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(54) Title: BIOMARKERS FOR VISCERAL LEISHMANIASIS

(57) Abstract: The present invention provides protein-based biomarkers and biomarker combinations that are useful in qualifying Leishmaniasis status in a patient. In particular, the biomarkers of this invention are useful to classify a subject sample as infected with Leishmaniasis or not infected with Leishmaniasis. The biomarkers can be detected by SELDI mass spectrometry.

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BIOMARKERS FOR VISCERAL LEISHMANIASIS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/985,611, filed November 5, 2007, which is incorporated by reference in its entirety.

FIELD

[0002] The invention relates generally to clinical diagnostics.

BACKGROUND

[0003] Leishmaniasis is a spectrum of diseases with important clinical and epidemiological diversity that exist in humans in three major forms, cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL). The diseases are caused by obligate intracellular protozoan parasites belonging to various species of the genus *Leishmania* (Saha *et al.*, 2006). Leishmaniasis is endemic in 88 countries, 72 of which are developing countries, including 13 of the least developed. Ninety per cent of the reported CL cases occur in seven countries- Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria, while 90 per cent of VL occurs in five countries- Bangladesh, India, Nepal, Sudan and Brazil (Desjeux P., 2004). Leishmaniasis is of concern in many other countries too because of military activities (e.g. US missions in Iraq) and adventure tourism. An estimated 1.5-2 million children and adults develop symptomatic disease every year (cutaneous 1-1.5 million; visceral 0.5 million). The incidence of infection is substantial when subclinical infections are included (Desjeux P., 2004). VL is also a substantial problem in HIV-positive individuals [it is the fourth most common opportunistic parasitic disease in people with HIV in Spain (Paredes *et al.*, 2003), in

Ethiopia, Sudan and Southern Europe as many as 70 per cent of adults with VL are also HIV positive (Desjeux, 2001)].

[0004] Cutaneous Leishmaniasis (CL) is caused by a wide range of species, including *Leishmania major*, *L. aethiopica* and *L. tropica* in the Old World, and *L. mexicana*, *L. braziliensis*, *L. amazonensis*, *L. pifanoi*, *L. garnhami*, *L. venezuelensis*, *L. guyanensis*, *L. peruviana*, and *L. panamensis* in the New World. Mucocutaneous Leishmaniasis (MCL) is most commonly caused by the New World species, *L. braziliensis* (Renee *et al.*, 2005, Wilson *et al.*, 2005), though *L. aethiopica* has also been reported to cause this syndrome. About 70 of around 1000 known sandfly species can transmit leishmaniasis (Murray *et al.*, 2005) including *L. lutomyia* in the Americas and *L. phlebotomous* elsewhere (Mandell *et al.*, 2005). Sandflies inoculate the skin with flagellated promastigotes, which invade or are phagocytosed by tissue macrophages or are immediately recruited by immune cells, including neutrophils. Within phagolysosomes of resident macrophages, surviving promastigotes transform and replicate as amastigotes, which can infect additional macrophages either locally or in distant tissues after dissemination (Murray *et al.*, 2005). Cutaneous leishmaniasis (CL) lesions can vary in severity (*e.g.*, lesion size), clinical appearance (*e.g.*, open ulcer versus flat plaques versus wart-like lesions), and duration [*e.g.*, time of evolution, time to spontaneous cure (Reithinger and Dujardin, 2007)].

[0005] Visceral leishmaniasis (VL), commonly known as kala-azar, is caused by *L. donovani* and *L. infantum* in the Old World and *L. chagasi* in the New World. Desjeux (1996) report that visceral leishmaniasis has an estimated yearly incidence of 500 000 cases. VL is characterized by fever, cachexia, hepatosplenomegaly, and hematologic cytopaenias, and is usually fatal without specific chemotherapy (Saha *et al.*, 2006). The name "kala azar" is thought to have originated from India, meaning "black fever", which refers to the hyperpigmentation of skin during the course of disease. Alternatively, the term might be derived from the word "kal" meaning "death", which signifies the fatality of the disease (Brahmachari UN, 1928). Rare patients, cured of VL in Sudan and India, can develop post-kala azar dermal leishmaniasis (PKDL), which appears as a dermatotropic form of *L. donovani* infection (Saha *et al.*, 2006).

[0006] The broad clinical spectrum of the leishmaniasis makes the diagnosis of present and past cases difficult. However, differential diagnosis is important because other diseases that produce lesions with a clinical appearance similar to those of leishmaniases (*e.g.*, leprosy, skin cancers, and tuberculosis for CL) are often present in areas of endemicity

(Reithinger and Dujardin, 2007). For example, Kala Azar can be confused with other similar conditions such as tropical splenomegaly, malaria, schistosomiasis or cirrhosis with portal hypertension, military tuberculosis, brucellosis, African trypanosomiasis, typhoid fever, bacterial endocarditis, histoplasmosis, malnutrition, leukaemia and lymphoma (Singh and Sivakumar, 2005).

[0007] Because of its high specificity, microscopic visualization or culture remain the gold standard diagnostic tests in leishmaniasis (Herwaldt, 1999). Importantly, the sensitivity of microscopy and culture tends to be low and can be highly variable (Herwaldt, 1999), depending on the number and dispersion of parasites in biopsy samples, the sampling procedure, and the technical skills of the personnel. Polymerase chain reaction (PCR) applied to lesion material can be highly sensitive and specific (Singh, 2006) but usually requires a biopsy for optimal sensitivity and the expensive machinery and reagents are not feasible for most endemic areas. There is currently no serologic assay that can reliably diagnose subjects with cutaneous leishmaniasis.

[0008] In cutaneous leishmaniasis, serum antileishmanial antibodies can be detected in many subjects using sensitive reference assays (Romero *et al.*, 2005). However, such tests are not widely available and, in practice, the diagnosis is made macroscopically by identification of amastigotes in biopsies, scrapings, or impression smears of lesions (Vega-Lopez, 2003). The highest yield is usually obtained from material from the ulcer base (Weina *et al.*, 2004; Ramirez *et al.*, 2000). Diagnostic sensitivity can be increased to more than 85% when microscopy and culture are combined (Blum *et al.*, 2004; Ramirez *et al.*, 2000). In addition culture (or DNA analysis) allows species identification (Vega-Lopez, 2003; Ramirez *et al.*, 2000). Detection of parasite DNA in lesion material by PCR is generally considered to be the most sensitive test for the diagnosis of both cutaneous and mucosal leishmaniasis (Vega-Lopez, 2003; Weina *et al.*, 2004; de Oliveira *et al.*, 2003; Faber *et al.*, 2003; Oliveira *et al.*, 2005), but is seldom the only test producing positive results (Weina *et al.*, 2004; Oliveira *et al.*, 2005). Culture and PCR testing are technically difficult laboratory techniques that are not currently practical in developing countries (Murray *et al.*, 2005).

[0009] For visceral leishmaniasis direct visualization of amastigotes in clinical specimens is the diagnostic gold standard in regions where deep tissue aspiration is routinely performed and the microscopy and technical skills are available. In immunocompetent patients, the best samples for aspiration are the ones from the spleen with a sensitivity > 94 per cent (Singh, 2006). But this procedure is risky, and even in the most experienced hands,

the risk of fatal bleeding cannot be zeroed (Bryceson, 1987). Another option is the demonstration of the parasites in liver aspirates and biopsies, but the sensitivity can be as low as 40 per cent. Additionally liver aspiration has to be performed as well very carefully to minimize bleeding risks for the patient (Singh, 2006). A much safer but painful method is marrow obtained from iliac or sternal crest puncture (Singh, 2006). In visceral leishmaniasis the hallmark is hyperimmunoglobulinaemia. Tests to measure the antibody level were developed but lack sensitivity and/or specificity (Singh and Sivakumar, 2003; Singh *et al.*, 2005; Boelaert *et al.*, 2004).

[0010] Diagnostic sensitivity for splenic, bone marrow, and lymph node aspirate smears is >95%, 55-97%, and 60%, respectively (Guerin *et al.*, 2002; Sundar *et al.*, 2002; Davidson, 1998; Sundar and Benjamin, 2003; Zijlstra and El-Hassan, 2001; Pagliano *et al.*, 2003). Elsewhere, including epidemic settings (Guerin *et al.*, 2002; Marlet *et al.*, 2003; Collin *et al.*, 2004; Davidson, 1998), the diagnostic standard for VL is serum antileishmanial immunoglobulin G in high titre, measured primarily with direct agglutination tests or other serological assays (Desjeux, 2004; Herwaldt, 1999; Guerin *et al.*, 2002; Gama *et al.*, 2004; Davidson, 1998; Sundar and Benjamin, 2003; Abdallah *et al.*, 2004). Freeze-dried antigen, which don't need refrigeration (Abdallah *et al.*, 2004) and rapid detection of anti-K39 antibody with fingerstick blood using an immunochromatographic strip test (Sundar *et al.*, 2002) have advanced field serodiagnosis. In symptomatic patients, the anti-K39 strip-test sensitivity is high (90-100%) (Sundar *et al.*, 2002; Vecken *et al.*, 2003; Boelaert *et al.*, 2004), but specificity varies by region (Sundar *et al.*, 2002; Davies *et al.*, 2003; Weina *et al.*, 2004; Boelaert *et al.*, 2004).

[0011] Accordingly, despite the worldwide prevalence and severity of Leishmaniasis, current diagnostic methods are lacking in sensitivity and/or specificity, and are often impractical in light of the available resources in affected areas. Thus, there is a present need for reliable and practical methods for diagnosing leishmaniasis. This invention is directed to this and other ends.

SUMMARY

[0012] The present invention provides polypeptide-based biomarkers that are differentially present in subjects with Leishmaniasis, and particularly that are differentially present in chronically infected subjects versus uninfected healthy individuals. In addition, the present invention provides methods of using the polypeptide-based biomarkers to qualify the

Leishmaniasis status of a subject, e.g., by measuring one or more biomarkers in a biological sample taken from a subject, such as a sample of serum, blood or other donated tissue.

[0013] In some aspects, the invention provides biomarkers that represent novel proteins or protein fragments expressed in infected individuals by a parasite of the genus *Leishmania*. In other aspects, biomarkers provided herein represent endogenous host proteins expressed, directly or indirectly, in response to infection by a parasite of the genus *Leishmania*.

[0014] In one aspect, the present invention provides a method for qualifying Leishmaniasis status in a subject, the method comprising: (a) measuring at least one biomarker in a biological sample from the subject, wherein the at least one biomarker is selected from the group consisting of the biomarkers set forth in Table 1, and the figures; and (b) correlating the measurement with Leishmaniasis status. In some aspects, the biological sample is a serum sample.

[0015] In some aspects, a method is provided for qualifying Visceral Leishmaniasis status in a subject, the method comprising: (a) measuring at least one biomarker of Table 1 in a biological sample from the subject; and (b) correlating the measurement with Visceral Leishmaniasis status. In some aspects, the biological sample is a serum sample. In some aspects, the at least one biomarker is selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

[0016] In some aspects, the at least one biomarker is selected from biomarkers having the following molecular weights: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa. In some aspects, the method comprises measuring biomarkers of each of the following molecular weights: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa. In some aspects, the method comprises measuring each of the biomarkers having the following molecular weights: about 5 kDa, about 13.9 kDa, about 28.2 kDa, about 47.7 kDa, about 3.3 kDa, about 12.4 kDa and about 51.3 kDa. In some aspects, the method further comprises measuring one or more of any of the biomarkers listed in Table 1 and/or in the figures.

