB	LYN	MAT2A					0.942
LDHB	MAT2A						0.931
LDHB	TPI1	MAT2A					0.927
MDH1	MMP9	TPI1					0.973
MDH1	MMP9	TPI1	LYN	MAT2A			0.970
MDH1	LYN	MAT2A					0.948
MDH1	TPI1	LYN	MAT2A				0.942
MDH1	TPI1	LYN					0.912
MMP9	LYN						0.976
MMP9	TPI1	LYN	MAT2A				0.954
MMP9	TPI1						0.939
TPI1	MAT2A						0.950
TPI1	LYN						0.809

Table 3C.

Bru vs control

	AUC
B4GALT1	0.763
CALU	0.642
HIST3H3	0.670
HIST4	0.939
ICAM1	0.862
LDHB	0.820
LYN	0.845
MAT2A	0.940
MDH1	0.899
MMP9	0.574
TPI1	0.698

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	HIST3H3	TPI1	LYN	MAT2A			0.934
MMP9	LYN	MAT2A					0.934
CALU	LYN	MAT2A					0.936
B4GALT1	CALU	TPI1	MAT2A				0.937
HIST3H3	LYN	MAT2A					0.937
B4GALT1	CALU	ICAM1	LDHB	TPI1	MAT2A		0.937
LDHB	MDH1	MMP9	TPI1	LYN	MAT2A		0.937

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	ICAM1	LDHB	MAT2A				0.938
HIST3H3	MDH1	LYN	MAT2A				0.938
B4GALT1	CALU	HIST3H3	ICAM1	MAT2A			0.938
B4GALT1	ICAM1	LDHB	MDH1	MAT2A			0.938
B4GALT1	ICAM1	MDH1	TPI1	MAT2A			0.938
CALU	MDH1	MMP9	TPI1	MAT2A			0.938
B4GALT1	ICAM1	LDHB	MDH1	LYN	MAT2A		0.938
CALU	HIST4	LDHB	TPI1				0.938
B4GALT1	CALU	HIST3H3	HIST4				0.938
B4GALT1	HIST3H3	HIST4	MMP9	TPI1			0.938
B4GALT1	TPI1	MAT2A					0.938
B4GALT1	CALU	HIST3H3	ICAM1	LYN	MAT2A		0.938
LDHB	MDH1	TPI1	LYN	MAT2A			0.938
CALU	LDHB	MDH1	MMP9	LYN	MAT2A		0.938
B4GALT1	CALU	ICAM1	LDHB	LYN	MAT2A		0.939
CALU	HIST3H3	LDHB	MDH1	TPI1	MAT2A		0.939
HIST3H3	ICAM1	MMP9	TPI1	LYN	MAT2A		0.939
HIST3H3	MMP9	MAT2A					0.939
CALU	HIST3H3	ICAM1	TPI1	MAT2A			0.939
B4GALT1	CALU	HIST4	ICAM1	MMP9	TPI1		0.939
B4GALT1	HIST4	TPI1					0.939
B4GALT1	HIST4	MMP9	TPI1				0.939
ICAM1	LDHB	MDH1	MMP9	TPI1	MAT2A		0.939
HIST3H3	ICAM1	TPI1	LYN	MAT2A			0.939
HIST3H3	LDHB	MDH1	LYN	MAT2A			0.939
ICAM1	LDHB	MDH1	MMP9	LYN	MAT2A		0.940
CALU	HIST4	LDHB					0.940
HIST3H3	LDHB	MAT2A					0.940
CALU	HIST3H3	ICAM1	MDH1	TPI1	MAT2A		0.940
HIST3H3	ICAM1	MDH1	MMP9	TPI1	MAT2A		0.940
ICAM1	MDH1	MMP9	TPI1	LYN	MAT2A		0.940
HIST3H3	HIST4						0.940
CALU	LDHB	MDH1	LYN	MAT2A			0.940
B4GALT1	CALU	LDHB	MMP9	LYN	MAT2A		0.940
CALU	MMP9	MAT2A					0.940
B4GALT1	ICAM1	LDHB	TPI1	LYN	MAT2A		0.940
B4GALT1	MDH1	TPI1	MAT2A				0.940
CALU	MDH1	LYN	MAT2A				0.940
MDH1	LYN	MAT2A					0.940
B4GALT1	HIST3H3	HIST4	ICAM1	MMP9	TPI1		0.940
HIST3H3	LDHB	TPI1	LYN	MAT2A			0.940
ICAM1	LDHB	LYN	MAT2A				0.940
LDHB	MMP9	LYN	MAT2A				0.940
B4GALT1	TPI1	LYN	MAT2A				0.940
B4GALT1	MDH1	LYN	MAT2A				0.941
B4GALT1	HIST3H3	HIST4	LDHB	TPI1			0.941
B4GALT1	LDHB	TPI1	MAT2A				0.941
HIST3H3	ICAM1	MDH1	LYN	MAT2A			0.941
B4GALT1	CALU	TPI1	LYN	MAT2A			0.941
B4GALT1	CALU	MDH1	LYN	MAT2A			0.941

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	HIST3H3	ICAM1	TPI1	LYN	MAT2A		0.941
B4GALT1	ICAM1	MMP9	TPI1	LYN	MAT2A		0.942
CALU	HIST3H3	LDHB	TPI1	MAT2A			0.942
LDHB	MDH1	MMP9	TPI1	MAT2A			0.942
CALU	HIST3H3	MAT2A					0.942
ICAM1	LDHB	MMP9	TPI1	MAT2A			0.942
CALU	HIST3H3	HIST4	ICAM1	LDHB	MMP9		0.942
HIST3H3	ICAM1	LDHB	MDH1	MMP9	MAT2A		0.942
B4GALT1	CALU	HIST4	ICAM1	MMP9			0.942
HIST3H3	MDH1	MMP9	MAT2A				0.942
B4GALT1	HIST4	ICAM1	LDHB	TPI1			0.943
B4GALT1	CALU	HIST4	LDHB				0.943
B4GALT1	LYN	MAT2A					0.943
CALU	ICAM1	MDH1	TPI1	LYN	MAT2A		0.943
B4GALT1	CALU	LDHB	LYN	MAT2A			0.943
HIST3H3	ICAM1	LDHB	MAT2A				0.943
ICAM1	MDH1	TPI1	LYN	MAT2A			0.943
B4GALT1	HIST3H3	HIST4	ICAM1	LDHB			0.943
B4GALT1	HIST3H3	ICAM1	MMP9	LYN	MAT2A		0.944
LDHB	LYN	MAT2A					0.944
CALU	LDHB	MAT2A					0.944
B4GALT1	CALU	ICAM1	TPI1	LYN	MAT2A		0.944
CALU	LDHB	MDH1	TPI1	MAT2A			0.944
B4GALT1	CALU	HIST4	ICAM1	LDHB			0.945
B4GALT1	HIST3H3	ICAM1	LYN	MAT2A			0.945
B4GALT1	HIST3H3	HIST4	LDHB				0.945
CALU	HIST3H3	HIST4	ICAM1	TPI1			0.945
CALU	MDH1	MMP9	MAT2A				0.945
B4GALT1	HIST3H3	HIST4	ICAM1	MMP9			0.946
B4GALT1	ICAM1	MMP9	LYN	MAT2A			0.946
CALU	HIST3H3	HIST4	TPI1	LYN	MAT2A		0.946
B4GALT1	CALU	HIST4	LDHB	MMP9	MAT2A		0.946
ICAM1	MMP9	MAT2A					0.946
CALU	HIST3H3	TPI1	MAT2A				0.946
CALU	HIST3H3	HIST4	ICAM1	LDHB			0.946
B4GALT1	CALU	HIST4	ICAM1	MDH1	LYN		0.946
CALU	HIST4	ICAM1	MMP9	TPI1			0.946
HIST3H3	LDHB	TPI1	MAT2A				0.947
B4GALT1	HIST3H3	HIST4					0.947
HIST3H3	ICAM1	MDH1	MMP9	LYN	MAT2A		0.947
CALU	ICAM1	MMP9	TPI1	MAT2A			0.947
CALU	ICAM1	LDHB	MDH1	TPI1	MAT2A		0.947
CALU	ICAM1	LDHB	MMP9	TPI1	MAT2A		0.948
CALU	LDHB	MDH1	MMP9	MAT2A			0.948
HIST3H3	ICAM1	LYN	MAT2A				0.948
B4GALT1	CALU	HIST3H3	HIST4	MMP9	MAT2A		0.948
ICAM1	MMP9	LYN	MAT2A				0.948
CALU	ICAM1	LYN	MAT2A				0.948
CALU	HIST3H3	HIST4	ICAM1	MMP9			0.948
B4GALT1	CALU	HIST4	ICAM1	LDHB	LYN		0.949

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	CALU	HIST3H3	HIST4	TPI1	MAT2A		0.949
HIST3H3	HIST4	ICAM1	LDHB	MMP9			0.949
HIST4	ICAM1	LDHB					0.949
HIST4	ICAM1	LDHB	TPI1				0.950
CALU	HIST4	MDH1	TPI1	LYN	MAT2A		0.950
B4GALT1	HIST3H3	HIST4	ICAM1	LDHB	MDH1		0.950
CALU	HIST3H3	HIST4	MMP9	TPI1	MAT2A		0.950
B4GALT1	CALU	HIST4	ICAM1	MDH1	MMP9		0.950
B4GALT1	CALU	HIST4	ICAM1	LDHB	MDH1		0.950
B4GALT1	CALU	HIST3H3	HIST4	LDHB	MAT2A		0.951
CALU	ICAM1	LDHB	MAT2A				0.951
CALU	HIST3H3	ICAM1	MDH1	MMP9	MAT2A		0.951
HIST3H3	ICAM1	MDH1	MAT2A				0.951
HIST3H3	TPI1	MAT2A					0.951
B4GALT1	CALU	HIST3H3	HIST4	LDHB	MDH1		0.951
CALU	HIST4	ICAM1	LDHB	MDH1	TPI1		0.951
B4GALT1	CALU	HIST4	ICAM1	MDH1	TPI1		0.951
ICAM1	LDHB	MDH1	MAT2A				0.951
CALU	ICAM1	TPI1	MAT2A				0.951
CALU	HIST3H3	HIST4	ICAM1				0.951
ICAM1	MDH1	MMP9	MAT2A				0.952
HIST4	LDHB	MMP9	TPI1	LYN	MAT2A		0.952
CALU	HIST4	LDHB	MDH1	MMP9	MAT2A		0.952
HIST3H3	HIST4	ICAM1	TPI1				0.952
ICAM1	MDH1	TPI1	MAT2A				0.952
B4GALT1	HIST4	ICAM1	MDH1	MMP9	TPI1		0.952
B4GALT1	CALU	HIST4	LDHB	TPI1	MAT2A		0.952
CALU	ICAM1	MDH1	MMP9	MAT2A			0.953
B4GALT1	HIST3H3	HIST4	LDHB	MMP9	MAT2A		0.953
B4GALT1	HIST4	ICAM1	LDHB	MDH1	MMP9		0.953
MDH1	TPI1	MAT2A					0.953
B4GALT1	HIST4	LDHB	MDH1	TPI1	MAT2A		0.953
CALU	HIST4	LDHB	MDH1	LYN	MAT2A		0.953
B4GALT1	HIST3H3	HIST4	LDHB	TPI1	MAT2A		0.953
CALU	HIST4	LDHB	LYN	MAT2A			0.954
B4GALT1	CALU	HIST4	MDH1	MMP9	MAT2A		0.954
CALU	HIST3H3	HIST4	LDHB	TPI1	LYN		0.954
CALU	HIST4	ICAM1					0.954
B4GALT1	HIST4	ICAM1	MDH1	TPI1	LYN		0.954
HIST4	ICAM1	LDHB	MMP9				0.954
HIST3H3	HIST4	LDHB	MDH1	MMP9	TPI1		0.955
HIST3H3	HIST4	ICAM1	MDH1	MMP9	TPI1		0.955
HIST4	ICAM1	MMP9					0.956
CALU	HIST4	ICAM1	MDH1	MMP9	TPI1		0.956
B4GALT1	HIST4	ICAM1	MDH1	LYN			0.956
HIST3H3	HIST4	MDH1	MMP9	TPI1	MAT2A		0.956
B4GALT1	HIST3H3	HIST4	ICAM1	MMP9	LYN		0.956
CALU	HIST4	ICAM1	TPI1	LYN	MAT2A		0.956
B4GALT1	HIST4	ICAM1	MMP9	TPI1	LYN		0.957
B4GALT1	CALU	HIST3H3	HIST4	ICAM1	MAT2A		0.957