[0017] In some aspects, the at least one biomarker is a protein or fragment thereof as provided in Table 1 and Table 5. In certain aspects, the at least one biomarker is represented by SEQ ID NOS: ____ in Table 5.

[0018] In some aspects, the at least one biomarker is measured by capturing the biomarker on an adsorbent of a SELDI probe and detecting the captured biomarkers by laser desorption-ionization mass spectrometry. In various aspects, the adsorbent is a cation exchange adsorbent or a metal chelation adsorbent. In some aspects, the at least one biomarker is measured by immunoassay or another method known in the art.

[0019] In some aspects, the correlating is performed by a software classification algorithm. In some aspects, the Leishmaniasis status is selected from chronic symptomatic, chronic asymptomatic, acute, and uninfected. In some aspects, the Leishmaniasis status is selected from chronically infected versus uninfected. In some aspects, the Leishmaniasis status is selected from chronically infected status versus acutely infected disease status, chronically infected asymptomatic status versus chronically affected with symptoms, or acutely infected status versus healthy uninfected status. In some aspects, the Leishmaniasis status is selected from Leishmaniasis versus healthy. In some preferred aspects, the at least one biomarker is selected from the biomarkers of Table 1. In some aspects, the Leishmaniasis status is selected from Leishmaniasis versus non-Leishmaniasis. In some preferred aspects, the at least one biomarker is selected from biomarkers of molecular weight : about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

[0020] In some aspects, one or more of the biomarkers measured according to methods described herein are differentially expressed in patients having a particular Leishmaniasis status relative to patients having one or more other Leishmaniasis statuses. In some aspects, measuring a differentially expressed biomarker in a subject according to a method herein qualifies the subject as having a Leishmaniasis status with a probability (P) of about 0.05 or lower, or about 0.01 or lower, or about 0.005 or lower. In some aspects, the measuring step comprises determining the level of expression of the at least one biomarker. In some aspects, the mean level of expression of the at least one biomarker in patients having a first Leishmaniasis status is about twice or more than the mean level of expression in patients having a second Leishmaniasis status.

[0021] In some aspects, the correlating step comprises comparing the level of expression of the at least one biomarker with a pre-determined level of expression. In various aspects, the pre-determined level of expression distinguishes between a first Leishmaniasis status and a second Leishmaniasis status with a probability (P) of about 0.05 or lower, or about 0.01 or lower, or about 0.005 or lower, and/or with a Receiver Operator Characteristic (ROC) of 0.7 or greater, 0.8 or greater, or 0.9 or greater. In some aspects, the pre-determined level of expression is less than the detection threshold, such that the measuring step comprises determining the presence or absence of the at least one biomarker.

[0022] In some aspects, the at least one biomarker is two or more biomarkers and the correlating step comprises comparing the level of expression of each biomarker with a pre-determined level of expression. In some aspects, the pre-determined level of expression of each biomarker distinguishes between a first Leishmaniasis status and a second Leishmaniasis status with an Receiver Operator Characteristic (ROC) of about 0.3 or less or about 0.7 or greater.

[0023] In various aspects, methods described herein distinguish between a first Leishmaniasis status and a second Leishmaniasis status with a probability (P) of about 0.05 or lower, or about 0.01 or lower, or about 0.005 or lower, and/or with a Receiver Operator Characteristic (ROC) of 0.7 or greater, 0.8 or greater, or 0.9 or greater wherein the at least one biomarker is a single biomarker.

[0024] In some aspects, the method further comprises managing subject treatment based on Leishmaniasis status. For example, if the measurement correlates with Leishmaniasis, then managing subject treatment can comprise administering sodium stibogluconate or another drug effective in treating Leishmaniasis.

[0025] In some aspects, the method further comprises measuring the at least one biomarker described herein after subject management.

[0026] In another aspect, the present invention provides a method comprising measuring at least one biomarker in a sample from a subject, wherein the at least one biomarker is selected from the group consisting of the biomarkers set forth in Table 1, and in the figures. In some aspects, the sample is a serum sample.

[0027] In some aspects, the present invention provides a kit comprising: (a) a solid support comprising at least one capture reagent attached thereto, wherein the capture reagent binds at least one biomarker from a first group consisting of the biomarkers set forth in Table

1 and in the figures; and (b) instructions for using the solid support to detect the at least one biomarker set forth in Table 1 and in the figures.

[0028] In some aspects, the kit provides instructions for using the solid support to detect a biomarker selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa. In some aspects, the kit provides instructions for using the solid support to detect each of the following biomarkers: biomarkers having molecular weights of about 5 kDa, about 13.9 kDa, about 28.2 kDa, about 47.7 kDa, about 3.3 kDa, about 12.4 kDa and about 51.3 kDa. In some aspects, the kit provides instructions for additionally measuring one or more of the biomarkers listed in Table 1 and in the figures.

[0029] In some aspects, the solid support comprising the capture reagent is a SELDI probe. In some aspects, the capture reagent is a cation exchange adsorbent. In other aspects, the kit additionally comprises (c) an anion exchange chromatography adsorbent. In other aspects, the kit additionally comprises (c) a container containing at least one of the biomarkers set forth in Table 1 or in the figures, preferably including one or more biomarkers of the following molecular masses: about : about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

[0030] In a further aspect, the present invention provides a kit comprising: (a) a solid support comprising at least one capture reagent attached thereto, wherein the capture reagent binds at least one biomarker from a first group consisting of the biomarkers set forth in Table 1 and in the figures; and (b) a container comprising at least one of the biomarkers set forth in Table 1 and in the figures.

[0031] In some aspects, the solid support comprising the capture reagent is a SELDI probe. In some aspects, the capture reagent is a cation exchange adsorbent or metal chelation adsorbent. In other aspects, the kit additionally comprises (c) an anion exchange chromatography adsorbent.

[0032] In yet a further aspect, the present invention provides a software product, the software product comprising: (a) code that accesses data attributed to a sample, the data comprising measurement of at least one biomarker in the sample, the biomarker selected from the group consisting of the biomarkers of Table 1 and in the figures; and (b) code that

executes a classification algorithm that classifies the Leishmaniasis status of the sample as a function of the measurement.

[0033] In some aspects, the classification algorithm classifies Visceral Leishmaniasis status of the sample as a function of the measurement of a biomarker selected from the biomarkers of Table 1. In some aspects, the classification algorithm classifies Visceral Leishmaniasis status of the sample as a function of the measurement of a biomarker selected from the group consisting of: biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa. In some aspects, the classification algorithm classifies Visceral Leishmaniasis status of the sample as a function of the measurement of one of the following sets of biomarkers: biomarkers having molecular weights of about 5 kDa, about 13.9 kDa, about 28.2 kDa, about 47.7 kDa, about 3.3 kDa, about 12.4 kDa and about 51.3 kDa

[0034] In some aspects, the classification algorithm classifies the Leishmaniasis status of the sample as a function of the additional measurement one or more of any of the biomarkers listed in Table 1 and in the figures.

[0035] In other aspects, the present invention provides purified biomolecules selected from the biomarkers set forth in Table 1 and in the figures and, additionally, methods comprising detecting one or more biomarkers set forth in Table 1 and in the figures by mass spectrometry, immunoassay, or other method known in the art. In some preferred aspects of both of the foregoing aspects, the one or more biomarkers are selected from the following group: biomarkers having molecular weights of about : about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa..

[0036] In some aspects, the method further comprises testing and qualifying stocks of blood based on the status of blood which has been tested according to the methods described herein. For example, if the measurements taken from blood samples correlate with Leishmaniasis, then the management of blood stocks can comprise decontamination of the infected blood by treatment of the infected blood with purification agents available to one skilled in the art including, but not limited to, agents such as sodium stibogluconate. Alternatively, the infected blood can be discarded or destroyed and only stocks of blood which have not tested positively for Leishmaniasis are retained.

[0037] In another aspect, the present invention provides a method of measuring at least two, three, four, or more biomarkers in a biological sample, wherein each of the at least two, three, four, or more biomarkers are selected from Table 1. In a preferred aspect, the at least two, three, four, or more biomarkers are selected from the following set of biomarkers: biomarkers having molecular weights of about 5 kDa, about 13.9 kDa, about 28.2 kDa, about 47.7 kDa, about 3.3 kDa, about 12.4 kDa and about 51.3 kDa.

[0038] In one aspect, the present invention provides a method for qualifying Leishmaniasis status in a subject in comparison to the status of a different parasitic disease (*i.e.*, a non-Leishmaniasis parasitic disease), the method comprising: (a) measuring at least one biomarker in a biological sample from the subject, wherein the at least one biomarker specifically indicates the presence of Leishmaniasis and does not indicate the presence of a different parasitic infection; and (b) correlating the measurement with Leishmaniasis status in comparison to the status of a different parasitic infection. In some aspects, the biological sample is a serum sample. In a preferred aspect of this method, the at least one biomarker is selected from the biomarkers set forth in Table 1. In another preferred aspect, the at least one biomarker is selected from the following biomarkers: biomarkers having molecular weights of about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa. In another preferred aspect, the parasitic infection comprises a kinetoplastidae infection. In still another preferred aspect, the parasitic infection includes, but is not limited to, African trypanosomiasis (sleeping sickness), toxoplasma, babesia, malaria and Chagas Disease.

[0039] In another aspect, the present invention provides a method for monitoring the course of progression of Leishmaniasis in a patient comprising: (a) measuring at least one biomarker in a first biological sample from the patient, wherein the at least one biomarker specifically indicates the presence of Leishmaniasis; (b) measuring the at least one biomarker in a second biological sample from the subject, wherein the second biological sample was obtained from the subject before or after the first biological sample; and (c) correlating the measurements with the progression or regression of Leishmaniasis in the subject. In some aspects, the at least one biomarker is selected from the group consisting of the biomarkers set forth in Table 1, and in the figures. In some preferred aspects, the at least one biomarker is selected from the group consisting of: biomarkers having molecular weights of about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

[0040] Other features, objects and advantages of the invention and its preferred aspects will become apparent from the detailed description, examples and claims that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] Figure 1 is a m/z scatter plot showing the differential signal intensity of the 3,375 Da biomarker in subjects with Visceral Leishmaniasis (positive) compared to healthy subjects (negative).

[0042] Figure 2 shows a scanned image of an SDS-PAGE gel along with the band intensity of the 3,375 Da biomarker in 4 pretreatment sera from subjects with Visceral Leishmaniasis and 4 healthy control subjects.

[0043] Figure 3 is a m/z scatter plot showing the differential signal intensity of the 12,463 Da biomarker in subjects with Visceral Leishmaniasis (positive) compared to healthy subjects (negative).

[0044] Figure 4 is a decision tree illustrating the ability 3 biomarkers (with MW: 51,351 Da, 28,238 Da and 3,378 Da) found in F4CL to correctly classify sera from subjects with Visceral Leishmaniasis from healthy control subjects.

[0045] Figure 5 is a decision tree illustrating the ability of the 12.4 kDa biomarker found in F6IH to correctly classify pretreatment sera from subjects with Visceral Leishmaniasis from healthy control subjects.

[0046] Figure 6 provides a scanned image of an SDS-PAGE gel showing biomarkers with MWs of 27,603 Da, 51,327 Da, 28,238 Da, 51,351 Da and 14,580 Da in total control versus total Visceral Leishmaniasis infected sera.