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST4	LDHB	MMP9	MAT2A			0.957
B4GALT1	HIST4	MDH1	MMP9	TPI1	MAT2A		0.957
B4GALT1	HIST4	ICAM1	MDH1	MMP9			0.957
B4GALT1	HIST3H3	HIST4	ICAM1	TPI1	LYN		0.957
CALU	HIST3H3	HIST4	ICAM1	MMP9	LYN		0.958
B4GALT1	CALU	HIST4	MDH1	MMP9	LYN		0.958
CALU	HIST3H3	HIST4	ICAM1	MDH1	MAT2A		0.958
CALU	HIST4	ICAM1	MDH1	MMP9			0.958
CALU	HIST4	MDH1	LYN	MAT2A			0.958
HIST4	ICAM1	LDHB	MDH1	MMP9			0.958
HIST3H3	HIST4	ICAM1	LDHB	LYN	MAT2A		0.958
B4GALT1	HIST4	ICAM1	LDHB	LYN			0.958
HIST4	ICAM1	LDHB	MDH1	TPI1	LYN		0.958
B4GALT1	HIST3H3	HIST4	TPI1	MAT2A			0.958
HIST3H3	HIST4	LDHB	MMP9	MAT2A			0.959
B4GALT1	CALU	HIST4	MDH1	LYN	MAT2A		0.959
B4GALT1	CALU	HIST4	MMP9	TPI1	LYN		0.959
CALU	HIST3H3	HIST4	LDHB	TPI1	MAT2A		0.959
HIST4	ICAM1	LDHB	TPI1	LYN	MAT2A		0.959
HIST3H3	HIST4	LDHB	MMP9	LYN	MAT2A		0.959
HIST4	ICAM1	MDH1					0.960
CALU	HIST3H3	HIST4	ICAM1	LDHB	LYN		0.960
CALU	HIST4	ICAM1	MDH1	TPI1	MAT2A		0.960
HIST3H3	HIST4	ICAM1	MMP9	LYN	MAT2A		0.960
B4GALT1	CALU	HIST4	ICAM1	MMP9	MAT2A		0.960
CALU	HIST3H3	HIST4	ICAM1	MAT2A			0.961
B4GALT1	HIST4	TPI1	MAT2A				0.961
HIST3H3	HIST4	ICAM1	TPI1	MAT2A			0.961
CALU	HIST4	MDH1	TPI1				0.961
B4GALT1	HIST3H3	HIST4	MMP9	TPI1	LYN		0.961
B4GALT1	HIST4	ICAM1	TPI1	LYN			0.961
B4GALT1	HIST4	LDHB	TPI1	LYN	MAT2A		0.961
CALU	HIST4	ICAM1	LDHB	MMP9	MAT2A		0.961
CALU	HIST3H3	HIST4	LDHB	MMP9	LYN		0.962
CALU	HIST4	ICAM1	MMP9	TPI1	MAT2A		0.962
CALU	HIST3H3	HIST4	ICAM1	LYN			0.963
CALU	HIST4	LDHB	MAT2A				0.963
HIST4	LDHB	MDH1	TPI1	MAT2A			0.963
HIST4	ICAM1	MDH1	LYN				0.964
B4GALT1	HIST4	ICAM1	TPI1	MAT2A			0.967
HIST4	ICAM1	LDHB	TPI1	MAT2A			0.967
HIST3H3	HIST4	MMP9	LYN	MAT2A			0.967
B4GALT1	HIST4	ICAM1	MDH1	MAT2A			0.967
CALU	HIST4	MDH1	MMP9	LYN			0.967
HIST4	TPI1	MAT2A					0.967
HIST3H3	HIST4	LDHB	TPI1	LYN			0.967
CALU	HIST3H3	HIST4	MMP9	LYN			0.967
CALU	HIST4	LYN					0.967
CALU	HIST4	MMP9	TPI1	LYN			0.967
HIST4	MDH1	LYN	MAT2A				0.967

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST4	LDHB	MDH1	MMP9	LYN		0.967
HIST4	ICAM1	MDH1	LYN	MAT2A			0.967
HIST4	ICAM1	TPI1	MAT2A				0.968
HIST3H3	HIST4	ICAM1	LDHB	MDH1	MAT2A		0.968
B4GALT1	HIST3H3	HIST4	TPI1	LYN			0.968
HIST4	ICAM1	LDHB	MMP9	MAT2A			0.968
HIST4	ICAM1	LDHB	MMP9	LYN			0.969
HIST4	ICAM1	MDH1	MMP9	LYN	MAT2A		0.969
HIST3H3	HIST4	MDH1	MMP9	MAT2A			0.969
HIST4	LDHB	MAT2A					0.969
B4GALT1	HIST4	MMP9	LYN				0.969
HIST4	LDHB	MDH1	MMP9	LYN	MAT2A		0.969
B4GALT1	HIST3H3	HIST4	ICAM1	LYN	MAT2A		0.969
B4GALT1	HIST4	MMP9	TPI1	LYN	MAT2A		0.969
HIST3H3	HIST4	ICAM1	LDHB	LYN			0.970
B4GALT1	HIST4	ICAM1	LDHB	LYN	MAT2A		0.970
HIST3H3	HIST4	MMP9	LYN				0.970
HIST3H3	HIST4	MAT2A					0.970
HIST4	ICAM1	LDHB	LYN				0.970
HIST3H3	HIST4	LDHB	MDH1	MMP9	LYN		0.970
B4GALT1	HIST3H3	HIST4	LYN	MAT2A			0.970
HIST3H3	HIST4	LYN	MAT2A				0.970
HIST4	LDHB	LYN					0.970
HIST3H3	HIST4	ICAM1	MDH1	MMP9	MAT2A		0.971
HIST4	LDHB	MDH1	MMP9	TPI1	LYN		0.971
B4GALT1	HIST3H3	HIST4	LYN				0.971
HIST3H3	HIST4	TPI1	LYN				0.971
HIST3H3	HIST4	MDH1					0.971
HIST4	LDHB	MDH1	LYN				0.971
B4GALT1	HIST4	ICAM1	MMP9	LYN	MAT2A		0.973
HIST4	ICAM1	LYN	MAT2A				0.973
HIST4	MDH1	MAT2A					0.974
HIST3H3	HIST4	LDHB	LYN				0.974
HIST3H3	HIST4	LDHB	MDH1	MAT2A			0.975
HIST3H3	HIST4	LYN					0.975
HIST4	MMP9	LYN					0.975
B4GALT1	HIST4	LDHB	MMP9	LYN	MAT2A		0.976
HIST3H3	HIST4	LDHB	MDH1	LYN			0.976
HIST3H3	HIST4	MDH1	MAT2A				0.978

Table 3D.

Bru vs salmonella

	AUC
B4GALT1	1.000
CALU	0.920
HIST3H3	0.845
HIST4	1.000
ICAM1	0.934
LDHB	0.953

(continued)

Bru vs salmonella

		AUC
LYN		0.887
MAT2A		0.958
MDH1		0.997
MMP9		0.732
TPI1		0.965

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	LDHB	MDH1	MMP9	MAT2A			0.997
B4GALT1	HIST3H3	HIST4	MDH1	LYN	MAT2A		0.997
B4GALT1	MDH1	LYN	MAT2A				0.997
B4GALT1	HIST3H3	ICAM1	MDH1	MAT2A			0.997
B4GALT1	MDH1	TPI1	LYN	MAT2A			0.997
B4GALT1	LDHB	MDH1	LYN	MAT2A			0.996
CALU	HIST4	LYN					0.996
CALU	HIST4	MMP9					0.996
B4GALT1	HIST4	MDH1	MMP9	TPI1	LYN		0.996
B4GALT1	HIST3H3	ICAM1	MDH1	MMP9	MAT2A		0.996
B4GALT1	CALU	HIST3H3	LDHB	MAT2A			0.996
B4GALT1	HIST4	ICAM1	TPI1	MAT2A			0.996
B4GALT1	HIST3H3	HIST4	LDHB	TPI1	LYN		0.996
B4GALT1	HIST4	TPI1	MAT2A				0.996
HIST4	ICAM1	MDH1	TPI1	LYN			0.996
CALU	HIST4	MDH1	LYN				0.996
HIST4	ICAM1	MMP9	TPI1				0.996
B4GALT1	HIST3H3	ICAM1	MMP9	TPI1	LYN		0.996
B4GALT1	CALU	ICAM1	MAT2A				0.996
CALU	HIST4	LDHB	MDH1	MMP9	LYN		0.996
B4GALT1	HIST4	MDH1	MMP9	MAT2A			0.996
B4GALT1	LDHB	MDH1	TPI1	MAT2A			0.995
B4GALT1	HIST4	MDH1	MAT2A				0.995
B4GALT1	HIST4	MDH1	TPI1	LYN	MAT2A		0.995
HIST3H3	HIST4	MDH1	TPI1	MAT2A			0.995
B4GALT1	HIST3H3	ICAM1	LDHB	MAT2A			0.995
B4GALT1	HIST3H3	LDHB	MDH1	MMP9	MAT2A		0.995
CALU	HIST4	LDHB	LYN				0.995
CALU	HIST4	ICAM1	TPI1				0.995
HIST4	ICAM1	LDHB	TPI1				0.995
CALU	HIST4	MDH1	MMP9	TPI1	LYN		0.995
B4GALT1	LDHB	MDH1	MAT2A				0.995
HIST3H3	HIST4	ICAM1	MDH1	MMP9	MAT2A		0.995
B4GALT1	LDHB	MDH1	MMP9	TPI1	MAT2A		0.995
B4GALT1	CALU	HIST3H3	TPI1	LYN	MAT2A		0.995
HIST3H3	HIST4	ICAM1	LDHB	MDH1	MAT2A		0.995
B4GALT1	ICAM1	LDHB	MAT2A				0.995
B4GALT1	CALU	LDHB	MDH1	MMP9	MAT2A		0.995
B4GALT1	CALU	HIST3H3	HIST4	LDHB	MAT2A		0.995
MDH1	MMP9						0.995
B4GALT1	CALU	LDHB	TPI1	LYN	MAT2A		0.995
ICAM1	MDH1	TPI1					0.995

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST4	LDHB	MMP9				0.995
B4GALT1	CALU	HIST3H3	HIST4	MDH1	MAT2A		0.995
CALU	HIST4	ICAM1	MMP9				0.995
B4GALT1	CALU	HIST4	LDHB	MDH1	LYN		0.995
HIST4	LYN						0.995
B4GALT1	CALU	HIST3H3	MDH1	MMP9	MAT2A		0.995
HIST3H3	ICAM1	MDH1					0.995
B4GALT1	MDH1	MAT2A					0.995
HIST3H3	HIST4	LDHB	MDH1	MMP9	MAT2A		0.995
B4GALT1	CALU	HIST4	MDH1	LYN	MAT2A		0.994
CALU	MDH1	LYN					0.994
HIST4	TPI1	LYN					0.994
B4GALT1	HIST4	MMP9	TPI1	MAT2A			0.994
B4GALT1	HIST3H3	MDH1	MMP9	MAT2A			0.994
B4GALT1	HIST3H3	MDH1	TPI1	LYN	MAT2A		0.994
B4GALT1	HIST3H3	ICAM1	MMP9	MAT2A			0.994
B4GALT1	ICAM1	MAT2A					0.994
HIST3H3	LDHB	MDH1	LYN				0.994
CALU	HIST3H3	ICAM1	MDH1				0.994
B4GALT1	CALU	HIST3H3	ICAM1	LDHB	MAT2A		0.994
HIST4	ICAM1	LDHB	MDH1	TPI1	LYN		0.994
B4GALT1	HIST3H3	LDHB	TPI1	MAT2A			0.994
B4GALT1	ICAM1	LDHB	MMP9	TPI1	MAT2A		0.993
ICAM1	MDH1	MMP9					0.993
CALU	ICAM1	MDH1	TPI1				0.993
CALU	HIST3H3	MDH1	TPI1				0.993
CALU	HIST4	LDHB	MMP9	TPI1			0.993
HIST3H3	LDHB	MDH1					0.993
CALU	HIST3H3	MDH1	LYN				0.993
ICAM1	LDHB	MDH1					0.993
HIST4	MDH1	MMP9	TPI1	LYN			0.993
CALU	HIST3H3	MDH1	MMP9				0.993
MDH1	MMP9	TPI1					0.993
HIST4	ICAM1	LDHB	MDH1	MAT2A			0.993
B4GALT1	ICAM1	LDHB	MDH1	MAT2A			0.993
CALU	HIST4	ICAM1	LDHB	MMP9			0.993
HIST3H3	HIST4	TPI1	MAT2A				0.992
B4GALT1	HIST3H3	LDHB	MDH1	MAT2A			0.992
CALU	ICAM1	MDH1	MMP9				0.992
CALU	ICAM1	LDHB	MDH1	LYN			0.992
HIST3H3	HIST4	ICAM1	TPI1	MAT2A			0.992
B4GALT1	HIST3H3	ICAM1	LDHB	TPI1	MAT2A		0.992
HIST3H3	HIST4	ICAM1	LDHB	MAT2A			0.992
CALU	LDHB	MDH1	MMP9				0.991
LDHB	MDH1	MMP9					0.991
CALU	HIST4	LDHB	MMP9	TPI1	LYN		0.991
HIST3H3	ICAM1	LDHB	MDH1				0.991
CALU	HIST3H3	ICAM1	MDH1	TPI1			0.991
B4GALT1	CALU	HIST4	ICAM1	MAT2A			0.991
B4GALT1	CALU	HIST4	MDH1	MAT2A			0.991

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
B4GALT1	CALU	HIST3H3	ICAM1	MDH1	MAT2A		0.991
LDHB	MDH1	TPI1					0.991
HIST3H3	HIST4	LDHB	MMP9	LYN	MAT2A		0.991
CALU	HIST3H3	LDHB	MDH1	LYN			0.991
HIST3H3	MDH1	MMP9	TPI1				0.990
CALU	HIST4	ICAM1	LDHB	MMP9	TPI1		0.990
HIST3H3	HIST4	MMP9	MAT2A				0.990
HIST3H3	HIST4	MDH1	LYN	MAT2A			0.990
HIST3H3	HIST4	MAT2A					0.990
HIST3H3	HIST4	MDH1	TPI1	LYN	MAT2A		0.990
HIST4	MDH1	MMP9	MAT2A				0.990
CALU	ICAM1	LDHB	MDH1	MMP9	LYN		0.990
MDH1	LYN						0.990
HIST4	ICAM1	LDHB	TPI1	MAT2A			0.989
HIST3H3	HIST4	ICAM1	MMP9	MAT2A			0.989
HIST3H3	HIST4	ICAM1	LDHB	LYN	MAT2A		0.989
CALU	HIST3H3	MDH1	TPI1	LYN			0.989
B4GALT1	CALU	MDH1	TPI1	LYN	MAT2A		0.988
CALU	HIST3H3	HIST4	MDH1	MAT2A			0.988
HIST4	MDH1	TPI1	MAT2A				0.988
LDHB	MDH1	TPI1	LYN				0.988
CALU	ICAM1	MDH1	TPI1	LYN			0.988
HIST3H3	ICAM1	LDHB	MDH1	MMP9			0.988
MDH1	TPI1	LYN					0.988
HIST4	MMP9	MAT2A					0.988
CALU	HIST3H3	HIST4	LDHB	MDH1	MAT2A		0.987
HIST3H3	HIST4	ICAM1	TPI1	LYN	MAT2A		0.987
B4GALT1	HIST3H3	TPI1	MAT2A				0.987
CALU	HIST4	ICAM1	LYN				0.987
ICAM1	MDH1	TPI1	LYN				0.987
HIST4	TPI1	MAT2A					0.987
CALU	LDHB	MDH1	TPI1	LYN			0.987
LDHB	MDH1	MAT2A					0.987
HIST3H3	MDH1	MAT2A					0.987
ICAM1	LDHB	MDH1	MMP9	LYN			0.987
HIST3H3	LDHB	MDH1	MMP9				0.986
HIST3H3	HIST4	MMP9	LYN	MAT2A			0.986
CALU	HIST3H3	HIST4	MDH1	MMP9	MAT2A		0.986
CALU	HIST4	LDHB	MDH1	MMP9	MAT2A		0.986
CALU	HIST3H3	LDHB	MDH1	MMP9	LYN		0.986
B4GALT1	CALU	ICAM1	LDHB	MDH1	MAT2A		0.986
B4GALT1	CALU	LDHB	MDH1	MAT2A			0.986
HIST4	ICAM1	LDHB	TPI1	LYN			0.985
HIST4	LDHB	MMP9	TPI1	MAT2A			0.985
CALU	ICAM1	MDH1	MMP9	MAT2A			0.985
B4GALT1	HIST3H3	MMP9	TPI1	MAT2A			0.985
CALU	HIST3H3	HIST4	LDHB	MMP9	MAT2A		0.985
CALU	HIST4	MDH1	MAT2A				0.985
B4GALT1	CALU	HIST4	ICAM1	TPI1	MAT2A		0.984
CALU	HIST3H3	HIST4	LDHB	MAT2A			0.984