[0047] Figure 7 provides a scanned image of an SDS-PAGE gel showing biomarkers with MWs of 9,309 Da, 9,478 Da, 12,610 Da, 51,111 Da and 12,463 Da in total control versus total Visceral Leishmaniasis infected sera.

DETAILED DESCRIPTION

1. INTRODUCTION

[0048] A 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 expression level of the biomarker (*e.g.*, as indicated by the mean, median, or other measure) 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. Therefore, they are useful as markers for disease (diagnostics), therapeutic effectiveness of a drug (theranostics), drug toxicity, and the like.

2. BIOMARKERS FOR LEISHMANIASIS

2.1. Biomarkers

[0049] This invention provides, *inter alia*, polypeptide-based biomarkers that are differentially present in subjects having Leishmaniasis, in particular, and particularly those biomarkers that are differentially expressed in subjects infected with Leishmaniasis versus non infected individuals (*e.g.*, control, healthy, benign condition or other disease state). The biomarkers are characterized by, *e.g.*, mass-to-charge ratio as determined by mass spectrometry, by the shape of their spectral peak in time-of-flight mass spectrometry, by their binding characteristics to adsorbent surfaces, and/or by other distinguishing characteristics. These characteristics provide various means to determine whether a particular detected biomolecule is useful as a biomarker of Leishmaniasis. These characteristics preferably represent inherent characteristics of the biomolecules rather than process limitations in the manner in which the biomolecules are discriminated. In one aspect, the biomarkers described herein are provided in isolated and/or purified form.

[0050] The biomarkers of Table 1 and the figures were discovered using SELDI technology employing ProteinChip® arrays from Ciphergen Biosystems, Inc. (Fremont, CA) ("Ciphergen"). The biomarkers were discovered in serum samples collected from subjects diagnosed with Visceral Leishmaniasis (Table 1), both prior to treatment (pre-treatment) and following treatment with sodium stibogluconate for 14 days and 20 days. The samples were fractionated by anion exchange chromatography. Fractionated samples were applied to SELDI biochips and spectra of polypeptides in the samples were generated by time-of-flight mass spectrometry. The spectra thus obtained were analyzed by a Software algorithm and subjected to scatter plot analysis. A Mann-Whitney test analysis was employed to compare Leishmaniasis and control groups for each protein cluster in the scatter plot, and proteins were selected that differed significantly ($p < 0.05$) between the two groups. This method is

described in more detail in the Examples Section, below. The identity of certain of the biomarkers of Table 1 of this invention has been determined and is indicated in Table 5 in the Examples section. For biomarkers whose identity has been determined, the presence of the biomarker can be determined by methods known in the art other than mass spectrometry.

[0051] The biomarkers of this invention can be further characterized by the shape of their spectral peak in time-of-flight mass spectrometry.

[0052] The biomarkers of this invention are further characterized by their binding properties on chromatographic surfaces.

[0053] The biomarkers thus discovered are presented in Table 1 which indicate the chromatographic fraction in which the biomarker was found (Fraction), the type of biochip to which the biomarker binds (Array Surface), the beam intensity applied (Beam Intensity), molecular weight (M/Z (kDa)), and p-values and ROC values as measures of statistical significance and diagnostic potential. Each of these parameters is described in more detail below.

TABLE 1

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
2898	0.0000139	0.901	Fraction 1 (pH 9+)	CM10	Low
2960	0.0000359	0.880	Fraction 1 (pH 9+)	CM10	Low
3248	0.0000160	0.901	Fraction 1 (pH 9+)	CM10	Low
3372	0.0001646	0.839	Fraction 1 (pH 9+)	CM10	Low
3518	0.0001004	0.860	Fraction 1 (pH 9+)	CM10	Low
3534	0.0000079	0.908	Fraction 1 (pH 9+)	CM10	Low
3893	0.0000021	0.056	Fraction 1 (pH 9+)	CM10	Low
4301	0.0008301	0.798	Fraction 1 (pH 9+)	CM10	Low
4388	0.0004750	0.819	Fraction 1 (pH 9+)	CM10	Low
4525	0.0000275	0.901	Fraction 1 (pH 9+)	CM10	Low
4593	0.0009258	0.819	Fraction 1 (pH 9+)	CM10	Low
4806	0.0000052	0.921	Fraction 1 (pH 9+)	CM10	Low
4826	0.0000033	0.942	Fraction 1 (pH 9+)	CM10	Low
4851	0.0000045	0.921	Fraction 1 (pH 9+)	CM10	Low
5176	0.0004750	0.178	Fraction 1 (pH 9+)	CM10	Low
5192	0.0000160	0.096	Fraction 1 (pH 9+)	CM10	Low
5264	0.0002991	0.158	Fraction 1 (pH 9+)	CM10	Low
6594	0.0001457	0.158	Fraction 1 (pH 9+)	CM10	Low
15152	0.0002362	0.137	Fraction 1 (pH 9+)	CM10	Low
15890	0.0001646	0.178	Fraction 1 (pH 9+)	CM10	Low

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
17762	0.0000531	0.137	Fraction 1 (pH 9+)	CM10	Low
17894	0.0008301	0.199	Fraction 1 (pH 9+)	CM10	Low
18584	0.0001138	0.137	Fraction 1 (pH 9+)	CM10	Low
39926	0.0008301	0.171	Fraction 1 (pH 9+)	CM10	Low
44285	0.0000069	0.056	Fraction 1 (pH 9+)	CM10	Low
44306	0.0000079	0.076	Fraction 1 (pH 9+)	CM10	Low
44308	0.0000060	0.076	Fraction 1 (pH 9+)	CM10	Low
44309	0.0000052	0.056	Fraction 1 (pH 9+)	CM10	Low
44309	0.0000033	0.056	Fraction 1 (pH 9+)	CM10	Low
44311	0.0000045	0.056	Fraction 1 (pH 9+)	CM10	Low
44312	0.0000060	0.076	Fraction 1 (pH 9+)	CM10	Low
44312	0.0000060	0.076	Fraction 1 (pH 9+)	CM10	Low
44316	0.0000060	0.076	Fraction 1 (pH 9+)	CM10	Low
44318	0.0000069	0.076	Fraction 1 (pH 9+)	CM10	Low
44331	0.0000060	0.076	Fraction 1 (pH 9+)	CM10	Low
10406	0.0007736	0.828	Fraction 1 (pH 9+)	CM10	High
10432	0.0003615	0.847	Fraction 1 (pH 9+)	CM10	High
10456	0.0005611	0.808	Fraction 1 (pH 9+)	CM10	High
10488	0.0006250	0.828	Fraction 1 (pH 9+)	CM10	High
10507	0.0009546	0.808	Fraction 1 (pH 9+)	CM10	High
10536	0.0007736	0.808	Fraction 1 (pH 9+)	CM10	High
11797	0.0004511	0.828	Fraction 1 (pH 9+)	CM10	High
12324	0.0001282	0.847	Fraction 1 (pH 9+)	CM10	High
12437	0.0002888	0.828	Fraction 1 (pH 9+)	CM10	High
12586	0.0004040	0.808	Fraction 1 (pH 9+)	CM10	High
12793	0.0002299	0.828	Fraction 1 (pH 9+)	CM10	High
15914	0.0000792	0.144	Fraction 1 (pH 9+)	CM10	High
16022	0.0004511	0.197	Fraction 1 (pH 9+)	CM10	High
16139	0.0003232	0.158	Fraction 1 (pH 9+)	CM10	High
16626	0.0001624	0.144	Fraction 1 (pH 9+)	CM10	High
16850	0.0001282	0.139	Fraction 1 (pH 9+)	CM10	High
27603	0.0000029	0.944	Fraction 1 (pH 9+)	CM10	High
29246	0.0000895	0.125	Fraction 1 (pH 9+)	CM10	High
32326	0.0006250	0.183	Fraction 1 (pH 9+)	CM10	High
33582	0.0000256	0.106	Fraction 1 (pH 9+)	CM10	High
38039	0.0005033	0.183	Fraction 1 (pH 9+)	CM10	High
39879	0.0001282	0.139	Fraction 1 (pH 9+)	CM10	High
41774	0.0000426	0.144	Fraction 1 (pH 9+)	CM10	High
44534	0.0000132	0.086	Fraction 1 (pH 9+)	CM10	High
55196	0.0000426	0.886	Fraction 1 (pH 9+)	CM10	High
58000	0.0000173	0.106	Fraction 1 (pH 9+)	CM10	High
59261	0.0000224	0.106	Fraction 1 (pH 9+)	CM10	High
66549	0.0000330	0.125	Fraction 1 (pH 9+)	CM10	High
79207	0.0002299	0.158	Fraction 1 (pH 9+)	CM10	High
124504	0.0004040	0.828	Fraction 1 (pH 9+)	CM10	High
157018	0.0000291	0.906	Fraction 1 (pH 9+)	CM10	High
2502	0.0001416	0.159	Fraction 2 (pH 7)	IMAC30	Low

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
2726	0.0000362	0.139	Fraction 2 (pH 7)	IMAC30	Low
3538	0.0006762	0.821	Fraction 2 (pH 7)	IMAC30	Low
3549	0.0001580	0.821	Fraction 2 (pH 7)	IMAC30	Low
3562	0.0001416	0.841	Fraction 2 (pH 7)	IMAC30	Low
3743	0.0003324	0.801	Fraction 2 (pH 7)	IMAC30	Low
3749	0.0000725	0.861	Fraction 2 (pH 7)	IMAC30	Low
4286	0.0002427	0.841	Fraction 2 (pH 7)	IMAC30	Low
4303	0.0009073	0.801	Fraction 2 (pH 7)	IMAC30	Low
4617	0.0004086	0.821	Fraction 2 (pH 7)	IMAC30	Low
4852	0.0000812	0.841	Fraction 2 (pH 7)	IMAC30	Low
5103	0.0000044	0.901	Fraction 2 (pH 7)	IMAC30	Low
3266	0.0001487	0.839	Fraction 3 (pH 5)	CM10	Low
3539	0.0002082	0.839	Fraction 3 (pH 5)	CM10	Low
3562	0.0000940	0.858	Fraction 3 (pH 5)	CM10	Low
4088	0.0006743	0.803	Fraction 3 (pH 5)	CM10	Low
4156	0.0007470	0.803	Fraction 3 (pH 5)	CM10	Low
4302	0.0001665	0.858	Fraction 3 (pH 5)	CM10	Low
4847	0.0000320	0.876	Fraction 3 (pH 5)	CM10	Low
4870	0.0000837	0.876	Fraction 3 (pH 5)	CM10	Low
5585	0.0002325	0.839	Fraction 3 (pH 5)	CM10	Low
5765	0.0002595	0.839	Fraction 3 (pH 5)	CM10	Low
5912	0.0000744	0.858	Fraction 3 (pH 5)	CM10	Low
15211	0.0000298	0.903	Fraction 3 (pH 5)	CM10	High
28474	0.0002226	0.125	Fraction 3 (pH 5)	CM10	High
51327	0.0000138	0.924	Fraction 3 (pH 5)	CM10	High
3371	0.0000577	0.841	Fraction 4 (pH 4)	CM10	Low
3379	0.0000322	0.861	Fraction 4 (pH 4)	CM10	Low
4139	0.0000176	0.881	Fraction 4 (pH 4)	CM10	Low
4531	0.0000577	0.861	Fraction 4 (pH 4)	CM10	Low
6018	0.0003687	0.841	Fraction 4 (pH 4)	CM10	Low
13901	0.0000199	0.119	Fraction 4 (pH 4)	CM10	Low
16431	0.0000225	0.119	Fraction 4 (pH 4)	CM10	Low
28238	0.0000176	0.119	Fraction 4 (pH 4)	CM10	Low
51351	0.0000026	0.941	Fraction 4 (pH 4)	CM10	Low
51774	0.0004526	0.781	Fraction 4 (pH 4)	CM10	Low
52093	0.0001760	0.841	Fraction 4 (pH 4)	CM10	Low
61850	0.0006762	0.199	Fraction 4 (pH 4)	CM10	Low
14580	0.0000002	0.020	Fraction 4 (pH 4)	CM10	High
14708	0.0000002	0.020	Fraction 4 (pH 4)	CM10	High
14870	0.0000008	0.039	Fraction 4 (pH 4)	CM10	High
16626	0.0005035	0.176	Fraction 4 (pH 4)	CM10	High
16736	0.0002882	0.151	Fraction 4 (pH 4)	CM10	High
16827	0.0001817	0.137	Fraction 4 (pH 4)	CM10	High
17339	0.0000010	0.039	Fraction 4 (pH 4)	CM10	High
17482	0.0000018	0.059	Fraction 4 (pH 4)	CM10	High
17991	0.0000252	0.112	Fraction 4 (pH 4)	CM10	High
19605	0.0005035	0.176	Fraction 4 (pH 4)	CM10	High

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
26371	0.0001275	0.846	Fraction 4 (pH 4)	CM10	High
28535	0.0000012	0.059	Fraction 4 (pH 4)	CM10	High
29387	0.0000057	0.078	Fraction 4 (pH 4)	CM10	High
50020	0.0006968	0.826	Fraction 4 (pH 4)	CM10	High
51367	0.0000003	0.983	Fraction 4 (pH 4)	CM10	High
56942	0.0002882	0.846	Fraction 4 (pH 4)	CM10	High
80097	0.0001436	0.165	Fraction 4 (pH 4)	CM10	High
81845	0.0001131	0.118	Fraction 4 (pH 4)	CM10	High
117781	0.0001436	0.866	Fraction 4 (pH 4)	CM10	High
4746	0.0009994	0.821	Fraction 5 (pH 3)	IMAC30	Low
9309	0.0001269	0.159	Fraction 5 (pH 3)	IMAC30	Low
9478	0.0000322	0.139	Fraction 5 (pH 3)	IMAC30	Low
13917	0.0000725	0.139	Fraction 5 (pH 3)	IMAC30	Low
33648	0.0000156	0.139	Fraction 5 (pH 3)	IMAC30	Low
66992	0.0000199	0.119	Fraction 5 (pH 3)	IMAC30	Low
71918	0.0001760	0.179	Fraction 5 (pH 3)	IMAC30	Low
12299	0.0003362	0.870	Fraction 5 (pH 3)	IMAC30	High
12333	0.0005954	0.808	Fraction 5 (pH 3)	IMAC30	High
12610	0.0000531	0.863	Fraction 5 (pH 3)	IMAC30	High
12678	0.0004750	0.822	Fraction 5 (pH 3)	IMAC30	High
12735	0.0004237	0.842	Fraction 5 (pH 3)	IMAC30	High
14586	0.0001858	0.140	Fraction 5 (pH 3)	IMAC30	High
14703	0.0002991	0.140	Fraction 5 (pH 3)	IMAC30	High
15675	0.0004750	0.808	Fraction 5 (pH 3)	IMAC30	High
26333	0.0002659	0.822	Fraction 5 (pH 3)	IMAC30	High
49192	0.0004750	0.822	Fraction 5 (pH 3)	IMAC30	High
49982	0.0000160	0.904	Fraction 5 (pH 3)	IMAC30	High
50581	0.0000139	0.883	Fraction 5 (pH 3)	IMAC30	High
51111	0.0000018	0.944	Fraction 5 (pH 3)	IMAC30	High
51555	0.0001646	0.842	Fraction 5 (pH 3)	IMAC30	High
52076	0.0001288	0.863	Fraction 5 (pH 3)	IMAC30	High
52721	0.0000052	0.924	Fraction 5 (pH 3)	IMAC30	High
54017	0.0000240	0.904	Fraction 5 (pH 3)	IMAC30	High
66719	0.0008301	0.202	Fraction 5 (pH 3)	IMAC30	High
108685	0.0009258	0.808	Fraction 5 (pH 3)	IMAC30	High
133227	0.0000105	0.099	Fraction 5 (pH 3)	IMAC30	High
3168	0.00011383	0.828	Fraction 6 (Wash)	IMAC30	Low
3375	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	Low
3582	0.00000218	0.944	Fraction 6 (Wash)	IMAC30	Low
3599	0.00000031	0.964	Fraction 6 (Wash)	IMAC30	Low
4261	0.00014436	0.847	Fraction 6 (Wash)	IMAC30	Low
4293	0.00036150	0.847	Fraction 6 (Wash)	IMAC30	Low
4363	0.00000058	0.983	Fraction 6 (Wash)	IMAC30	Low
4474	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	Low
4544	0.00004259	0.906	Fraction 6 (Wash)	IMAC30	Low
4631	0.00000078	0.964	Fraction 6 (Wash)	IMAC30	Low
4745	0.00000218	0.944	Fraction 6 (Wash)	IMAC30	Low

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
5073	0.00001158	0.906	Fraction 6 (Wash)	IMAC30	Low
6200	0.00000586	0.911	Fraction 6 (Wash)	IMAC30	Low
6405	0.00003304	0.911	Fraction 6 (Wash)	IMAC30	Low
7295	0.00003304	0.886	Fraction 6 (Wash)	IMAC30	Low
7629	0.00000443	0.086	Fraction 6 (Wash)	IMAC30	Low
7950	0.00010095	0.125	Fraction 6 (Wash)	IMAC30	Low
11551	0.00000020	0.983	Fraction 6 (Wash)	IMAC30	Low
11740	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	Low
13765	0.00004259	0.125	Fraction 6 (Wash)	IMAC30	Low
13907	0.00001513	0.086	Fraction 6 (Wash)	IMAC30	Low
14094	0.00000510	0.067	Fraction 6 (Wash)	IMAC30	Low
15178	0.00002555	0.081	Fraction 6 (Wash)	IMAC30	Low
15370	0.00010095	0.139	Fraction 6 (Wash)	IMAC30	Low
15905	0.00002555	0.106	Fraction 6 (Wash)	IMAC30	Low
16113	0.00001727	0.086	Fraction 6 (Wash)	IMAC30	Low
17317	0.00032323	0.164	Fraction 6 (Wash)	IMAC30	Low
28184	0.00001012	0.067	Fraction 6 (Wash)	IMAC30	Low
29018	0.00000290	0.067	Fraction 6 (Wash)	IMAC30	Low
50985	0.00000122	0.944	Fraction 6 (Wash)	IMAC30	Low
51355	0.00001513	0.906	Fraction 6 (Wash)	IMAC30	Low
53784	0.00004259	0.886	Fraction 6 (Wash)	IMAC30	Low
60791	0.00003753	0.886	Fraction 6 (Wash)	IMAC30	Low
62393	0.00000443	0.925	Fraction 6 (Wash)	IMAC30	Low
91819	0.00028877	0.847	Fraction 6 (Wash)	IMAC30	Low
10959	0.00095457	0.808	Fraction 6 (Wash)	IMAC30	High
11053	0.00085970	0.808	Fraction 6 (Wash)	IMAC30	High
11074	0.00040398	0.808	Fraction 6 (Wash)	IMAC30	High
11128	0.00040398	0.828	Fraction 6 (Wash)	IMAC30	High
11161	0.00085970	0.789	Fraction 6 (Wash)	IMAC30	High
11730	0.00018248	0.828	Fraction 6 (Wash)	IMAC30	High
12238	0.00000189	0.944	Fraction 6 (Wash)	IMAC30	High
12307	0.00000290	0.925	Fraction 6 (Wash)	IMAC30	High
12393	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	High
12463	0.00000014	0.983	Fraction 6 (Wash)	IMAC30	High
12584	0.00000014	0.983	Fraction 6 (Wash)	IMAC30	High
12625	0.00000014	0.983	Fraction 6 (Wash)	IMAC30	High
12666	0.00000014	0.983	Fraction 6 (Wash)	IMAC30	High
12786	0.00000058	0.964	Fraction 6 (Wash)	IMAC30	High
12851	0.00000036	0.964	Fraction 6 (Wash)	IMAC30	High
12931	0.00000027	0.983	Fraction 6 (Wash)	IMAC30	High
13004	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	High
13076	0.00000078	0.964	Fraction 6 (Wash)	IMAC30	High
13210	0.00018248	0.828	Fraction 6 (Wash)	IMAC30	High
14711	0.00022992	0.158	Fraction 6 (Wash)	IMAC30	High
14883	0.00003753	0.100	Fraction 6 (Wash)	IMAC30	High
15358	0.00001969	0.906	Fraction 6 (Wash)	IMAC30	High
15558	0.00011383	0.847	Fraction 6 (Wash)	IMAC30	High

Marker M/Z (kDa)	P-Value	ROC	Fraction	Array Surface	Beam Intensity
16626	0.00056108	0.197	Fraction 6 (Wash)	IMAC30	High
16767	0.00014436	0.158	Fraction 6 (Wash)	IMAC30	High
16825	0.00022992	0.158	Fraction 6 (Wash)	IMAC30	High
28543	0.00002907	0.106	Fraction 6 (Wash)	IMAC30	High
29326	0.00001969	0.106	Fraction 6 (Wash)	IMAC30	High
50252	0.00000020	0.983	Fraction 6 (Wash)	IMAC30	High
50627	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	High
51218	0.00000017	0.983	Fraction 6 (Wash)	IMAC30	High
59346	0.00001012	0.906	Fraction 6 (Wash)	IMAC30	High
60195	0.00000163	0.964	Fraction 6 (Wash)	IMAC30	High
61361	0.00000443	0.944	Fraction 6 (Wash)	IMAC30	High
89621	0.00000031	0.983	Fraction 6 (Wash)	IMAC30	High
94758	0.00001012	0.086	Fraction 6 (Wash)	IMAC30	High
133020	0.00050329	0.164	Fraction 6 (Wash)	IMAC30	High

2.2. Detection and Characterization of Biomarkers

[0054] The biomarkers of this invention can be detected by any suitable method.

Detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, *e.g.*, multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (*e.g.*, surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

[0055] In some aspects, a sample is analyzed by means of a biochip. Biochips generally comprise solid substrates and have a generally planar surface, to which a capture reagent (also called an adsorbent or affinity reagent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there.

[0056] Protein biochips are biochips adapted for the capture of polypeptides. Many protein biochips are described in the art. These include, for example, protein biochips produced by Ciphergen Biosystems, Inc. (Fremont, CA), Zyomyx (Hayward, CA), Invitrogen (Carlsbad, CA), Biacore (Uppsala, Sweden) and Procognia (Berkshire, UK).