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
HIST3H3	HIST4	LYN	MAT2A				0.984
B4GALT1	CALU	TPI1	MAT2A				0.984
HIST4	ICAM1	TPI1	MAT2A				0.984
CALU	MDH1	MMP9	MAT2A				0.984
HIST3H3	HIST4	TPI1	LYN	MAT2A			0.983
B4GALT1	CALU	HIST4	MDH1	TPI1	MAT2A		0.983
CALU	HIST3H3	HIST4	MDH1	LYN	MAT2A		0.982
HIST3H3	MDH1	MMP9	MAT2A				0.982
LDHB	MDH1	MMP9	TPI1	LYN			0.981
CALU	LDHB	MDH1	MMP9	TPI1			0.981
B4GALT1	CALU	LDHB	MDH1	TPI1	MAT2A		0.981
B4GALT1	CALU	ICAM1	MDH1	TPI1	MAT2A		0.981
CALU	HIST3H3	HIST4	ICAM1	MAT2A			0.981
LDHB	MDH1	MMP9	TPI1	LYN	MAT2A		0.980
CALU	HIST3H3	ICAM1	MDH1	MAT2A			0.980
B4GALT1	CALU	HIST3H3	MDH1	TPI1	MAT2A		0.980
HIST4	LDHB	MDH1	MMP9	LYN	MAT2A		0.980
HIST3H3	LDHB	MDH1	MMP9	TPI1	LYN		0.980
LDHB	TPI1						0.979
LDHB	MDH1	MMP9	LYN	MAT2A			0.979
CALU	HIST4	ICAM1	MAT2A				0.979
LDHB	MDH1	MMP9	TPI1	MAT2A			0.979
HIST3H3	MDH1	TPI1	MAT2A				0.979
MDH1	MMP9	TPI1	LYN	MAT2A			0.978
CALU	HIST4	ICAM1	MDH1	TPI1	MAT2A		0.978
HIST4	ICAM1	LDHB	MMP9	TPI1	LYN		0.978
CALU	LDHB	MAT2A					0.978
HIST4	ICAM1	LDHB	TPI1	LYN	MAT2A		0.978
MDH1	TPI1	LYN	MAT2A				0.978
ICAM1	LDHB	MDH1	MMP9	TPI1	MAT2A		0.978
ICAM1	LDHB	MDH1	TPI1	LYN			0.977
MDH1	LYN	MAT2A					0.976
CALU	HIST3H3	HIST4	LYN	MAT2A			0.976
HIST3H3	ICAM1	LDHB	MDH1	MAT2A			0.976
CALU	HIST3H3	LDHB	MDH1	MAT2A			0.976
ICAM1	LDHB	MDH1	MMP9	LYN	MAT2A		0.975
CALU	HIST3H3	ICAM1	LDHB	MDH1	MAT2A		0.975
HIST4	MDH1	MMP9	TPI1	LYN	MAT2A		0.975
ICAM1	LDHB	MDH1	TPI1	LYN	MAT2A		0.974
CALU	ICAM1	TPI1	LYN				0.974
HIST4	LYN	MAT2A					0.974
ICAM1	MMP9	TPI1	LYN				0.974
HIST4	LDHB	LYN	MAT2A				0.974
CALU	LDHB	MDH1	LYN	MAT2A			0.973
ICAM1	MDH1	MMP9	TPI1	LYN	MAT2A		0.973
CALU	HIST4	ICAM1	MMP9	LYN	MAT2A		0.973
HIST4	ICAM1	LDHB	MMP9	LYN	MAT2A		0.973
LDHB	MMP9	LYN					0.971
ICAM1	LDHB	MMP9	LYN				0.971
HIST3H3	ICAM1	LDHB	TPI1				0.971

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	ICAM1	LDHB	MAT2A				0.969
CALU	HIST3H3	MDH1	LYN	MAT2A			0.968
ICAM1	TPI1	MAT2A					0.968
CALU	LDHB	MMP9	LYN				0.967
CALU	HIST3H3	ICAM1	LDHB	TPI1	LYN		0.962
CALU	HIST3H3	ICAM1	LDHB	LYN			0.962
CALU	ICAM1	LDHB	TPI1	LYN	MAT2A		0.962
CALU	HIST3H3	ICAM1	TPI1	LYN			0.962
ICAM1	LDHB	MMP9	TPI1	MAT2A			0.962
CALU	HIST3H3	ICAM1	LDHB				0.962
HIST3H3	ICAM1	MMP9	TPI1				0.962
CALU	HIST3H3	MMP9	TPI1	LYN	MAT2A		0.961
CALU	LDHB	TPI1	MAT2A				0.961
CALU	LDHB	LYN	MAT2A				0.961
CALU	HIST4	TPI1	LYN	MAT2A			0.960
CALU	ICAM1	MMP9	TPI1	LYN			0.960
CALU	HIST3H3	LDHB	TPI1	LYN	MAT2A		0.960
HIST3H3	ICAM1	MMP9	TPI1	LYN			0.960
HIST3H3	ICAM1	MAT2A					0.960
CALU	HIST3H3	ICAM1	TPI1	MAT2A			0.958
HIST3H3	ICAM1	LDHB	MMP9	TPI1	MAT2A		0.958
HIST3H3	LDHB						0.958
ICAM1	LDHB	MMP9	LYN	MAT2A			0.958
CALU	HIST3H3	ICAM1	TPI1				0.958
MMP9	TPI1	MAT2A					0.958
HIST3H3	LDHB	MMP9	MAT2A				0.957
CALU	HIST3H3	TPI1	LYN	MAT2A			0.957
CALU	ICAM1	MMP9	LYN	MAT2A			0.957
CALU	ICAM1	LYN					0.957
CALU	LYN						0.957
ICAM1	MMP9	MAT2A					0.957
HIST3H3	LDHB	LYN	MAT2A				0.956
CALU	HIST3H3	MMP9	LYN				0.956
ICAM1	MMP9	TPI1	LYN	MAT2A			0.956
CALU	HIST3H3	ICAM1	MMP9	TPI1	LYN		0.954
CALU	LDHB	MMP9					0.954
CALU	HIST3H3	LDHB					0.954
HIST3H3	ICAM1	LYN					0.954
LDHB	MMP9	LYN	MAT2A				0.954
HIST3H3	ICAM1	TPI1	LYN	MAT2A			0.954
CALU	ICAM1	LYN	MAT2A				0.953
CALU	HIST3H3	ICAM1	LDHB	MMP9	LYN		0.952
CALU	HIST3H3	LYN					0.950
HIST3H3	ICAM1	MMP9	TPI1	LYN	MAT2A		0.950
CALU	HIST3H3	ICAM1	LYN				0.947
HIST3H3	MMP9	LYN	MAT2A				0.945
HIST3H3	ICAM1	MMP9	LYN	MAT2A			0.943
CALU	ICAM1						0.933
ICAM1	MMP9						0.930
HIST3H3	ICAM1	MMP9					0.924

(continued)

prot 1	prot 2	prot 3	prot 4	prot 5	prot 6	prot 7	AUC 1
CALU	HIST3H3	ICAM1	MMP9				0.923
CALU	HIST3H3						0.908
HIST3H3	MMP9	LYN					0.906
HIST3H3	MMP9						0.832

[0466] Further analysis of the markers identified the best performing markers and panels of markers for Q-fever comparisons to Brucellosis, Lyme disease and to healthy controls that have good individual performance and that were also able to complement each other. Similarly,the best performing markers and panels of markers for Lyme Disease comparisons to Brucellosis, Q-Fever and to healthy controls that have good individual performance and that were also able to complement each other were identified. (Tables 4A-4G).

Table 4A.

Protein	Transition	Disease Group	Reference Group	DI
B4GALT1	186	Q-fever	Healthy Control	0.67
CALU	249	Q-fever	Healthy Control	0.42
HIST3H3	470	Q-fever	Healthy Control	0.50
HIST4	475	Q-fever	Healthy Control	0.13
ICAM1	541	Q-fever	Healthy Control	0.82
LDHB	599	Q-fever	Healthy Control	0.66
LYN	-	Q-fever	Healthy Control	5.08
MAT2A	-	Q-fever	Healthy Control	1.25
MDH1	652	Q-fever	Healthy Control	0.64
MMP9	654	Q-fever	Healthy Control	0.54
TPI1	1056	Q-fever	Healthy Control	0.56

B4GALT1	186	Healthy Control	Q-fever	1.49
CALU	249	Healthy Control	Q-fever	2.37
HIST3H3	470	Healthy Control	Q-fever	1.99
HIST4	475	Healthy Control	Q-fever	7.95
ICAM1	541	Healthy Control	Q-fever	1.21
LDHB	599	Healthy Control	Q-fever	1.52
LYN	-	Healthy Control	Q-fever	0.20
MAT2A	-	Healthy Control	Q-fever	0.80
MDH1	652	Healthy Control	Q-fever	1.55
MMP9	654	Healthy Control	Q-fever	1.85
TPI1	1056	Healthy Control	Q-fever	1.78

Table 4B.

Protein	Transition	Disease Group	Reference Group	DI
B4GALT1	186	Brucellosis	Q-Fever	2.73
CALU	249	Brucellosis	Q-Fever	3.17
HIST3H3	470	Brucellosis	Q-Fever	4.56
HIST4	475	Brucellosis	Q-Fever	130.92
ICAM1	541	Brucellosis	Q-Fever	3.18
LDHB	599	Brucellosis	Q-Fever	3.94
LYN	-	Brucellosis	Q-Fever	1.14
MAT2A	-	Brucellosis	Q-Fever	0.19
MDH1	652	Brucellosis	Q-Fever	4.28
MMP9	654	Brucellosis	Q-Fever	2.59
TPI1	1056	Brucellosis	Q-Fever	3.15
B4GALT1	186	Q-Fever	Brucellosis	0.37
CALU	249	Q-Fever	Brucellosis	0.32
HIST3H3	470	Q-Fever	Brucellosis	0.22
HIST4	475	Q-Fever	Brucellosis	0.01
ICAM1	541	Q-Fever	Brucellosis	0.31
LDHB	599	Q-Fever	Brucellosis	0.25
LYN	-	Q-Fever	Brucellosis	0.87
MAT2A	-	Q-Fever	Brucellosis	5.29
MDH1	652	Q-Fever	Brucellosis	0.23
MMP9	654	Q-Fever	Brucellosis	0.39
TPI1	1056	Q-Fever	Brucellosis	0.32

Table 4C.

Protein	Transition	Disease Group	Reference Group	DI
B4GALT1	186	Lyme disease	Healthy Control	0.70
CALU	249	Lyme disease	Healthy Control	1.54
HIST3H3	470	Lyme disease	Healthy Control	0.95
HIST4	475	Lyme disease	Healthy Control	0.29
ICAM1	541	Lyme disease	Healthy Control	0.90
LDHB	599	Lyme disease	Healthy Control	0.98
LYN	-	Lyme disease	Healthy Control	1.25
MAT2A	-	Lyme disease	Healthy Control	1.18
MDH1	652	Lyme disease	Healthy Control	0.84
MMP9	654	Lyme disease	Healthy Control	4.48
TPI1	1056	Lyme disease	Healthy Control	0.96
B4GALT1	186	Healthy Control	Lyme disease	1.43
CALU	249	Healthy Control	Lyme disease	0.65
HIST3H3	470	Healthy Control	Lyme disease	1.05
HIST4	475	Healthy Control	Lyme disease	3.41
ICAM1	541	Healthy Control	Lyme disease	1.12
LDHB	599	Healthy Control	Lyme disease	1.02
LYN	-	Healthy Control	Lyme disease	0.80
MAT2A	-	Healthy Control	Lyme disease	0.85
MDH1	652	Healthy Control	Lyme disease	1.19
MMP9	654	Healthy Control	Lyme disease	0.22
TPI1	1056	Healthy Control	Lyme disease	1.04

Table 4D.

Protein	Transition	Disease Group	Reference Group	DI
B4GALT1	186	Brucellosis	Lyme	2.62
CALU	249	Brucellosis	Lyme	0.87
HIST3H3	470	Brucellosis	Lyme	2.41
HIST4	475	Brucellosis	Lyme	56.14
ICAM1	541	Brucellosis	Lyme	2.92
LDHB	599	Brucellosis	Lyme	2.65
LYN	-	Brucellosis	Lyme	4.66
MAT2A	-	Brucellosis	Lyme	0.20
MDH1	652	Brucellosis	Lyme	3.26
MMP9	654	Brucellosis	Lyme	0.31
TPI1	1056	Brucellosis	Lyme	1.84
B4GALT1	186	Lyme	Brucellosis	0.38
CALU	249	Lyme	Brucellosis	1.15
HIST3H3	470	Lyme	Brucellosis	0.42
HIST4	475	Lyme	Brucellosis	0.02
ICAM1	541	Lyme	Brucellosis	0.34
LDHB	599	Lyme	Brucellosis	0.38
LYN	-	Lyme	Brucellosis	0.21
MAT2A	-	Lyme	Brucellosis	5.02
MDH1	652	Lyme	Brucellosis	0.31
MMP9	654	Lyme	Brucellosis	3.19
TPI1	1056	Lyme	Brucellosis	0.54

Table 4E.

Protein	Transition	Disease Group	Reference Group	DI
B4GALT1	186	Lyme disease	Q-fever	1.04
CALU	249	Lyme disease	Q-fever	3.65
HIST3H3	470	Lyme disease	Q-fever	1.89
HIST4	475	Lyme disease	Q-fever	2.33

ICAM1	541	Lyme disease	Q-fever	1.09
LDHB	599	Lyme disease	Q-fever	1.49
LYN	-	Lyme disease	Q-fever	0.25
MAT2A	-	Lyme disease	Q-fever	0.95
MDH1	652	Lyme disease	Q-fever	1.31
MMP9	654	Lyme disease	Q-fever	8.29
TPI1	1056	Lyme disease	Q-fever	1.71

B4GALT1	186	Q-fever	Lyme disease	0.96
CALU	249	Q-fever	Lyme disease	0.27
HIST3H3	470	Q-fever	Lyme disease	0.53
HIST4	475	Q-fever	Lyme disease	0.43
ICAM1	541	Q-fever	Lyme disease	0.92
LDHB	599	Q-fever	Lyme disease	0.67
LYN	-	Q-fever	Lyme disease	4.07
MAT2A	-	Q-fever	Lyme disease	1.05
MDH1	652	Q-fever	Lyme disease	0.76
MMP9	654	Q-fever	Lyme disease	0.12
TPI1	1056	Q-fever	Lyme disease	0.58

Table 4F.

		Brucellosis vs		
Minimal optimal panel		Control	Q-fever	Lyme
HIST4	LYN	0.974		1.000
HIST4	MAT2A	0.974	0.992	
HIST4	MDH1		0.998	
HIST4	LDHB		0.996	
HIST4	MMP9			0.999
HIST4	ICAM1			0.998

Table 4G.

		Lyme vs		
Minimal optimal panel		Control	Q-fever	Brucella
MMP9		0.950		
MMP9	MDH1	0.964		
CALU	B4GALT1		0.961	
HIST4				0.995
HIST4	LYN			1.000
HIST 4	MMP9			0.999

Table 4H.

Minimal optimal panel		Q fever vs Control	Q fever vs Lyme	Q fever vs Brucella
LYN	LDHB	0.908		
CALU			0.941	
CALU	B4GALT1		0.961	
HIST4				0.999
HIST 4	LDHB			0.996
HIST 4	MDH1			0.998

Discussion

[0467] A rational approach for identification of a new class of candidate biomarkers of the zoonosis, brucellosis, has been described herein. Currently brucellosis can be diagnosed either by recovering viable bacteria from patient cultures or, more typically, by measuring the relative reactivity of anti-Brucella immunoglobulin from patient sera. Perturbations to the secretome and secretome-associated compartments of infected host cells were focused on and identified the, and then many of those changes were followed into the periphery.

[0468] Proteomic analysis to the secretome 24 hours after infection was focused on. By that time the *Brucella* bacteria that survived the early stages of infection have established their intracellular niche, and have begun to significantly impact the host cell physiology as they begin to expand their numbers (Celli J, et al. (2003) J Exp Med 198: 545-556; Lamontagne J, et al. (2009) J Proteome Res 8: 1594-1609). To reflect *Brucella* cell tropism *in vivo* both macrophage and trophoblast cell lines were used as infection models. The bone marrow-derived and cell line macrophage infection models of *Brucella* infection have been extensively described. Epithelial placental trophoblasts, which are in direct contact with the fetus, also constitute an important Brucella abortus target. Brucella can attain significant numbers within trophoblasts without damaging the chorionic layer. When infected trophoblasts separate from the basal membrane and die, they can release large amounts of bacteria into the fetal layers (Anderson TD, et al. (1986) Vet Pathol 23: 227-239; Anderson TD, et al. (1986) Vet Pathol 23: 219-226; Ushizawa K, et al. (2005) J Reprod Dev 51: 211-220). Thus, Brucella infected trophoblast cell lines are a physiologically relevant complement to macrophage infections and given the secretory functions of these cells, they are an excellent source for identifying candidate secreted protein biomarkers. Bovine (Miyazaki H, et al. (2002) Placenta 23: 613-630) caprine (Ramsoondar J, et al. (1993) Biol Reprod 49: 681-694), porcine (Halwachs-Baumann G, et al. (2006) J Clin Virol 37: 91-97) as well as a few human trophoblastic cell lines derived from placental tumors have been used as in vitro infection models (Pfaff EW, et al. (2005) Int J Parasitol 35: 1569-1576; Vidricaire G, et al. (2003) J Biol Chem 278: 15832-15844; Gross A, et al. (2004) Microb Pathog 36: 75-82), although these models are not as well characterized as the macrophage models. The response of professional phagocytes and epithelial cells to *Brucella* infection appeared to differ, and using both cell models a rich dataset of differentially expressed proteins was identified. The vast majority appeared to be potentially secreted, reflecting the multiple mechanisms available to mammalian cells for protein secretion (Chua CE, et al. (2012) J Cell Physiol 227: 3722-3730; Glebov K, Walter J (2012) Neurodegener Dis 10: 309-312; Giuliani F, et al. (2011) Curr Opin Cell Biol 23: 498-504).

Biomarkers

[0469] The incubation period for Brucella infection in humans may last several weeks. During this period the bacteria distribute mainly in regional lymphatics close to the site of infection, typically the oropharyngeal, tonsils, cervical or retro orbital lymphatics (Sauret JM, Vilissova N (2002) J Am Board Fam Med 15(5); Spink WW (1956) The Nature of Brucellosis. University of Minnesota Press. pp. 114-115). A transient local inflammation may be observed. Symptoms generally manifest 3-8 weeks after infection, when the bacteria have dispersed through the reticuloendothelial system to the liver and spleen (Rodriguez-Zapata M, et al. (2010) Infect Immun 78: 3272-3279). The symptoms appear to be related more to the host Th1 response and chronic inflammatory reactions than to toxic effects mediated directly by bacterial proteins or components, such as exotoxins or LPS. The sera that was tested were most likely collected when the subjects had reached this latter stage of disease progression, after the manifestation of symptoms and likely after major organs such as the liver and spleen had become infected. Biomarker discovery using the cell line models, however, was done at 24 hours of infection. A subset of the secreted proteins we observed after a 24 hour infection of macrophages and trophoblasts appeared specifically associated with acute brucellosis in these presumably later stage subjects. This indicates that the changes to the secretory pathways of infected host cells at the onset of infection remained relevant at the later stages

as well.

[0470] Brucellosis presents clinically as recurring fever typically with joint and abdominal pain. Lyme disease has a similar clinical presentation and epidemiology as brucellosis, but a dissimilar infection mechanism. Q-fever also has a similar presentation and epidemiology as brucellosis, and a similar infection mechanism. Salmonellosis, in contrast, has a different clinical presentation and epidemiology than brucellosis but a similar infection mechanism. These three additional diseases, therefore, would be useful in assessing the specificity of the candidate biomarkers as well as their physiological relevance.

[0471] Host proteins defined panels able to distinguish sera from subjects with brucellosis from those infected with Lyme disease, Q-fever, or salmonellosis. They represented various biological functions identified in the secretome of the Brucella-infected cells that seemed to complement each other in the panel calculations.

Equivalents

[0472] In describing exemplary embodiments, specific terminology is used for the sake of clarity. For purposes of description, each specific term is intended to at least include all technical and functional equivalents that operate in a similar manner to accomplish a similar purpose. Additionally, in some instances where a particular exemplary embodiment includes a plurality of system elements or method steps, those elements or steps may be replaced with a single element or step. Likewise, a single element or step may be replaced with a plurality of elements or steps that serve the same purpose. Further, where parameters for various properties are specified herein for exemplary embodiments, those parameters may be adjusted up or down by 1/20th, 1/10th, 1/5th, 1/3rd, ½, *etc.,* or by rounded-off approximations thereof, unless otherwise specified. Moreover, while exemplary embodiments have been shown and described with references to particular embodiments thereof, those of ordinary skill in the art will understand that various substitutions and alterations in form and details may be made therein without departing from the scope of the invention. Further still, other aspects, functions and advantages are also within the scope of the invention.

[0473] Exemplary flowcharts are provided herein for illustrative purposes and are nonlimiting examples of methods. One of ordinary skill in the art will recognize that exemplary methods may include more or fewer steps than those illustrated in the exemplary flowcharts, and that the steps in the exemplary flowcharts may be performed in a different order than shown.

Claims

1. A method for determining whether a subject has Brucellosis or Q-Fever, the method comprising
   determining the level of HIST4H4, LDHB, and MDH1 in a blood sample(s) obtained from the subject;
   comparing the level of HIST4H4, LDHB, and MDH1 in the subject sample(s) with a control level of HIST4H4, LDHB, and MDH1,
   wherein a higher level of HIST4H4, a higher level of LDHB, and a higher level of MDH1 in the subject sample(s) as compared to the level of the corresponding marker in a control sample(s) from a subject(s) that has Q-Fever; or to an average level of HIST4H4, LDHB, and MDH in a sample(s) from a subject(s) that has Q-Fever indicates that the subject has Brucellosis, and
   wherein a lower level of HIST4H4, a lower level of LHDB, and a lower level of MDH1 in the subject sample(s) as compared to the level of the corresponding marker in the control sample(s) from a healthy control subject; or to an average level of HIST4H4, LDHB, and MDH in a sample(s) from a healthy control subject indicates that the subject has Q-Fever.

2. The method of claim 1 wherein the level in the subject sample(s) is determined by mass spectrometry.

3. The method of claim 1, further comprising determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDHI, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

4. The method of claim 1, further comprising determining the level of one or more markers selected from the group consisting of SLC3A2, GOT1, GOT2, ACADVL, DBI, ACOXI, AHNAK, AIMP1, AKRIBI, ANPEP, ANXA2, ANXA5, ANXA6, AKR7A2, ARPC3, ASAHI, B4GALT1, BCAM, BLOC1S5, CALU, CAPG, CAPZB, CAPZA2, CBX1, CDC37, HSPE1, CHMP1A, CHMP1B, CHMP2A, CHMP4A, CHMP4B, CHMP5, CLIO, CNDP2, CNPY2, COP A, COPB2, COROIA, COROIB, COROIC, CPVL, C19orfI0, CXorf26, CXCL10, CACYBP, DSP, HSD17B4, DLG1, DNASE2, DDT, DYNLL1, EPB41L3, EEF1A1, EEF1B2, EEF1D, EEF2, ENG, EHD1, ELAVL1, EMC2, EMR2, ENO1, EN03, HSP90B1, EROIL, ESYT2, EVL, ST13, F5, FKBP4, FLNA, FH, H6PD, GPI, GDI2, ARHGDIA, GGH, GBA, PRKCSH,

GNS, HSPA9, HSPA5, GSTP1, GLT25D1, HIST1H1D, HIST1H1B, HIST3H2A, H2AFY, HIST2H2BE, HIST2H2BF, HIST3H3, HIST1H4A, HSD17B10, HEXA, HMGB3, HNRNPC, HNRNPD, HNRNPK, HNRNPR, HNRNPU, HSP90AA1, HSP90AB1, HSPAIA, HSPA8, ICAM1, EIF5A2, ILIB, IMPDH2, ISYNA1, ISOC1, ITGA5, ITGAM, ITPR1, KRT9, KRT7, KRT8, CAMK2D, PKM, LASP1, LDHB, LIMA1, LMNA, LMNB1, LMNB2, LRCH1, GAA, LYN, MDH1, MDH2, MESDC2, MAT2A, MYL12A, MMP9, RABIF, MYH10, MYH9, MY01E, MY06, PPP1R12A, NAGA, POR, NME1, NME2, NDRG1, NDUFA2, NAP1L1, NPM1, NUTF2, NCL, NUDC, OAS2, PAFAH1B2, PA2G4, PARK7, PARP1, PRCP, PDCD61P, P4HB, PDIA5, PEBP1, PECAM1, PFDN1, PFDN2, PGAM1, PGK1, PIP4K2A, PIP5K1A, PLEKH02, PLEC, PLOD3, LCP1, PNP, ALPP, PPIA, PPIB, PRDX1, PRDX2, PRDX3, PRDX6, PRPF19, PSMC6, PSMC3, PSMC2, PRSS8, NPEPPS, PSMA7, PSMB5, PSMDIO, PSMD11, PSMD14, PSMD1, PSMD2, PSMD4, PTBP3, PAICS, ATIC, PXMP2, RAB6A, RANGAP1, RA114, RAN, RANBP1, RBBP4, RAD23B, RPL14, RPL23A, RPL24, RPL26, RPL29, RPL3, RPL31, RPL7A, RPL8, RPLP2, MRPL39, HNRNPA1, HNRNPA2B1, RPS23, RPS8, RSAD2, RPSA, RUVBL1, RUVBL2, S100P, S100A6, S100A11, SEC61B, SEPTI1, SEPT2, SEPT6, SEPT7, SEPT9, PHGDH, SF3B3, SFPQ, SIAE, SNRPD3, NAPA, SOD2, SORT1, SGSH, SPRED1, SPTBN1, SPG11, SPTAN1, SRSF3, SRSF8, STIP1, STMN1, STRAP, STX11, SUM03, EPRS, IARS2, TAGLN2, TALDO1, TUBA4A, TUBB4B, TUBB, TBL2, CCT4, CCT8, TDRD6, VCP, TKT, TLN1, TMOD3, TPI1, TRA2B, TXNRD1, USP14, UGGT1, ATP6V1A, ATP6V1B2, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, VPS4A, XRCC6, and YBX1.