Examples of such protein biochips are described in the following patents or published patent applications: U.S. Patent No. 6,225,047 (Hutchens & Yip); U.S. Patent No. 6,537,749 (Kuimelis and Wagner); U.S. Patent No. 6,329,209 (Wagner *et al.*); PCT International Publication No. WO 00/56934 (Englert *et al.*); PCT International Publication No. WO 03/048768 (Boutell *et al.*) and U.S. Patent No. 5,242,828 (Bergstrom *et al.*).

[0057] In a preferred aspect, the biomarkers of this invention are detected by mass spectrometry, a method that employs a mass spectrometer to detect gas phase ions. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these.

[0058] In a further preferred method, the mass spectrometer is a laser desorption/ionization mass spectrometer. In laser desorption/ionization mass spectrometry, the analytes are placed on the surface of a mass spectrometry probe, a device adapted to engage a probe interface of the mass spectrometer and to present an analyte to ionizing energy for ionization and introduction into a mass spectrometer. A laser desorption mass spectrometer employs laser energy, typically from an ultraviolet laser, but also from an infrared laser, to desorb analytes from a surface, to volatilize and ionize them and make them available to the ion optics of the mass spectrometer.

[0059] A preferred mass spectrometric technique for use in the invention is "Surface Enhanced Laser Desorption and Ionization" or "SELDI," as described, for example, in U.S. Patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip. This refers to a method of desorption/ionization gas phase ion spectrometry (*e.g.*, mass spectrometry) in which an analyte (here, one or more of the biomarkers) is captured on the surface of a SELDI mass spectrometry probe. There are several versions of SELDI, which are described in more detail below.

[0060] One version of SELDI is called "affinity capture mass spectrometry." It also is called "Surface-Enhanced Affinity Capture" or "SEAC". This version involves the use of probes that have a material on the probe surface that captures analytes through a non-covalent affinity interaction (adsorption) between the material and the analyte. The material is variously called an "adsorbent," a "capture reagent," an "affinity reagent" or a "binding moiety." Such probes can be referred to as "affinity capture probes" and as having an "adsorbent surface." The capture reagent can be any material capable of binding an analyte. The capture reagent is attached to the probe surface by physisorption or chemisorption. In

certain aspects the probes have the capture reagent already attached to the surface. In other aspects, the probes are pre-activated and include a reactive moiety that is capable of binding the capture reagent, *e.g.*, through a reaction forming a covalent or coordinate covalent bond. Epoxide and acyl-imidazole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitrilotriacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. Adsorbents are generally classified as chromatographic adsorbents and biospecific adsorbents.

[0061] "Chromatographic adsorbent" refers to an adsorbent material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators (*e.g.*, nitrilotriacetic acid or iminodiacetic acid), immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (*e.g.*, nucleotides, amino acids, simple sugars and fatty acids) and mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents).

[0062] "Biospecific adsorbent" refers to an adsorbent comprising a biomolecule, *e.g.*, a nucleic acid molecule (*e.g.*, an aptamer), a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid, a nucleic acid (*e.g.*, DNA)-protein conjugate). In certain instances, the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than chromatographic adsorbents. Further examples of adsorbents for use in SELDI can be found in U.S. Patent No. 6,225,047. A "bioselective adsorbent" refers to an adsorbent that binds to an analyte with an affinity of at least 10^{-8} M.

[0063] Protein biochips produced by CIPHERGEN Biosystems, Inc. comprise surfaces having chromatographic or biospecific adsorbents attached thereto at addressable locations. CIPHERGEN ProteinChip® arrays include NP20 (hydrophilic); H4 and H50 (hydrophobic); SAX-2, Q-10 and LSAX-30 (anion exchange); WCX-2, CM-10 and LWCX-30 (cation exchange); IMAC-3, IMAC-30 and IMAC 40 (metal chelate); and PS-10, PS-20 (reactive surface with acyl-imidazole, epoxide) and PG-20 (protein G coupled through acyl-imidazole). Hydrophobic ProteinChip arrays have isopropyl or nonylphenoxy-poly(ethylene glycol)methacrylate functionalities. Anion exchange ProteinChip arrays have quaternary ammonium functionalities. Cation exchange ProteinChip arrays have carboxylate

functionalities. Immobilized metal chelate ProteinChip arrays have nitrilotriacetic acid functionalities that adsorb transition metal ions, such as copper, nickel, zinc, and gallium, by chelation. Preactivated ProteinChip arrays have acyl-imidazole or epoxide functional groups that can react with groups on proteins for covalent binding.

[0064] Such biochips are further described in: U.S. Patent No. 6,579,719 (Hutchens and Yip, "Retentate Chromatography," June 17, 2003); U.S. Patent 6,897,072 (Rich *et al.*, "Probes for a Gas Phase Ion Spectrometer," Can 24, 2005); U.S. Patent No. 6,555,813 (Beecher *et al.*, "Sample Holder with Hydrophobic Coating for Gas Phase Mass Spectrometer," April 29, 2003); U.S. Patent Application No. U.S. 2003 0032043 A1 (Pohl and Papanu, "Latex Based Adsorbent Chip," July 16, 2002); and PCT International Publication No. WO 03/040700 (Um *et al.*, "Hydrophobic Surface Chip," Can 15, 2003); U.S. Patent Application No. US 2003/0218130 A1 (Boschetti *et al.*, "Biochips With Surfaces Coated With Polysaccharide-Based Hydrogels," April 14, 2003) and U.S. Patent Application No. 60/448,467, entitled "Photocrosslinked Hydrogel Surface Coatings" (Huang *et al.*, filed February 21, 2003).

[0065] In general, a probe with an adsorbent surface is contacted with the sample for a period of time sufficient to allow the biomarker or biomarkers that can be present in the sample to bind to the adsorbent. After an incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed. The extent to which molecules remain bound can be manipulated by adjusting the stringency of the wash. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature. Unless the probe has both SEAC and SEND properties (as described herein), an energy absorbing molecule then is applied to the substrate with the bound biomarkers.

[0066] The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

[0067] Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface ("SEND probe"). The phrase "energy absorbing molecules" (EAM) denotes molecules that are capable of absorbing energy from a laser desorption/ionization source and, thereafter, contribute to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI, frequently referred to as "matrix," and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyaceto-phenone derivatives. In certain aspects, the energy absorbing molecule is incorporated into a linear or cross-linked polymer, e.g., a polymethacrylate. For example, the composition can be a co-polymer of α -cyano-4-methacryloyloxy-cinnamic acid and acrylate. In some aspects, the composition is a co-polymer of α -cyano-4-methacryloyloxy-cinnamic acid, acrylate and 3-(tri-ethoxy)silyl propyl methacrylate. In some aspects, the composition is a co-polymer of α -cyano-4-methacryloyloxy-cinnamic acid and octadecylmethacrylate ("C18 SEND"). SEND is further described in U.S. Patent No. 6,124,137 and PCT International Publication No. WO 03/64594 (Kitagawa, "Monomers And Polymers Having Energy Absorbing Moieties Of Use In Desorption/Ionization Of Analytes," August 7, 2003).

[0068] SEAC/SEND is a version of SELDI in which both a capture reagent and an energy absorbing molecule are attached to the sample presenting surface. SEAC/SEND probes therefore allow the capture of analytes through affinity capture and ionization/desorption without the need to apply external matrix. The C18 SEND biochip is a version of SEAC/SEND, comprising a C18 moiety which functions as a capture reagent, and a CHCA moiety which functions as an energy absorbing moiety.

[0069] Another version of SELDI, called Surface-Enhanced Photolabile Attachment and Release (SEPAR), involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, e.g., to laser light (see, U.S. Patent No. 5,719,060). SEPAR and other forms of SELDI are readily adapted to detecting a biomarker or biomarker profile, pursuant to the present invention.

[0070] In another mass spectrometry method, the biomarkers are first captured on a chromatographic resin having chromatographic properties that bind the biomarkers. In the present example, this could include a variety of methods. For example, one could capture the

biomarkers on a cation exchange resin, such as CM Ceramic HyperD F resin, wash the resin, elute the biomarkers and detect by MALDI. Alternatively, this method could be preceded by fractionating the sample on an anion exchange resin before application to the cation exchange resin. In another alternative, one could fractionate on an anion exchange resin and detect by MALDI directly. In yet another method, one could capture the biomarkers on an immuno-chromatographic resin that comprises antibodies that bind the biomarkers, wash the resin to remove unbound material, elute the biomarkers from the resin and detect the eluted biomarkers by MALDI or by SELDI. In yet another method, one could isolate the biomarkers using gel electrophoresis and detect the biomarkers by MALDI OR SELDI.

[0071] Analysis of analytes by time-of-flight mass spectrometry generates a time-of-flight spectrum. The time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's ProteinChip® software, data processing typically includes TOF-to-M/Z transformation to generate a mass spectrum, baseline subtraction to eliminate instrument offsets and high frequency noise filtering to reduce high frequency noise.

[0072] Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of biomarkers detected, and optionally the strength of the signal and the determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference.

[0073] The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling biomarkers with nearly identical molecular weights to be more easily seen. In another useful format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

[0074] Analysis generally involves the identification of peaks in the spectrum that represent signal from an analyte. Peak selection can be done visually, but software is available, as part of Ciphergen's ProteinChip® software package, that can automate the detection of peaks. In general, this software functions by identifying signals having a signal-to-noise ratio above a selected threshold and labeling the mass of the peak at the centroid of the peak signal. In one useful application, many spectra are compared to identify identical peaks present in some selected percentage of the mass spectra. One version of this software clusters all peaks appearing in the various spectra within a defined mass range, and assigns a mass (M/Z) to all the peaks that are near the mid-point of the mass (M/Z) cluster.

[0075] Software used to analyze the data can include code that applies an algorithm to the analysis of the signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention. The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates the status of the particular clinical parameter under examination. Analysis of the data can be "keyed" to a variety of parameters that are obtained, either directly or indirectly, from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the shape of a peak or group of peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

[0076] A preferred protocol for the detection of the biomarkers of this invention is as follows. The biological sample to be tested, *e.g.*, serum, preferably is subject to pre-fractionation before SELDI analysis. This simplifies the sample and improves sensitivity. A preferred method of pre-fractionation involves contacting the sample with an anion exchange chromatographic material, such as Q HyperD (BioSeptra, SA). The bound materials are then subject to stepwise pH elution using buffers at pH 9, pH 7, pH 5 and pH 4. (The fractions in which the biomarkers are eluted also are indicated in Table 1.) Various fractions containing the biomarker are collected.

[0077] The sample to be tested (preferably pre-fractionated) is then contacted with an affinity capture probe comprising an cation exchange adsorbent (preferably a WCX ProteinChip array (Ciphergen Biosystems, Inc.)) or an IMAC adsorbent (preferably an IMAC3 ProteinChip array (Ciphergen Biosystems, Inc.)), again as indicated in Table 1. The probe is washed with a buffer that will retain the biomarker while washing away unbound

molecules. A suitable wash for each biomarker is the buffer identified in Table 1. The biomarkers are detected by laser desorption/ionization mass spectrometry.

[0078] Alternatively, if antibodies that recognize the biomarker are available, these can be attached to the surface of a probe, such as a pre-activated PS10 or PS20 ProteinChip array (CIPHERGEN Biosystems, Inc.). These antibodies can capture the biomarkers from a sample onto the probe surface. Then the biomarkers can be detected by, *e.g.*, laser desorption/ionization mass spectrometry.