5. Use of a kit comprising reagents for determining the level of HIST4H4, LDHB, and MDH1 in a blood sample(s) obtained from a subject and instructions for use in a diagnostic method according to claim 1.

6. The use of claim 5, wherein the kit further comprises reagents for determining the level of any one or more of the markers listed in Table 1 in a sample(s) from the subject.

7. The use of the kit of claim 5, wherein the kit further comprises reagents for determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT, and TPI1.

8. The use of the kit of claim 5, wherein the kit further comprises reagents for obtaining a sample(s) from a subject or a control fluid sample(s).

9. The method of claim 1, further comprising determining the level of one or more additional markers selected from the group consisting of LYN, MAT2A, ICAM1, MMP9, CALU, and B4GALT1.

10. The method of claim 1, further comprising determining the level of one or more additional markers selected from the group consisting of HIST3H3, ICAM1, FLNA, H6PD, and MMP9.

11. The method of claim 1, wherein the blood sample(s) is a serum sample.

12. The method of claim 2, wherein the mass spectrometry comprises ionizing the HIST4H4, LDHB, and MDH1 in the subject sample(s).

13. The method of claim 1, wherein the level in the subject sample(s) is determined by immunoassay.

14. The use of the kit of claim 5, wherein the kit further comprises a control sample(s).

15. The method of claim 1, wherein the subject is a veterinary subject.

16. The method of claim 1, wherein the subject is human.

Patentansprüche

1. Verfahren zum Bestimmen, ob ein Subjekt Brucellose oder Q-Fieber hat, wobei das Verfahren umfasst:

Bestimmen des Pegels an HIST4H4, LDHB und MDH1 in einer Blutprobe/in Blutproben, die von dem Subjekt erhalten wurde(n) ;
Vergleichen des Pegels an HIST4H4, LDHB und MDH1 in der Subjektprobe/den Subjektproben mit einem

Kontrollpegel an HIST4H4, LDHB und MDH1,
wobei ein höherer Pegel an HIST4H4, ein höherer Pegel an LDHB und ein höherer Pegel an MDH1 in der Subjektprobe/den Subjektprobem im Vergleich zum Pegel des entsprechenden Markers in einer Kontrollprobe/in Kontrollproben aus einem Subjekt/den Subjekten, das/die Q-Fieber hat/haben, oder zu einem durchschnittlichen Pegel an HIST4H4, LDHB und MDH in einer Probe/in Proben aus einem Subjekt/Subjekten, das/die Q-Fieber hat/haben, anzeigt, dass das Subjekt Brucellose hat, und
wobei ein niedrigerer Pegel an HIST4H4, ein niedrigerer Pegel an LDHB und ein niedrigerer Pegel an MDH1 in der Subjektprobe/den Subjektproben im Verbleich zum Pegel des entsprechenden Markers in der Kontrollprobe/in Kontrollproben aus einem gesunden Kontrollsubjekt; oder zu einem durchschnittlichen Pegel von HIST4H4, LDHB und MDH in einer Probe/in Proben aus einem gesunden Kontrollsubjekt anzeigt, dass das Subjekt Q-Fieber hat.

2. Verfahren aus Anspruch 1, wobei der Pegel in der Subjektprobe/den Subjektproben durch Massenspektrometrie bestimmt wird.

3. Verfahren aus Anspruch 1, ferner umfassend Bestimmen des Pegels eines oder mehrerer zusätzlichen/zusätzlicher Marker(s), ausgewählt aus der Gruppe, bestehend aus LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDHI, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT und TPI1.

4. Verfahren aus Anspruch, ferner umfassend Bestimmen des Pegels eines oder mehrerer Marker(s), ausgewählt aus der Gruppe, bestehend aus SLC3A2, GOT1, GOT2, ACADVL, DBI, ACOXI, AHNAK, AIMP1, AKRIBI, ANPEP, ANXA2, ANXA5, ANXA6, AKR7A2, ARPC3, ASAHI, B4GALT1, BCAM, BLOC1S5, CALU, CAPG, CAPZB, CAPZA2, CBX1, CDC37, HSPE1, CHMP1A, CHMP1B, CHMP2A, CHMP4A, CHMP4B, CHMP5, CLIO, CNDP2, CNPY2, COP A, COPB2, COROIA, COROIB, COROIC, CPVL, C19orf10, CXorf26, CXCL10, CACYBP, DSP, HSD17B4, DLG1, DNASE2, DDT, DYNLL1, EPB41L3, EEF1A1, EEF1B2, EEF1D, EEF2, ENG, EHD1, ELAVL1, EMC2, EMR2, ENO1, EN03, HSP90B1, EROIL, ESYT2, EVL, ST13, F5, FKBP4, FLNA, FH, H6PD, GPI, GDI2, ARHGDIA, GGH, GBA, PRKCSH, GNS, HSPA9, HSPA5, GSTP1, GLT25D1, HIST1H1D, HIST1H1B, HIST3H2A, H2AFY, HIST2H2BE, HIST2H2BF, HIST3H3, HIST1H4A, HSD17B10, HEXA, HMGB3, HNRNPC, HNRNPD, HNRNPK, HNRNPR, HNRNPU, HSP90AA1, HSP90AB1, HSPAIA, HSPA8, ICAM1, EIF5A2, ILIB, IMPDH2, ISYNA1, ISOC1, ITGA5, ITGAM, ITPR1, KRT9, KRT7, KRT8, CAMK2D, PKM, LASP1, LDHB, LIMA1, LMNA, LMNB1, LMNB2, LRCH1, GAA, LYN, MDH1, MDH2, MESDC2, MAT2A, MYL12A, MMP9, RABIF, MYH10, MYH9, MY01E, MY06, PPP1R12A, NAGA, POR, NME1, NME2, NDRG1, NDUFA2, NAP1L1, NPM1, NUTF2, NCL, NUDC, OAS2, PAFAH1B2, PA2G4, PARK7, PARP1, PRCP, PDCD6IP, P4HB, PDIA5, PEBP1, PECAM1, PFDN1, PFDN2, PGAM1, PGK1, PIP4K2A, PIP5K1A, PLEKH02, PLEC, PLOD3, LCP1, PNP, ALPP, PPIA, PPIB, PRDX1, PRDX2, PRDX3, PRDX6, PRPF19, PSMC6, PSMC3, PSMC2, PRSS8, NPEPPS, PSMA7, PSMB5, PSMDIO, PSMD11, PSMD14, PSMD1, PSMD2, PSMD4, PTBP3, PAICS, ATIC, PXMP2, RAB6A, RANGAP1, RAI14, RAN, RANBP1, RBBP4, RAD23B, RPL14, RPL23A, RPL24, RPL26, RPL29, RPL3, RPL31, RPL7A, RPL8, RPLP2, MRPL39, HNRNPA1, HNRNPA2B1, RPS23, RPS8, RSAD2, RPSA, RUVBL1, RUVBL2, S100P, S100A6, S100A11, SEC61B, SEPTI1, SEPT2, SEPT6, SEPT7, SEPT9, PHGDH, SF3B3, SFPQ, SIAE, SNRPD3, NAPA, SOD2, SORT1, SGSH, SPRED1, SPTBN1, SPG11, SPTAN1, SRSF3, SRSF8, STIP1, STMN1, STRAP, STX11, SUM03, EPRS, IARS2, TAGLN2, TALDO1, TUBA4A, TUBB4B, TUBB, TBL2, CCT4, CCT8, TDRD6, VCP, TKT, TLN1, TMOD3, TPI1, TRA2B, TXNRD1, USP14, UGGT1, ATP6V1A, ATP6V1B2, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, VPS4A, XRCC6 und YBX1.

5. Verwendung eines Kits, umfassend Reagenzien zum Bestimmen des Pegels an HIST4H4, LDHB und MDH1 in einer Blutprobe/in Blutproben, die aus einem Subjekt erhalten wurde(n), und Anweisungen zur Verwendung in einem diagnostischen Verfahren gemäß Anspruch 1.

6. Verwendung von Anspruch 5, wobei das Kit ferner Reagenzien zum Bestimmen eines oder mehrerer der Marker(s), der/die in Tabelle 1 gelistet ist/sind, in einer Probe/in Proben aus dem Subjekt umfasst.

7. Verwendung des Kits von Anspruch 5, wobei das Kit ferner Reagenzien zum Bestimmen des Pegels eines oder mehrerer zusätzlichen/zusätzlicher Marker(s), ausgewählt aus der Gruppe, bestehend aus LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT und TPI1, umfasst.

8. Verwendung des Kits von Anspruch 5, wobei das Kit ferner Reagenzien zum Erhalten einer Probe/von Proben aus

einem Subjekt oder einer flüssigen Kontrollprobe/von flüssigen Kontrollproben umfasst.

9. Verfahren von Anspruch 1, ferner umfassend Bestimmen des Pegels eines oder mehrerer zusätzlicher/zusätzlichen Marker(s), ausgewählt aus der Gruppe, bestehend aus LYN, MAT2A, ICAM1, MMP9, CALU und B4GALT1.

10. Verfahren von Anspruch 1, ferner umfassend Bestimmen des Pegels eines oder mehrerer zusätzlichen/zusätzlicher Marker(s), ausgewählt aus der Gruppe, bestehend aus HIST3H3, ICAM1, FLNA, H6PD und MMP9.

11. Verfahren von Anspruch 1, wobei die Blutprobe(n) eine Serumprobe ist/sind.

12. Verfahren von Anspruch 2, wobei die Massenspektrometrie Ionisieren des/der HIST4H4, LDHB und MDH1 in der Subjektprobe/den Subjektproben umfasst.

13. Verfahren von Anspruch 1, wobei der Pegel in der Subjektprobe/den Subjektproben durch Immunoassay bestimmt wird.

14. Verwendung des Kits von Anspruch 5, wobei das Kit ferner eine Kontrollprobe(n) umfasst.

15. Verfahren von Anspruch 1, wobei das Subjekt ein Veterinärsubjekt ist.

16. Verfahren von Anspruch 1, wobei das Subjekt ein Mensch ist.

Revendications

1. Procédé permettant de déterminer si un sujet a une brucellose ou une fièvre Q, le procédé comprenant
la détermination du niveau d'HIST4H4, de LDHB et de MDH1 dans un/des échantillon(s) de sang obtenu(s) du sujet ;
la comparaison du taux d'HIST4H4, de LDHB et de MDH1 dans le/les échantillon(s) du sujet avec un taux témoin d'HIST4H4, de LDHB et de MDH1,
dans lequel un taux supérieur d'HIST4H4, un taux supérieur de LDHB et un taux supérieur de MDH1 dans le/les échantillon(s) du sujet par rapport au taux du marqueur correspondant dans un/des échantillon(s) témoin(s) d'un/de sujet(s) qui a/ont une fièvre Q ; ou
un taux moyen d'HIST4H4, de LDHB et de MDH dans un/des échantillon(s) d'un/de sujet(s) qui a/ont la fièvre Q indique que le sujet à une brucellose, et
dans lequel un taux inférieur d'HIST4H4, un taux inférieur de LHDB et un taux inférieur de MDH1 dans le/les échantillon(s) du sujet par rapport au taux du marqueur correspondant dans le/les échantillon(s) témoin(s) d'un sujet témoin sain ; ou
un taux moyen d'HIST4H4, de LDHB et de MDH dans un/des échantillon(s) d'un sujet témoin sain indique que le sujet a une fièvre Q.

2. Procédé selon la revendication 1, dans lequel le taux dans le/les échantillon(s) du sujet est déterminé par spectrométrie de masse.

3. Procédé selon la revendication 1, comprenant en outre la détermination du taux d'un ou plusieurs marqueurs supplémentaires sélectionnés dans le groupe consistant en LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDHI, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT et TPI1.

4. Procédé selon la revendication 1, comprenant en outre la détermination du taux d'un ou plusieurs marqueurs sélectionnés dans le groupe consistant en SLC3A2, GOT1, GOT2, ACADVL, DBI, ACOXI, AHNAK, AIMP1, AKRIBI, ANPEP, ANXA2, ANXA5, ANXA6, AKR7A2, ARPC3, ASAHI, B4GALT1, BCAM, BLOC1S5, CALU, CAPG, CAPZB, CAPZA2, CBX1, CDC37, HSPE1, CHMP1A, CHMP1B, CHMP2A, CHMP4A, CHMP4B, CHMP5, CLIO, CNDP2, CNPY2, COP A, COPB2, COROIA, COROIB, COROIC, CPVL, C19orfl0, CXorf26, CXCL10, CACYBP, DSP, HSD17B4, DLG1, DNASE2, DDT, DYNLL1, EPB41L3, EEF1A1, EEF1B2, EEF1D, EEF2, ENG, EHD1, ELAVL1, EMC2, EMR2, ENO1, EN03, HSP90B1, EROIL, ESYT2, EVL, ST13, F5, FKBP4, FLNA, FH, H6PD, GPI, GDI2, ARHGDIA, GGH, GBA, PRKCSH, GNS, HSPA9, HSPA5, GSTP1, GLT25D1, HIST1H1D, HIST1H1B, HIST3H2A, H2AFY, HIST2H2BE, HIST2H2BF, HIST3H3, HIST1H4A, HSD17B10, HEXA, HMGB3, HNRNPC, HNRNPD, HNRNPK, HNRNPR, HNRNPU, HSP90AA1, HSP90AB1, HSPAIA, HSPA8, ICAM1, EIF5A2, ILIB, IMPDH2, ISYNA1,

ISOC1, ITGA5, ITGAM, ITPR1, KRT9, KRT7, KRT8, CAMK2D, PKM, LASP1, LDHB, LIMA1, LMNA, LMNB1, LMNB2, LRCH1, GAA, LYN, MDH1, MDH2, MESDC2, MAT2A, MYL12A, MMP9, RABIF, MYH10, MYH9, MY01E, MY06, PPP1R12A, NAGA, POR, NME1, NME2, NDRG1, NDUFA2, NAP1L1, NPM1, NUTF2, NCL, NUDC, OAS2, PAFAH1B2, PA2G4, PARK7, PARP1, PRCP, PDCD6IP, P4HB, PDIA5, PEBP1, PECAM1, PFDN1, PFDN2, PGAM1, PGK1, PIP4K2A, PIP5K1A, PLEKH02, PLEC, PLOD3, LCP1, PNP, ALPP, PPIA, PPIB, PRDX1, PRDX2, PRDX3, PRDX6, PRPF19, PSMC6, PSMC3, PSMC2, PRSS8, NPEPPS, PSMA7, PSM85, PSMDIO, PSMD11, PSMD14, PSMD1, PSMD2, PSMD4, PTBP3, PAICS, ATIC, PXMP2, RAB6A, RANGAP1, RA114, RAN, RANBP1, RBBP4, RAD23B, RPL14, RPL23A, RPL24, RPL26, RPL29, RPL3, RPL31, RPL7A, RPL8, RPLP2, MRPL39, HNRNPA1, HNRNPA2B1, RPS23, RPS8, RSAD2, RPSA, RUVBL1, RUVBL2, S100P, S100A6, S100A11, SEC61B, SEPTI1, SEPT2, SEPT6, SEPT7, SEPT9, PHGDH, SF3B3, SFPQ, SIAE, SNRPD3, NAPA, SOD2, SORT1, SGSH, SPRED1, SPTBN1, SPG11, SPTAN1, SRSF3, SRSF8, STIP1, STMN1, STRAP, STX11, SUM03, EPRS, IARS2, TAGLN2, TALDO1, TUBA4A, TUBB4B, TUBB, TBL2, CCT4, CCT8, TDRD6, VCP, TKT, TLN1, TMOD3, TPI1, TRA2B, TXNRD1, USP14, UGGT1, ATP6V1A, ATP6V1B2, ATP6V1D, ATP6V1E1, ATP6V1F, ATP6V1G1, VPS4A, XRCC6 et YBX1.

5. Utilisation d'un kit comprenant des réactifs pour déterminer le taux d'HIST4H4, de LDHB et de MDH1 dans un/des échantillon(s) de sang obtenu(s) d'un sujet et des instructions pour une utilisation dans un procédé de diagnostic selon la revendication 1.

6. Utilisation selon la revendication 5, dans lequel le kit comprend en outre des réactifs pour déterminer le taux de l'un quelconque ou plusieurs des marqueurs énumérés dans le Tableau 1 dans un/des échantillon(s) du sujet.

7. Utilisation du kit selon la revendication 5, dans lequel le kit comprend en outre des réactifs pour déterminer le taux d'un ou plusieurs marqueurs supplémentaires sélectionnés dans le groupe consistant en LYN, MAT2A, B4GALT1, HIST3H3, ICAM1, LDHB, MDH1, CALU, COROIA, ENO1, EPB41L3, FLNA, GSTP1, H6PD, HISTH2BE, HIST4H4, ITGAM, MMP9, PRKCSH, RPSA, TKT et TPI1.

8. Utilisation du kit selon la revendication 5, dans lequel le kit comprend en outre des réactifs pour obtenir un/des échantillon(s) d'un sujet ou un/des échantillon(s) de fluide témoin(s).

9. Procédé selon la revendication 1, comprenant en outre la détermination du taux d'un ou plusieurs marqueurs supplémentaires sélectionnés dans le groupe consistant en LYN, MAT2A, ICAM1, MMP9, CALU et B4GALT1.

10. Procédé selon la revendication 1, comprenant en outre la détermination du taux d'un ou plusieurs marqueurs supplémentaires sélectionnés dans le groupe consistant en HIST3H3, ICAM1, FLNA, H6PD et MMP9.

11. Procédé selon la revendication 1, dans lequel le/les échantillon(s) de sang est/sont un échantillon sérique.

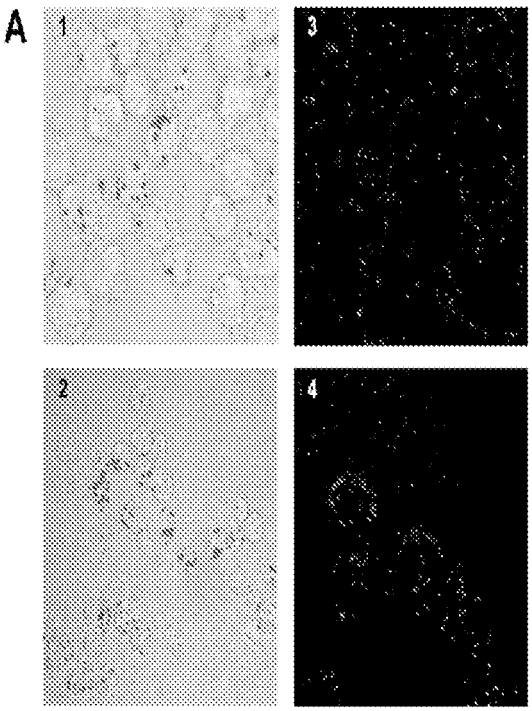
12. Procédé selon la revendication 2, dans lequel la spectrométrie de masse comprend l'ionisation des HIST4H4, LDHB et MDH1 dans le/les échantillon(s) du sujet.

13. Procédé selon la revendication 1, dans lequel le taux dans le/les échantillon(s) du sujet est déterminé par un essai immunologique.

14. Utilisation du kit selon la revendication 5, dans lequel le kit comprend en outre un/des échantillon(s) témoin(s).

15. Procédé selon la revendication 1, dans lequel le sujet est un sujet vétérinaire.

16. Procédé selon la revendication 1, dans lequel le sujet est un être humain.


A
1
3
2
4

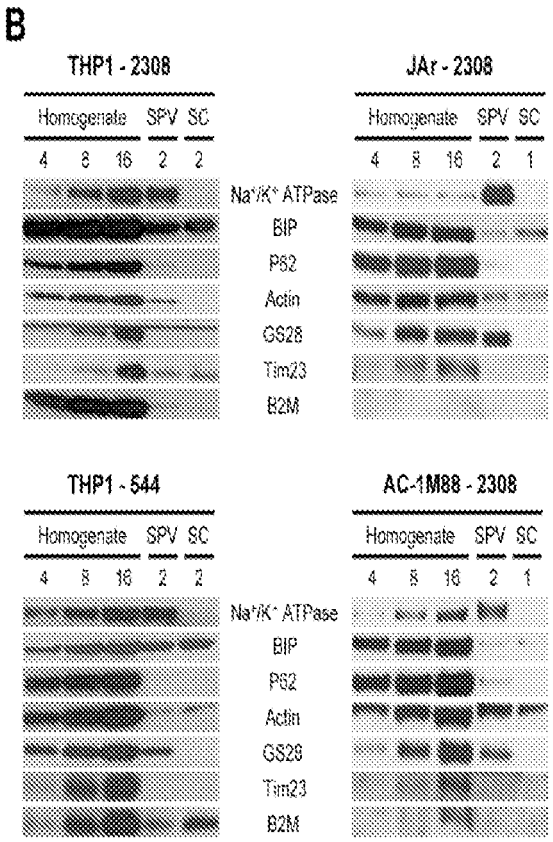

B
THP1 - 2308
Homogenate
SPV
SC
4
8
16
2
2
JAr - 2308
Homogenate
SPV
SC
4
8
16
2
1
Na+/K+ ATPase
BIP
PS2
Actin
GS28
Tim23
B2M
THP1 - 544
Homogenate
SPV
SC
4
8
16
2
2
AC-1M88 - 2308
Homogenate
SPV
SC
4
8
16
2
1
Na+/K+ ATPase
BIP
PS2
Actin
GS28
Tim23
B2M

Figure 1

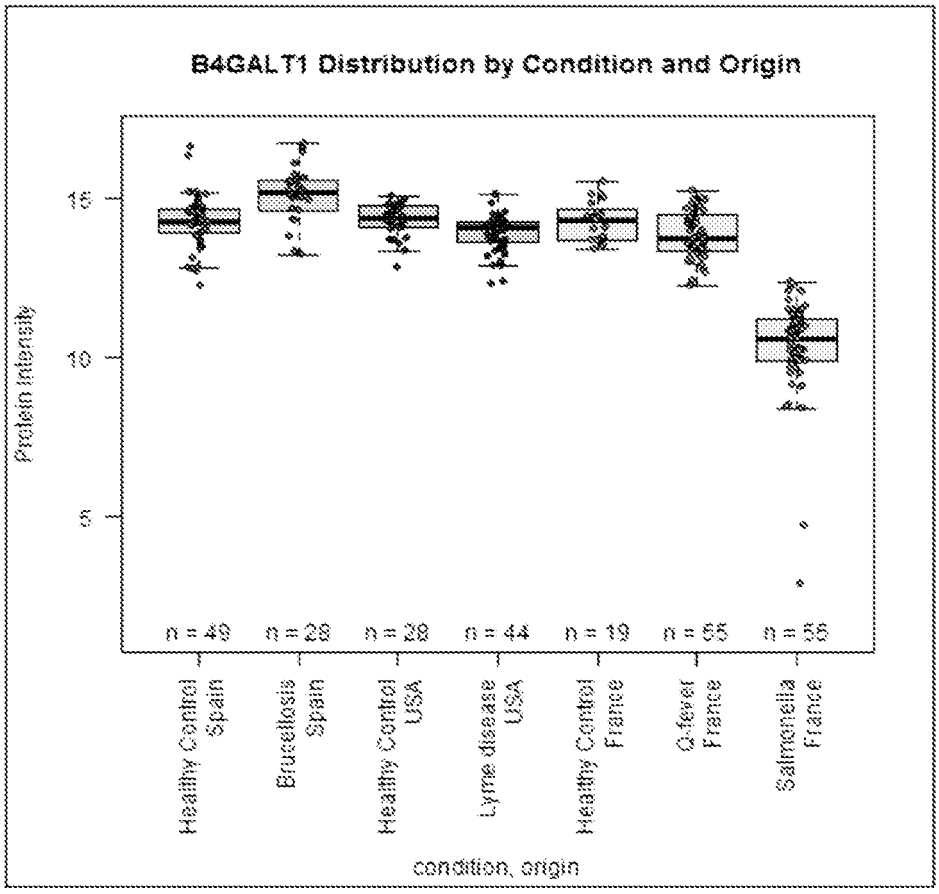

B4GALT1 Distribution by Condition and Origin
Protein Intensity
15
10
5
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 2

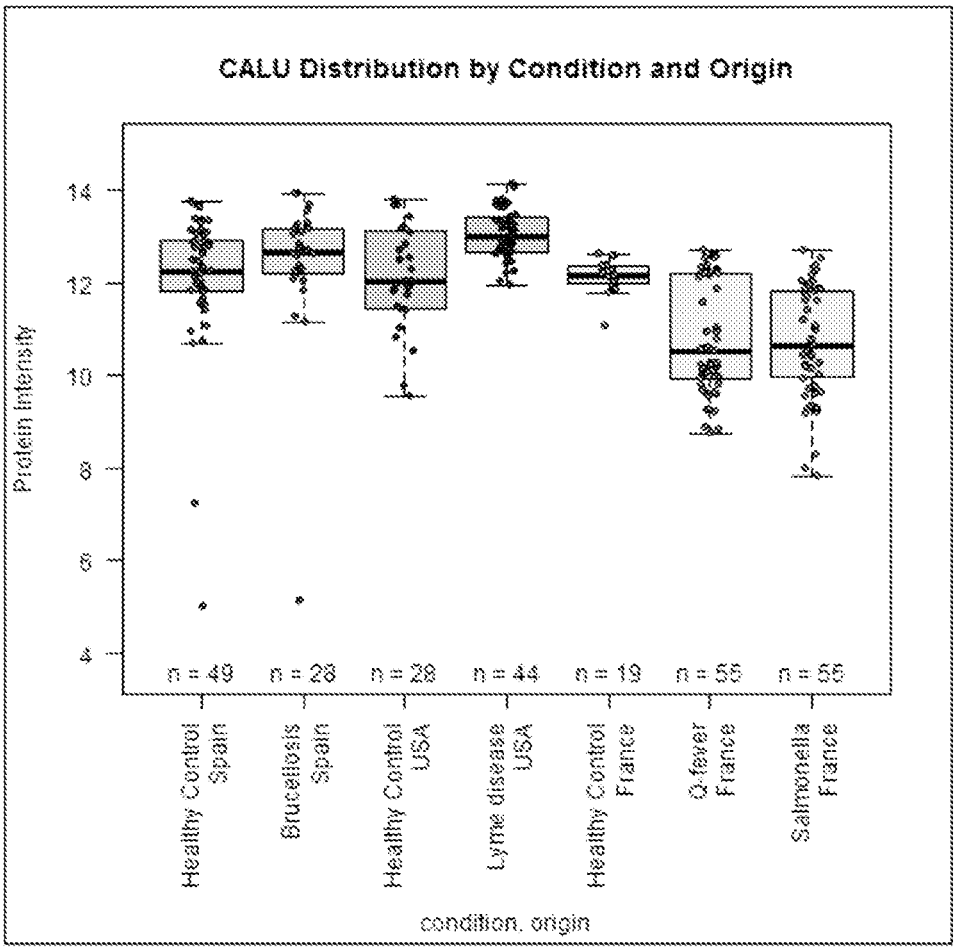

CALU Distribution by Condition and Origin
Protein Intensity
14
12
10
8
6
4
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 3