2.3. Detection by Immunoassay

[0079] In some aspects of the invention, the biomarkers of the invention are measured by a method other than mass spectrometry or other than methods that rely on a measurement of the mass of the biomarker. In one such aspect that does not rely on mass, the biomarkers of this invention are measured by immunoassay. Immunoassay requires biospecific capture reagents, such as antibodies, to capture the biomarkers. Antibodies can be produced by methods well known in the art, *e.g.*, by immunizing animals with the biomarkers. Biomarkers can be isolated from samples based on their binding characteristics. Alternatively, if the amino acid sequence of a polypeptide biomarker is known, the polypeptide can be synthesized and used to generate antibodies by methods well known in the art.

[0080] This invention contemplates traditional immunoassays including, for example, sandwich immunoassays including ELISA or fluorescence-based immunoassays, as well as other enzyme immunoassays. Nephelometry is an assay done in liquid phase which measures the binding of an antigen to an antibody in solution by measuring changes in absorbance upon antibody-antigen binding. In the SELDI-based immunoassay, a biospecific capture reagent for the biomarker is attached to the surface of an MS probe, such as a pre-activated ProteinChip array. The biomarker is then specifically captured on the biochip through this reagent, and the captured biomarker is detected by mass spectrometry.

3. DETERMINATION OF SUBJECT LEISHMANIASIS STATUS

3.1. Single Markers

[0081] The biomarkers of the invention can be used in diagnostic tests to assess Leishmaniasis status in a subject, *e.g.*, to diagnose Leishmaniasis. The phrase "Leishmaniasis status" includes any distinguishable manifestation of the disease, including non-disease. For example, disease status includes, without limitation, the presence or absence of disease (*e.g.*,

Leishmaniasis v. non Leishmaniasis or Leishmaniasis v. other parasitic disease (*e.g.*, African sleeping sickness, malaria)), the risk of developing disease, the stage of the disease, the progress of disease (*e.g.*, progress of disease or remission of disease over time) and the effectiveness or response to treatment of disease. The status of the subject can inform the practitioner about what status set is being distinguished. For example, a subject that presents with signs of a parasitic disease could be classed into Leishmaniasis v. non-Leishmaniasis parasitic disease, while a person exposed to a situation in which Leishmaniasis infection is possible and who is presenting with signs of Leishmaniasis infection could be classified into Leishmaniasis v. non-Leishmaniasis. Based on this status, further procedures can be indicated, including additional diagnostic tests or therapeutic procedures or regimens.

[0082] The power of a diagnostic test to correctly predict status is commonly measured as the sensitivity of the assay, the specificity of the assay or the area under a receiver operated characteristic ("ROC") curve. Sensitivity is the percentage of true positives that are predicted by a test to be positive, while specificity is the percentage of true negatives that are predicted by a test to be negative. An ROC curve provides the sensitivity of a test as a function of 1-specificity. The greater the area under the ROC curve, the more powerful the predictive value of the test. Other useful measures of the utility of a test are positive predictive value and negative predictive value. Positive predictive value is the percentage of people who test positive that are actually positive. Negative predictive value is the percentage of people who test negative that are actually negative.

[0083] The biomarkers of this invention show a statistical difference in different Leishmaniasis statuses of at least $p \leq 0.05$, $p \leq 10^{-2}$, $p \leq 10^{-3}$, $p \leq 10^{-4}$ or $p \leq 10^{-5}$. Diagnostic tests that use these biomarkers alone or in combination show a sensitivity and specificity of at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% and about 100%.

[0084] Certain biomarkers listed in Table I are differentially present in Leishmaniasis, and, therefore, each is individually useful in aiding in the determination of Leishmaniasis status. The method involves, first, measuring the selected biomarker in a subject sample using the methods described herein, *e.g.*, capture on a SELDI biochip followed by detection by mass spectrometry and, second, comparing the measurement with a diagnostic amount or cut-off that distinguishes a positive Leishmaniasis status from a negative Leishmaniasis status. The diagnostic amount represents a measured amount of a biomarker above which or below which a subject is classified as having a particular

Leishmaniasis status. For example, if the biomarker is up-regulated compared to normal during Leishmaniasis, then a measured amount above the diagnostic cutoff provides a diagnosis of Leishmaniasis. Alternatively, if the biomarker is down-regulated during Leishmaniasis, then a measured amount below the diagnostic cutoff provides a diagnosis of Leishmaniasis. As is well understood in the art, by adjusting the particular diagnostic cut-off used in an assay, one can increase sensitivity or specificity of the diagnostic assay depending on the preference of the diagnostician. The particular diagnostic cut-off can be determined, for example, by measuring the amount of the biomarker in a statistically significant number of samples from subjects with the different Leishmaniasis statuses, as was done here, and drawing the cut-off to suit the diagnostician's desired levels of specificity and sensitivity.

3.2. Combinations of Markers

[0085] While individual biomarkers are useful diagnostic biomarkers, it has been found that a combination of biomarkers can provide greater predictive value of a particular status than single biomarkers alone. Specifically, the detection of a plurality of biomarkers in a sample can increase the sensitivity and/or specificity of the test. A combination of at least two biomarkers is sometimes referred to as a "biomarker profile" or "biomarker fingerprint."

3.3. Presence of Leishmaniasis

[0086] In some aspects, this invention provides methods for determining the presence or absence of Leishmaniasis in a subject (status: Leishmaniasis v. non-Leishmaniasis). The presence or absence of Leishmaniasis is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular risk level.

3.4. Determining Risk of Developing Disease

[0087] In some aspects, this invention provides methods for determining the risk of developing disease in a subject. Biomarker amounts or patterns are characteristic of various risk states, e.g., high, medium or low. The risk of developing a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular risk level

3.5. Determining Stage of Disease

[0088] In some aspects, this invention provides methods for determining the stage of disease in a subject. Each stage of the disease has a characteristic amount of a biomarker or relative amounts of a set of biomarkers (a pattern). The stage of a disease is determined by measuring the relevant biomarker or biomarkers and then either submitting them to a classification algorithm or comparing them with a reference amount and/or pattern of biomarkers that is associated with the particular stage.

3.6. Determining Course (Progression/Remission) of Disease

[0089] In some aspects, this invention provides methods for determining the course of disease in a subject. Disease course refers to changes in disease status over time, including disease progression (worsening) and disease regression (improvement). Over time, the amounts or relative amounts (*e.g.*, the pattern) of the biomarkers changes. Therefore, the trend of these markers, either increased or decreased over time toward diseased or non-diseased indicates the course of the disease. Accordingly, this method involves measuring one or more biomarkers in a subject at least two different time points, *e.g.*, a first time and a second time, and comparing the change in amounts, if any. The course of disease is determined based on these comparisons.

3.7. Subject Management

[0090] In certain aspects of the methods of qualifying Leishmaniasis status, the methods further comprise managing subject treatment based on the status. Such management includes the actions of the physician or clinician subsequent to determining Leishmaniasis status. For example, if a physician makes a diagnosis of Leishmaniasis, then a certain regime of treatment, such as prescription or administration of sodium stibogluconate might follow. Alternatively, a diagnosis of non- Leishmaniasis might be followed with further testing to determine a specific disease that might the patient might be suffering from. Also, if the diagnostic test gives an inconclusive result on Leishmaniasis status, further tests can be called for.

[0091] Additional aspects of the invention relate to the communication of assay results or diagnoses or both to technicians, physicians or patients, for example. In certain aspects, computers will be used to communicate assay results or diagnoses or both to interested parties, *e.g.*, physicians and their patients. In some aspects, the assays will be

performed or the assay results analyzed in a country or jurisdiction which differs from the country or jurisdiction to which the results or diagnoses are communicated.

[0092] In a preferred aspect of the invention, a diagnosis based on the presence or absence in a test subject of any the biomarkers of Table 1 is communicated to the subject as soon as possible after the diagnosis is obtained. The diagnosis can be communicated to the subject by the subject's treating physician. Alternatively, the diagnosis can be sent to a test subject by email or communicated to the subject by phone. A computer can be used to communicate the diagnosis by email or phone. In certain aspects, the message containing results of a diagnostic test can be generated and delivered automatically to the subject using a combination of computer hardware and software which will be familiar to artisans skilled in telecommunications. One example of a healthcare-oriented communications system is described in U.S. Patent Number 6,283,761; however, the present invention is not limited to methods which utilize this particular communications system. In certain aspects of the methods of the invention, all or some of the method steps, including the assaying of samples, diagnosing of diseases, and communicating of assay results or diagnoses, can be carried out in diverse (*e.g.*, foreign) jurisdictions.

3.8. Determining Therapeutic Efficacy of Pharmaceutical Drug

[0093] In some aspects, this invention provides methods for determining the therapeutic efficacy of a pharmaceutical drug. These methods are useful in performing clinical trials of the drug, as well as monitoring the progress of a patient on the drug. Therapy or clinical trials involve administering the drug in a particular regimen. The regimen can involve a single dose of the drug or multiple doses of the drug over time. The doctor or clinical researcher monitors the effect of the drug on the patient or subject over the course of administration. If the drug has a pharmacological impact on the condition, the amounts or relative amounts (*e.g.*, the pattern or profile) of the biomarkers of this invention changes toward a non-disease profile. One can follow the course of the amounts of these biomarkers in the subject during the course of treatment. Accordingly, this method involves measuring one or more biomarkers in a subject receiving drug therapy, and correlating the amounts of the biomarkers with the disease status of the subject. Some aspects of this method involves determining the levels of the biomarkers at least two different time points during a course of drug therapy, *e.g.*, a first time and a second time, and comparing the change in amounts of the biomarkers, if any. For example, the biomarkers can be measured before and after drug administration or at two different time points during drug administration. The effect of

therapy is determined based on these comparisons. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications. If a treatment is effective, then the biomarkers will trend toward normal, while if treatment is ineffective, the biomarkers will trend toward disease indications.

3.9. Biomarkers and Modified Forms of a Protein

[0094] Proteins frequently exist in a sample in a plurality of different forms. These forms can result from either, or both, of pre- and post-translational modification. Pre-translational modified forms include allelic variants, splice variants and RNA editing forms. Post-translationally modified forms include forms resulting from proteolytic cleavage (*e.g.*, fragments of a parent protein), glycosylation, phosphorylation, lipidation, oxidation, methylation, cysteinylolation, sulphonation and acetylation. When detecting or measuring a protein in a sample, the ability to differentiate between different forms of a protein depends upon the nature of the difference and the method used to detect or measure. For example, immunological methods of detection typically cannot distinguish between different forms of a protein that contain the same epitope or epitopes to which the antibody or antibodies are directed. In diagnostic assays, the inability to distinguish different forms of a protein has little impact when the forms detected by the particular method used are equally good biomarkers as any particular form. However, when a particular form (or a subset of particular forms) of a protein is a better biomarker than the collection of modified forms detected together by a particular method, the power of the assay can suffer. In this case, it is useful to employ an assay method that distinguishes between forms of a protein and that specifically detects and measures a desired modified form or forms of the protein. Distinguishing different forms of an analyte or specifically detecting a particular form of an analyte is referred to as "resolving" the analyte.