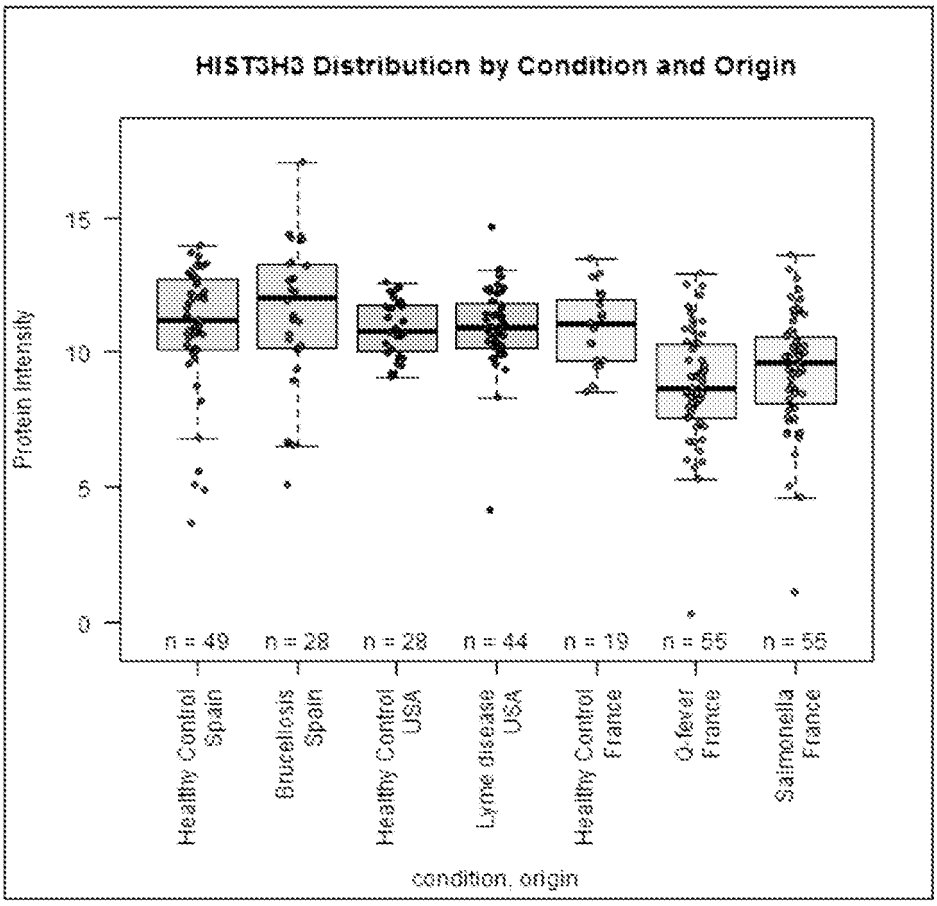

HIST3H3 Distribution by Condition and Origin
Protein Intensity
0
5
10
15
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q fever France
Salmonella France
condition, origin

Figure 4

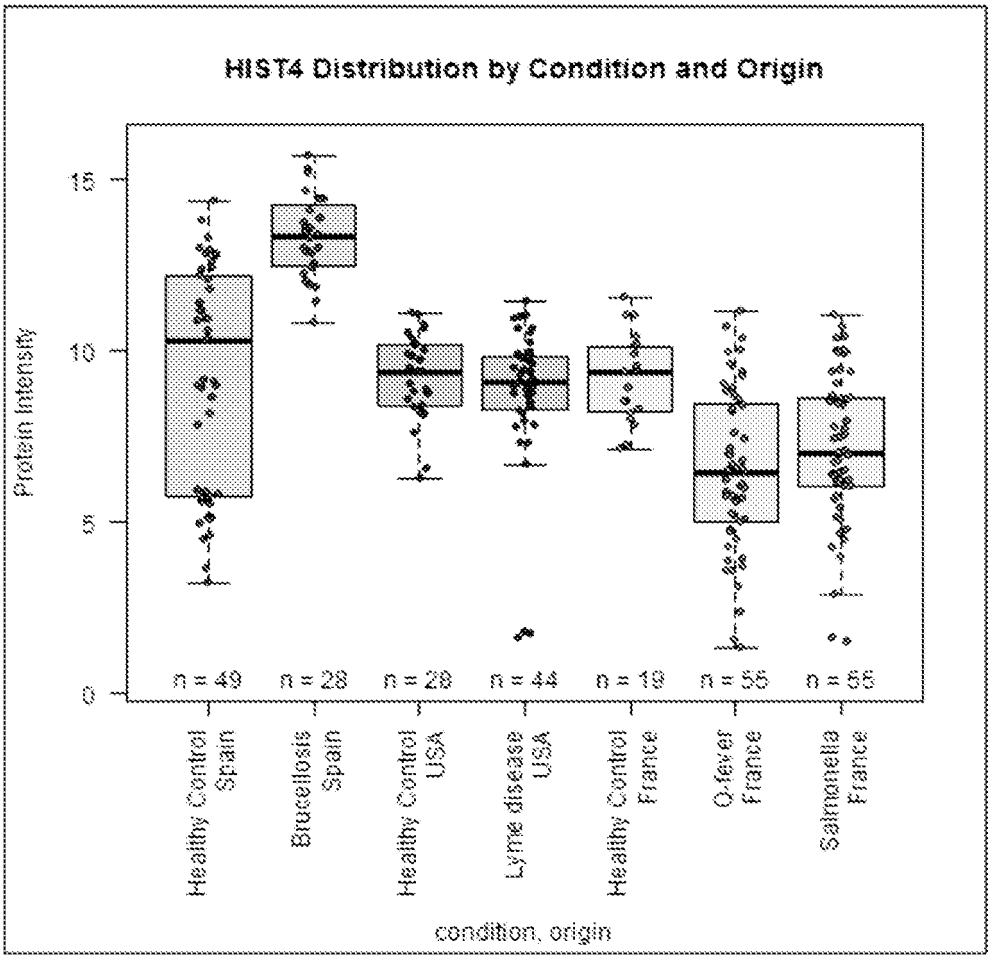

HIST4 Distribution by Condition and Origin
Protein Intensity
0
5
10
15
n = 49
n = 28
n = 29
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 5

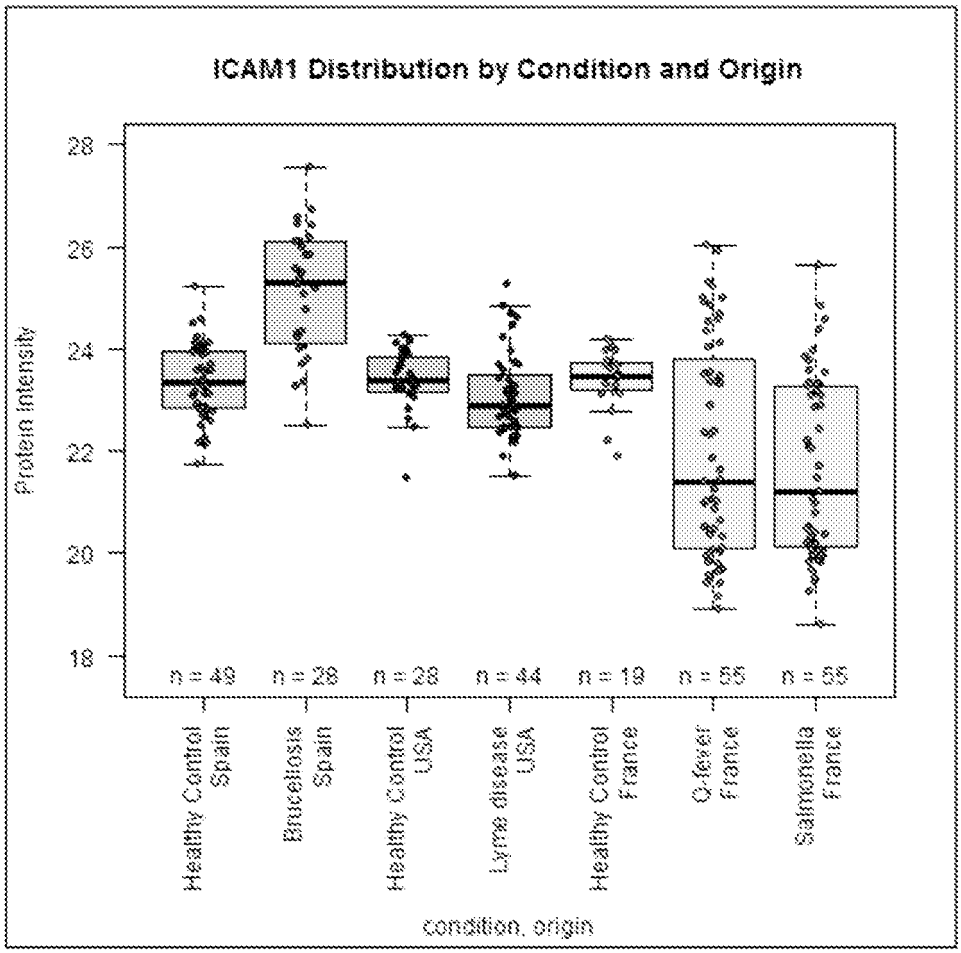

ICAM1 Distribution by Condition and Origin
Protein intensity
28
26
24
22
20
18
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 6

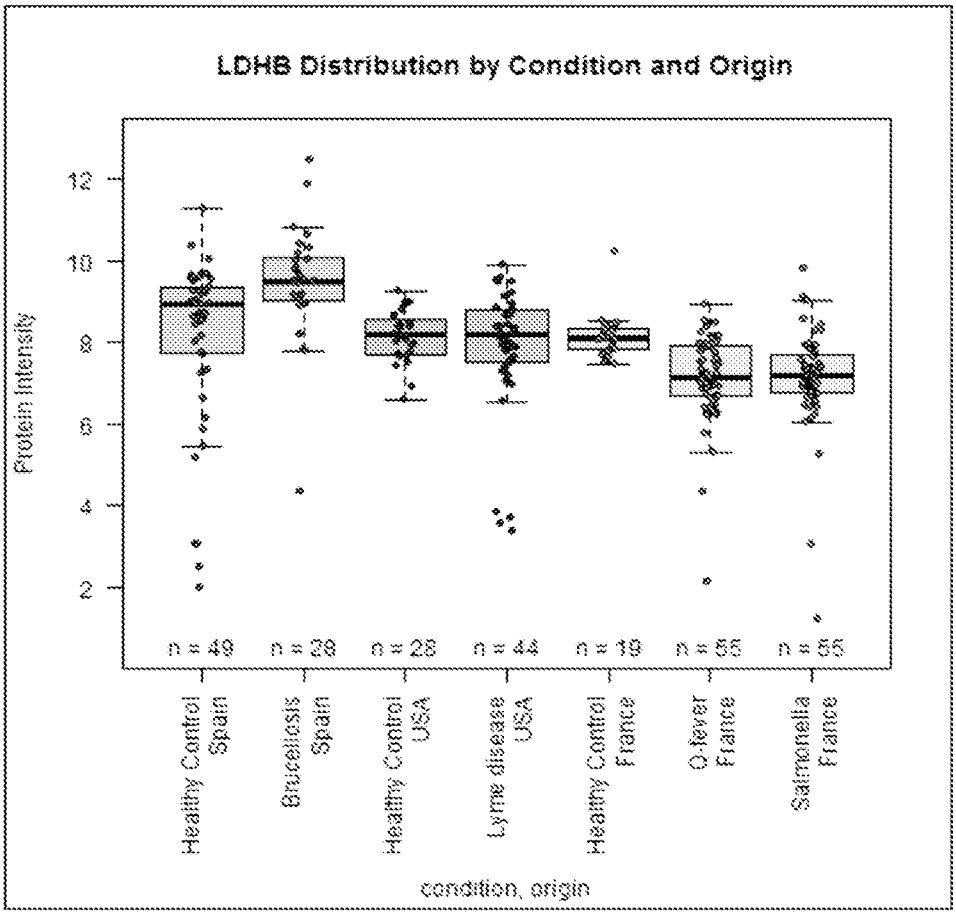

LDHB Distribution by Condition and Origin
Protein Intensity
12
10
8
6
4
2
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 7

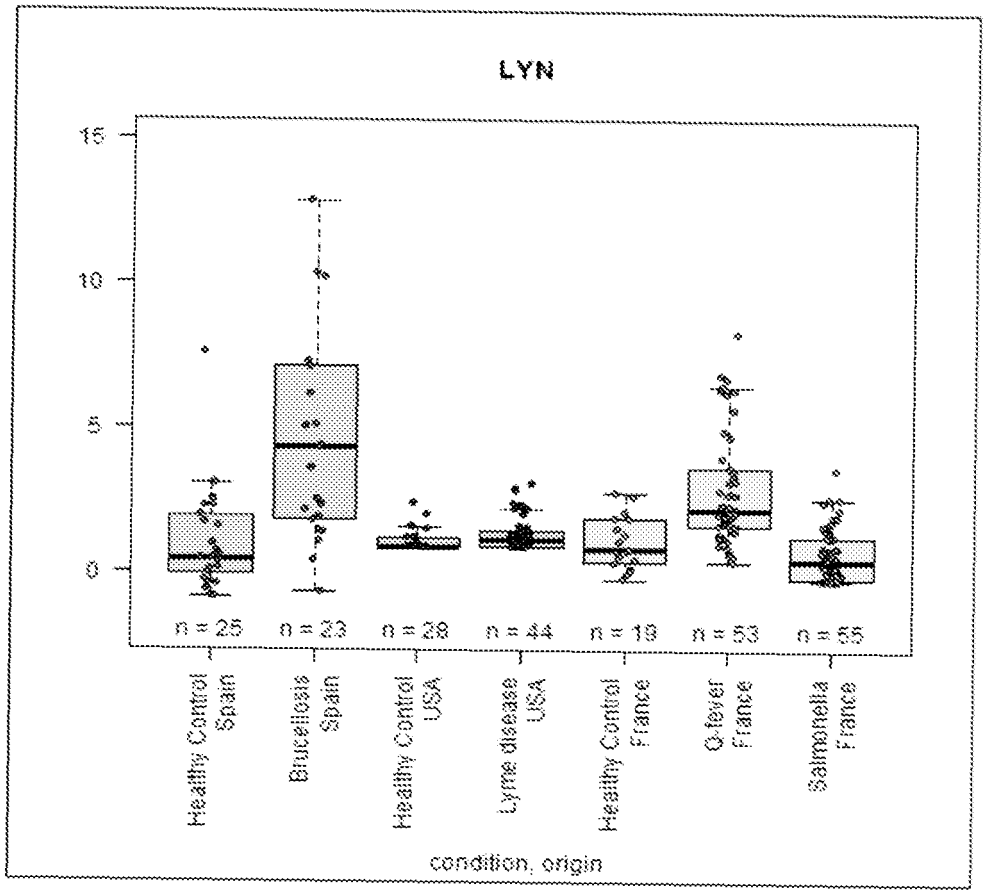

LYN
15
10
5
0
n = 25
n = 33
n = 28
n = 44
n = 19
n = 53
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 8