[0095] The collection of analytes detected in an assay and the ability to resolve modified forms of a protein of course depends on the methodology used. For example, an immunoassay using a monoclonal antibody will detect all forms of a protein containing the epitope and will not distinguish between them. However, a sandwich immunoassay that uses two antibodies directed against different epitopes on a protein will detect all forms of the protein that contain both epitope and will not detect those forms that contain only one of the epitopes. Accordingly this method can be useful when the modified forms differ in a terminal amino acid and one of the antibodies is directed to the terminus of one of these forms.

[0096] Preferably, the biospecific capture reagent is bound to a solid phase, such as a bead, a plate, a membrane or a chip. Methods of coupling biomolecules, such as antibodies, to a solid phase are well known in the art. They can employ, for example, bifunctional linking agents, or the solid phase can be derivatized with a reactive group, such as an epoxide or an imidazole, that will bind the molecule on contact. Biospecific capture reagents against different target proteins can be mixed in the same place, or they can be attached to solid phases in different physical or addressable locations. For example, one can load multiple columns with derivatized beads, each column able to capture a single protein cluster. Alternatively, one can pack a single column with different beads derivatized with capture reagents against a variety of protein clusters, thereby capturing all the analytes in a single place. Accordingly, antibody-derivatized bead-based technologies, such as xMAP technology of Luminex (Austin, TX) can be used to detect the protein clusters. However, the biospecific capture reagents must be specifically directed toward the members of a cluster in order to differentiate them.

[0097] Mass spectrometry is a particularly powerful resolving methodology because different forms of a protein typically have different masses and can be differentiated by mass spectrometry. One useful methodology combines mass spectrometry with immunoassay. First, a biospecific capture reagent (*e.g.*, an antibody, aptamer or Affibody that recognizes the biomarker and modified forms of it) is used to capture the biomarker of interest. Preferably, the biospecific capture reagent is bound to a solid phase, such as a bead, a plate, a membrane or a chip. After unbound materials are washed away, the captured analytes are detected and/or measured by mass spectrometry. (This method also will also result in the capture of protein interactors that are bound to the proteins or that are otherwise recognized by antibodies and that, themselves, can be biomarkers.) Then, the captured proteins can be detected by SELDI mass spectrometry or by eluting the proteins from the capture reagent and detecting the eluted proteins by traditional MALDI, SELDI or any other ionization method for mass spectrometry (*e.g.*, electrospray).

[0098] Thus, when reference is made herein to detecting a particular protein or to measuring the amount of a particular protein, it means detecting and measuring the protein with or without resolving modified forms of protein. For example, the step of "measuring Apolipoprotein A-IV precursor" includes measuring Apolipoprotein A-IV precursor by means that do not differentiate between various forms of the protein (*e.g.*, certain immunoassays) as well as by means that differentiate some forms from other forms or that

measure a specific form of the protein. In contrast, when it is desired to measure a particular form or forms of a protein, the particular form (or forms) is specified. For example, "measuring M7.065159" means measuring M7.065159 in a way that distinguishes it from forms of the protein that do not have the characteristic properties identified in Table 1.

4. GENERATION OF CLASSIFICATION ALGORITHMS FOR QUALIFYING LEISHMANIASIS STATUS

[0099] In some aspects, data derived from the spectra (*e.g.*, mass spectra or time-of-flight spectra) that are generated using samples such as "known samples" can then be used to "train" a classification model. A "known sample" is a sample that has been pre-classified. The data that are derived from the spectra and are used to form the classification model can be referred to as a "training data set." Once trained, the classification model can recognize patterns in data derived from spectra generated using unknown samples. The classification model can then be used to classify the unknown samples into classes. This can be useful, for example, in predicting whether or not a particular biological sample is associated with a certain biological condition (*e.g.*, diseased versus non-diseased).

[0100] The training data set that is used to form the classification model can comprise raw data or pre-processed data. In some aspects, raw data can be obtained directly from time-of-flight spectra or mass spectra, and then can be optionally "pre-processed" as described above.

[0101] Classification models can be formed using any suitable statistical classification (or "learning") method that attempts to segregate bodies of data into classes based on objective parameters present in the data. Classification methods can be either supervised or unsupervised. Examples of supervised and unsupervised classification processes are described in Jain, "Statistical Pattern Recognition: A Review", IEEE Transactions on Pattern Analysis and Machine Intelligence, Vol. 22, No. 1, January 2000, the teachings of which are incorporated by reference.

[0102] In supervised classification, training data containing examples of known categories are presented to a learning mechanism, which learns one or more sets of relationships that define each of the known classes. New data can then be applied to the learning mechanism, which then classifies the new data using the learned relationships. Examples of supervised classification processes include linear regression processes (*e.g.*, multiple linear regression (MLR), partial least squares (PLS) regression and principal

components regression (PCR)), binary decision trees (*e.g.*, recursive partitioning processes such as CART - classification and regression trees), artificial neural networks such as back propagation networks, discriminant analyses (*e.g.*, Bayesian classifier or Fischer analysis), logistic classifiers, and support vector classifiers (support vector machines).

[0103] A preferred supervised classification method is a recursive partitioning process. Recursive partitioning processes use recursive partitioning trees to classify spectra derived from unknown samples. Further details about recursive partitioning processes are provided in U.S. Patent Application No. 2002 0138208 A1 to Paulse *et al.*, "Method for analyzing mass spectra."

[0104] In other aspects, the classification models that are created can be formed using unsupervised learning methods. Unsupervised classification attempts to learn classifications based on similarities in the training data set, without pre-classifying the spectra from which the training data set was derived. Unsupervised learning methods include cluster analyses. A cluster analysis attempts to divide the data into "clusters" or groups that ideally should have members that are very similar to each other, and very dissimilar to members of other clusters. Similarity is then measured using some distance metric, which measures the distance between data items, and clusters together data items that are closer to each other. Clustering techniques include the MacQueen's K-means algorithm and the Kohonen's Self-Organizing Map algorithm.

[0105] Learning algorithms asserted for use in classifying biological information are described, for example, in PCT International Publication No. WO 01/31580 (Barnhill *et al.*, "Methods and devices for identifying patterns in biological systems and methods of use thereof"), U.S. Patent Application No. 2002 0193950 A1 (Gavin *et al.*, "Method or analyzing mass spectra"), U.S. Patent Application No. 2003 0004402 A1 (Hitt *et al.*, "Process for discriminating between biological states based on hidden patterns from biological data"), and U.S. Patent Application No. 2003 0055615 A1 (Zhang and Zhang, "Systems and methods for processing biological expression data").

[0106] The classification models can be formed on and used on any suitable digital computer. Suitable digital computers include micro, mini, or large computers using any standard or specialized operating system, such as a Unix, Windows™ or Linux™ based operating system. The digital computer that is used can be physically separate from the mass

spectrometer that is used to create the spectra of interest, or it can be coupled to the mass spectrometer.

[0107] The training data set and the classification models according to aspects of the invention can be embodied by computer code that is executed or used by a digital computer. The computer code can be stored on any suitable computer readable media including optical or magnetic disks, sticks, tapes, and the like, and can be written in any suitable computer programming language including C, C++, visual basic, and the like

[0108] The learning algorithms described above are useful both for developing classification algorithms for the biomarkers already discovered, and for finding new biomarkers for Leishmaniasis. The classification algorithms, in turn, form the base for diagnostic tests by providing diagnostic values (*e.g.*, cut-off points) for biomarkers used singly or in combination.

5. COMPOSITIONS OF MATTER

[0109] In another aspect, this invention provides compositions of matter based on the biomarkers of this invention.

[0110] In some aspects, this invention provides biomarkers of this invention in purified form. Purified biomarkers have utility as antigens to raise antibodies. Purified biomarkers also have utility as standards in assay procedures. As used herein, a "purified biomarker" is a biomarker that has been isolated from other proteins and peptides, and/or other material from the biological sample in which the biomarker is found. Biomarkers can be purified using any method known in the art, including, but not limited to, mechanical separation (*e.g.*, centrifugation), ammonium sulphate precipitation, dialysis (including size-exclusion dialysis), size-exclusion chromatography, affinity chromatography, anion-exchange chromatography, cation-exchange chromatography, and metal-chelate chromatography. Such methods can be performed at any appropriate scale, for example, in a chromatography column, or on a biochip.

[0111] In some aspects, this invention provides a biospecific capture reagent, optionally in purified form, that specifically binds a biomarker of this invention. In some aspects, the biospecific capture reagent is an antibody. Such compositions are useful for detecting the biomarker in a detection assay, *e.g.*, for diagnostics.

[0112] In some aspects, this invention provides an article comprising a biospecific capture reagent that binds a biomarker of this invention, wherein the reagent is bound to a

solid phase. For example, this invention contemplates a device comprising bead, chip, membrane, monolith or microtiter plate derivatized with the biospecific capture reagent. Such articles are useful in biomarker detection assays.

[0113] In another aspect this invention provides a composition comprising a biospecific capture reagent, such as an antibody, bound to a biomarker of this invention, the composition optionally being in purified form. Such compositions are useful for purifying the biomarker or in assays for detecting the biomarker.

[0114] In some aspects, this invention provides an article comprising a solid substrate to which is attached an adsorbent, *e.g.*, a chromatographic adsorbent or a biospecific capture reagent, to which is further bound a biomarker of this invention. In some aspects, the article is a biochip or a probe for mass spectrometry, *e.g.*, a SELDI probe. Such articles are useful for purifying the biomarker or detecting the biomarker.

6. KITS FOR DETECTION OF BIOMARKERS FOR LEISHMANIASIS

[0115] In another aspect, the present invention provides kits for qualifying Leishmaniasis status, which kits are used to detect biomarkers according to the invention. In some aspects, the kit comprises a solid support, such as a chip, a microtiter plate or a bead or resin having a capture reagent attached thereon, wherein the capture reagent binds a biomarker of the invention. Thus, for example, the kits of the present invention can comprise mass spectrometry probes for SELDI, such as ProteinChip[®] arrays. In the case of biospecific capture reagents, the kit can comprise a solid support with a reactive surface, and a container comprising the biospecific capture reagent.

[0116] The kit can also comprise a washing solution or instructions for making a washing solution, in which the combination of the capture reagent and the washing solution allows capture of the biomarker or biomarkers on the solid support for subsequent detection by, *e.g.*, mass spectrometry. The kit can include more than type of adsorbent, each present on a different solid support.

[0117] In a further aspect, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions can inform a consumer about how to collect the sample, how to wash the probe or the particular biomarkers to be detected.

[0118] In some aspects, the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

7. USE OF BIOMARKERS FOR LEISHMANIASIS IN SCREENING ASSAYS AND METHODS OF TREATING LEISHMANIASIS

[0119] The methods of the present invention have other applications as well. For example, the biomarkers can be used to screen for compounds that modulate the expression of the biomarkers *in vitro* or *in vivo*, which compounds in turn can be useful in treating or preventing Leishmaniasis in patients. In another example, the biomarkers can be used to monitor the response to treatments for Leishmaniasis. In yet another example, the biomarkers can be used in heredity studies to determine if the subject is at risk for developing Leishmaniasis.

[0120] Thus, for example, the kits of this invention could include a solid substrate having a hydrophobic function, such as a protein biochip (*e.g.*, a CIPHERGEN H50 ProteinChip array, *e.g.*, ProteinChip array) and a sodium acetate buffer for washing the substrate, as well as instructions providing a protocol to measure the biomarkers of this invention on the chip and to use these measurements to diagnose Leishmaniasis.