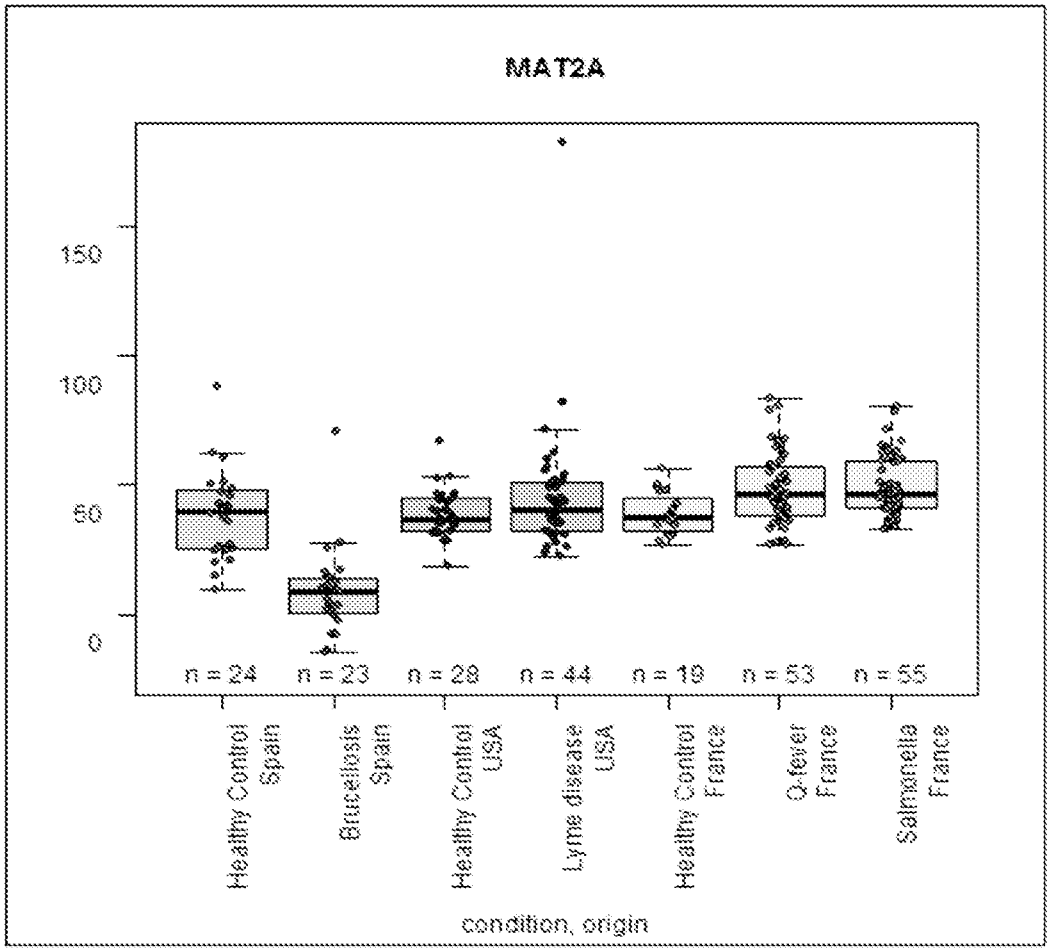

MAT2A
150
100
50
0
n = 24
n = 23
n = 28
n = 44
n = 19
n = 53
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 9

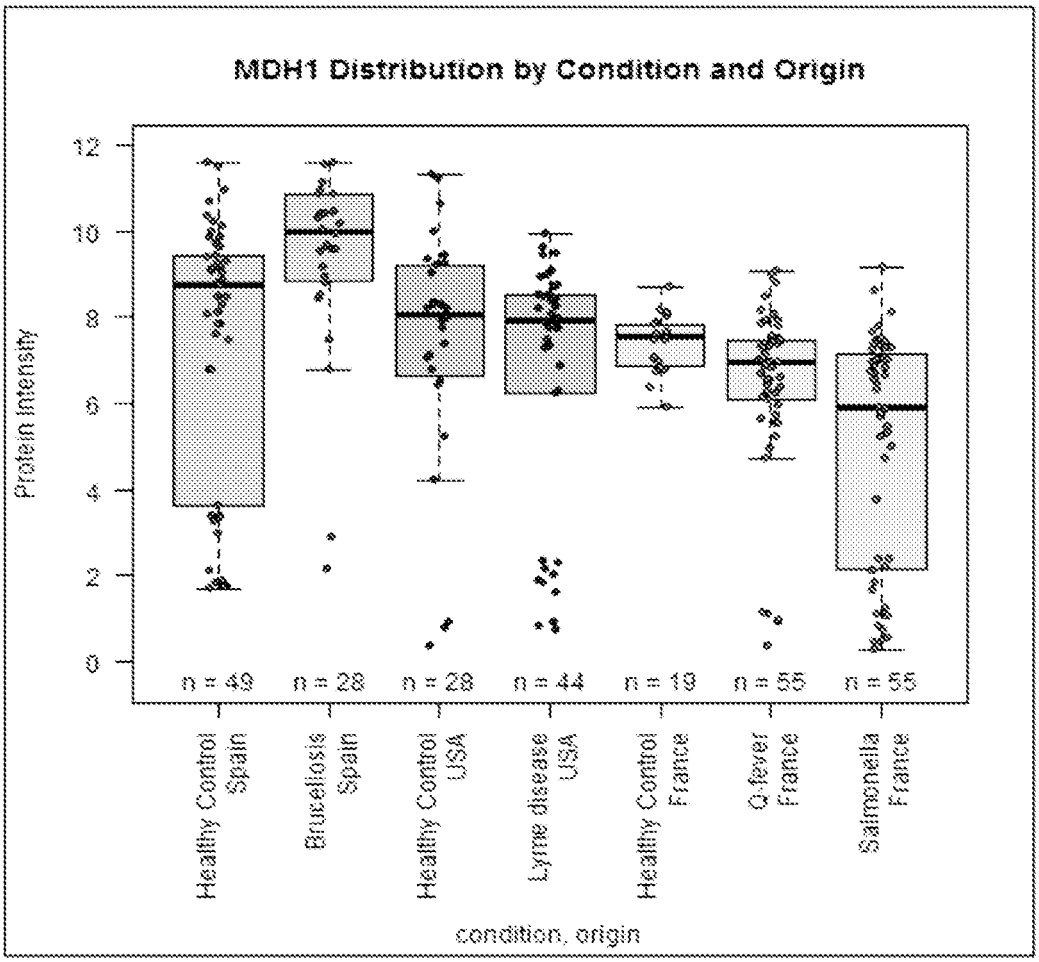

MDH1 Distribution by Condition and Origin
Protein Intensity
0
2
4
6
8
10
12
n = 49
n = 28
n = 29
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 10

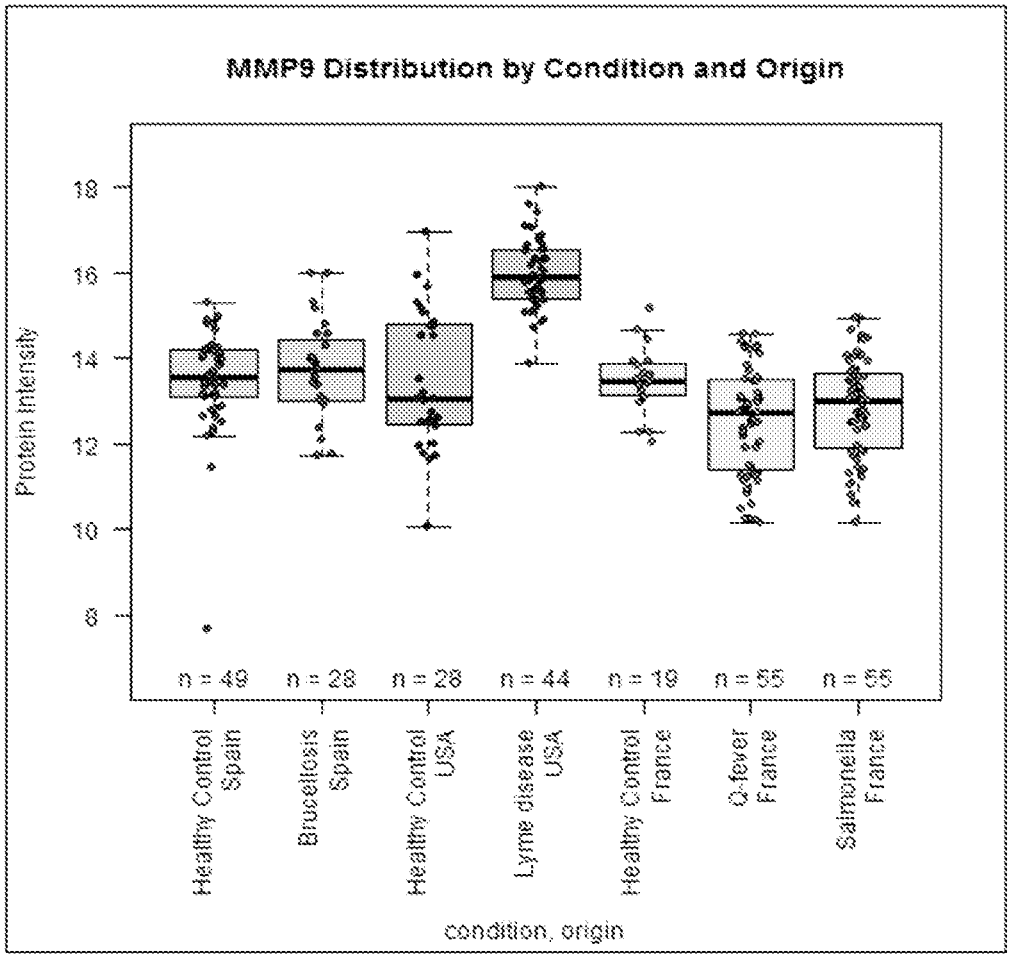

MMP9 Distribution by Condition and Origin
Protein Intensity
18
16
14
12
10
8
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 11

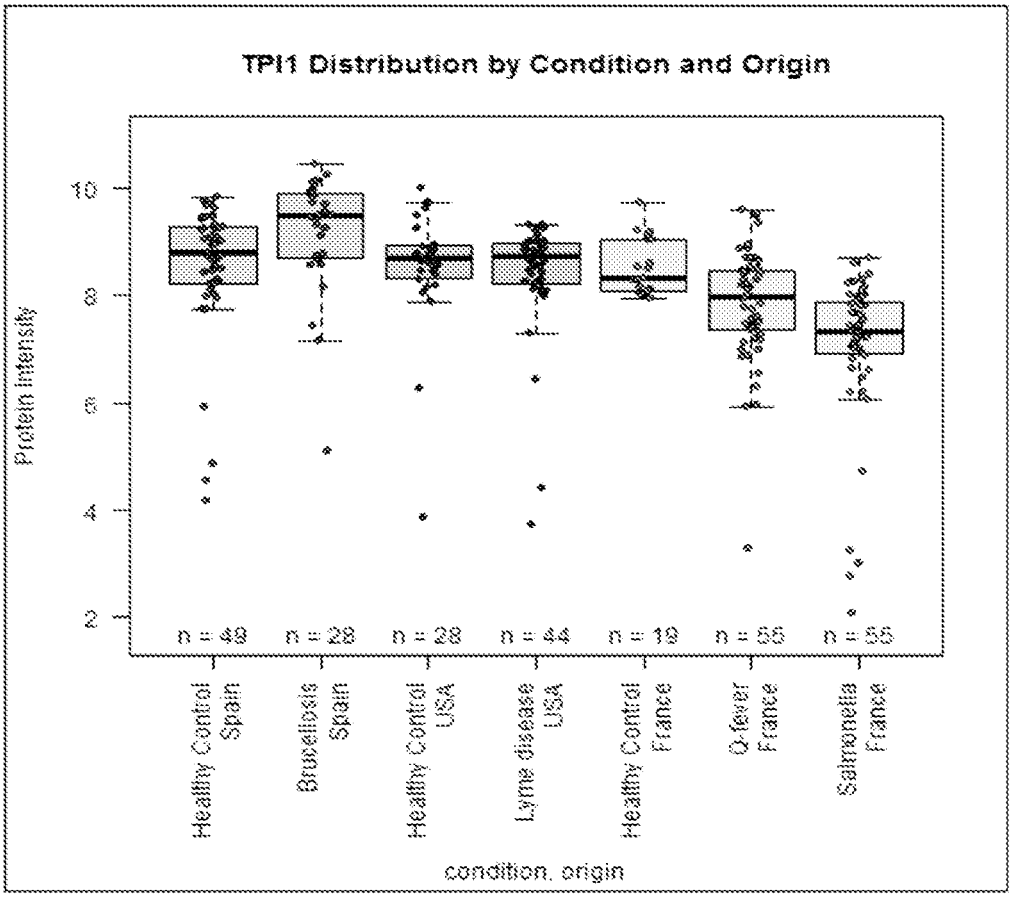

TPI1 Distribution by Condition and Origin
Protein intensity
2
4
6
8
10
n = 49
n = 28
n = 28
n = 44
n = 19
n = 55
n = 55
Healthy Control Spain
Brucellosis Spain
Healthy Control USA
Lyme disease USA
Healthy Control France
Q-fever France
Salmonella France
condition, origin

Figure 12

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	布鲁氏菌病，q-热和莱姆病生物标志物及其用途		
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摘要(译)

本发明提供了用于诊断布鲁氏菌病，Q-发热和/或莱姆病的生物标记，方法和试剂盒，用于监测治疗布鲁氏菌病，Q-发热或莱姆病的有效性的方法和试剂盒，以及用于鉴定可以治疗布鲁氏菌病，Q-发热和/或莱姆病的化合物减少或抑制受试者中与疾病相关的并发症的发展，以及治疗布鲁氏菌病，Q-发热和/或莱姆病的方法。