[0121] Compounds suitable for therapeutic testing can be screened initially by identifying compounds which interact with one or more biomarkers listed in Table 1 and 5. By way of example, screening might include recombinantly expressing a biomarker listed in Table 1 and 5, purifying the biomarker, and affixing the biomarker to a substrate. Test compounds would then be contacted with the substrate, typically in aqueous conditions, and interactions between the test compound and the biomarker are measured, for example, by measuring elution rates as a function of salt concentration. Certain proteins can recognize and cleave one or more biomarkers of Table 1 and 5, in which case the proteins can be detected by monitoring the digestion of one or more biomarkers in a standard assay, *e.g.*, by gel electrophoresis of the proteins.

[0122] In a related aspect, the ability of a test compound to inhibit the activity of one or more of the biomarkers of Table 1 and 5 can be measured. One of skill in the art will recognize that the techniques used to measure the activity of a particular biomarker will vary depending on the function and properties of the biomarker. For example, an enzymatic activity of a biomarker can be assayed provided that an appropriate substrate is available and provided that the concentration of the substrate or the appearance of the reaction product is readily measurable. The ability of potentially therapeutic test compounds to inhibit or enhance the activity of a given biomarker can be determined by measuring the rates of

catalysis in the presence or absence of the test compounds. The ability of a test compound to interfere with a non-enzymatic (*e.g.*, structural) function or activity of one of the biomarkers of Table 1 and 5 can also be measured. For example, the self-assembly of a multi-protein complex which includes one of the biomarkers of Table I can be monitored by spectroscopy in the presence or absence of a test compound. Alternatively, if the biomarker is a non-enzymatic enhancer of transcription, test compounds which interfere with the ability of the biomarker to enhance transcription can be identified by measuring the levels of biomarker-dependent transcription *in vivo* or *in vitro* in the presence and absence of the test compound.

[0123] Test compounds capable of modulating the activity of any of the biomarkers of Table 1 and 5 can be administered to patients who are suffering from or are at risk of developing Leishmaniasis. For example, the administration of a test compound which increases the activity of a particular biomarker can decrease the risk of Leishmaniasis in a patient if the activity of the particular biomarker *in vivo* prevents the accumulation of proteins for Leishmaniasis. Conversely, the administration of a test compound which decreases the activity of a particular biomarker can decrease the risk of Leishmaniasis in a patient if the increased activity of the biomarker is responsible, at least in part, for the onset of Leishmaniasis.

[0124] In an additional aspect, the invention provides a method for identifying compounds useful for the treatment of disorders such as Leishmaniasis which are associated with increased levels of modified forms of the biomarkers in Table 1 and 5. For example, in some aspects, cell extracts or expression libraries can be screened for compounds which catalyze the cleavage of a full-length biomarker to form truncated forms of the biomarker. In some aspects of such a screening assay, cleavage of the biomarker can be detected by attaching a fluorophore to the biomarker which remains quenched when the biomarker is uncleaved but which fluoresces when the protein is cleaved. Alternatively, a version of full-length biomarker modified so as to render the amide bond between amino acids x and y uncleavable can be used to selectively bind or "trap" the cellular protease which cleaves full-length biomarker at that site *in vivo*. Methods for screening and identifying proteases and their targets are well-documented in the scientific literature, *e.g.*, in Lopez-Otin *et al.* (Nature Reviews, 3:509-519 (2002)).

[0125] In some aspects, the invention provides a method for treating or reducing the progression or likelihood of a disease, *e.g.*, Leishmaniasis, which is associated with the increased levels of a truncated biomarker. For example, after one or more proteins have been

identified which cleave the full-length biomarker, combinatorial libraries can be screened for compounds which inhibit the cleavage activity of the identified proteins. Methods of screening chemical libraries for such compounds are well-known in art. See, *e.g.*, Lopez-Otin *et al.* (2002). Alternatively, inhibitory compounds can be intelligently designed based on the structure of the biomarker.

[0126] At the clinical level, screening a test compound includes obtaining samples from test subjects before and after the subjects have been exposed to a test compound. The levels in the samples of one or more of the biomarkers listed in Tables 1 or Table 5 can be measured and analyzed to determine whether the levels of the biomarkers change after exposure to a test compound. The samples can be analyzed by mass spectrometry, as described herein, or the samples can be analyzed by any appropriate means known to one of skill in the art. For example, the levels of one or more of the biomarkers listed in Tables 1, 2, or 3 can be measured directly by Western blot using radio- or fluorescently-labeled antibodies which specifically bind to the biomarkers. Alternatively, changes in the levels of mRNA encoding the one or more biomarkers can be measured and correlated with the administration of a given test compound to a subject. In a further aspect, the changes in the level of expression of one or more of the biomarkers can be measured using *in vitro* methods and materials. For example, human tissue cultured cells which express, or are capable of expressing, one or more of the biomarkers of Table 1 or Table 5 can be contacted with test compounds. Subjects who have been treated with test compounds will be routinely examined for any physiological effects which can result from the treatment. In particular, the test compounds will be evaluated for their ability to decrease disease likelihood in a subject. Alternatively, if the test compounds are administered to subjects who have previously been diagnosed with Leishmaniasis, test compounds will be screened for their ability to slow or stop the progression of the disease.

8. EXEMPLARY ASPECTS

[0127] It is understood that the exemplary aspects described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1. Biomarkers for Visceral Leishmaniasis

Methods

Sera

[0128] A total of 64 sera were available for this study which were split into set 1 (consisting of 21 pretreatment VL sera and 19 healthy controls from the same geographic region) used to find preliminary biomarkers and set 2 (24 pretreatment sera) as a validation set. The material was obtained from Dr Nirmal Baral, Koirala Institute, Nepal.

[0129] Samples were aliquoted (30 µl/tube) and stored at -20°C until further use. All fractionated serum samples had been thawed a maximum of 3 times between collection and analysis.

[0130] The following clinical information was available from the positive samples: duration of fever and cough, hepatomegaly (size), splenomegaly (size), BM aspirate, albumin level, bilirubin level and Hb.

Fractionation and Binding of Sera

[0131] For fractionation, Cypherger's Expression Difference Mapping™ Kit- Serum Fractionation was used allowing high throughput processing on anion exchange beads in a 96-well microplate format. To increase the number of proteins visualized, sera were separated into six different fractions (pH 9+ flow through, pH7, pH5, pH4, pH3, and organic wash). This fractionation procedure significantly increases the number of peaks detectable from each sample (Fung and Enderwick, 2002). For the anion exchange fractionation, we used 20 µl of serum and 30 µl of U9 buffer as starting material according to the CIPHERGEN protocols.

[0132] Pretreatment sera and controls were bound on 2 different array surfaces (ProteinChip™: Ciphergen Biosystems Inc.), the weak cation exchange chip (CM10) and immobilized metal affinity capture coupled with copper (IMAC-Cu²⁺) chip arrays.

[0133] To find the optimal fractions for each array type (CM10 and IMAC30), all 6 fractions from 8 randomly chosen sera (pretreatment and controls) were loaded on both array surfaces (ProteinChip™: Ciphergen Biosystems Inc.). The best 3 fractions (that produced the highest number of peaks with good intensity) for each array type were chosen to be bound from all samples (on CM10 fraction 1, 3 and 4; on IMAC30 fraction 2, 5 and 6).

[0134] CM10 ProteinChip Arrays incorporate a negatively charged carboxylate chemistry that makes them act as a weak cationic exchanger. Their surface binds proteins that are positively charged at a given pH (from the product folder CM10 ProteinChip® Array, CIPHERGEN Biosystems, Inc.). IMAC30 ProteinChip Arrays reversibly bind proteins to the surface through a coordinated metal interaction. These ProteinChip Arrays incorporate Nitrilotriacetic Acid (NTA) groups and form stable octahedral complexes with polyvalent metal ions including Cu^{2+} , Ni^{2+} , Fe^{3+} , Ga^{3+} , and the like. After applying the desired metal ion to the array surface, two free sites from the formed octahedral complex can interact with specific amino acid residues (such as histidine) or post-translational modifications such as phosphate groups (from the product folder IMAC30 ProteinChip® Array, CIPHERGEN Biosystems Inc.).

[0135] For ProteinChip Array binding, the spots on the IMAC arrays were preloaded with 50 μl CuSO_4 (0.1 M). The CM10 and IMAC arrays were equilibrated with 150 μl binding buffer. 10 μl of each fraction was diluted in 90 μl of specific binding buffer, added to each well of the bioprocessor and mixed for 30 minutes with vigorous shaking on a MicroMix. After removing the remaining sample, the arrays were washed and mixed three times with 150 μl binding buffer for 5 min followed by two short washes with 150 μl de-ionized water. All steps were performed at room temperature.

[0136] An energy absorbing matrix containing sinapinic acid (SPA) was prepared according to the recommendations of the manufacturer [5 mg/vial dissolved in 400 μl solution (200 μl Acetonitrile; 200 μl of 1% Trifluoroacetic Acid in HPLC grade H_2O)] and, after the arrays were dry, 1 μl SPA solution applied on each spot. This was repeated once and the ProteinChip arrays were stored at room temperature in the dark until used.

Reading of the Arrays

[0137] The arrays were read on a Protein Biosystem IIc (PBS IIc) instrument, which was calibrated externally using the Protein MW standards kit E100-0001 (CIPHERGEN Biosystems) loaded on an NP-20 array. Each array was run twice with two different laser intensities to achieve better resolution for low- and high-molecular-mass proteins.

Data Analysis

[0138] Serum protein profiles of visceral leishmaniasis patients and healthy controls were compared with each other to detect biomarkers for visceral leishmaniasis. Peaks were first auto detected using CIPHERGEN Express Data manager 2.1 [1st pass analysis calculates p-

values ($p < 0.05$) and ROC (Receiver Operator Characteristic: calculation used to indicate how good the peak is to distinguish between infected and non-infected samples) from autodetected peaks] and each peak visually inspected for the 2nd pass analysis (p -values < 0.001 , recalculates p -values and ROC from the peaks manually relabeled).

[0139] Spectra were baseline subtracted and normalized to the total ion current from 2000 Da to 100 000 Da (low energy settings) and 10 000 Da to 200 000 Da (high energy settings). Spectra that were not within the "twice the average" QC rule were deleted. For peak cluster detection EDM (expression difference mapping) analysis was performed. Peaks were autodetected in a 0.3% cluster mass window for low energy settings and 2% cluster mass window for high energy settings. Biomarker Patterns Software (BPS; Ciphergen Biosystems) was used to generate diagnostic classification trees (algorithms) for patients and controls.

Identification

[0140] 80 μ l of the fractioned sample that showed the highest concentration of targeted candidate biomarkers as well as 80 μ l of the fractionated control sample that showed the lowest concentration of the same particular biomarker were desalted and 10 μ l of each sample loaded on a 12% SDS-PAGE Bis-Tris gel. Each positive sample was compared to its control. Gels were fixed with fixer solution for 2 hours and afterwards stained with Coomassie overnight to visualize the bands. De-staining with distilled water was performed until the bands were clearly visible.

Sequencing of the Proteins

[0141] Bands were cut from the gels and sequenced using LC-MS-MS using procedures known in the art, followed by a Mascot database search.

Results

[0142] The tested patients consisted of 25 men and 20 women with an age between 10 and 50 years. Their liver measured between 2 and 10 cm, the spleen between 3 and 25 cm.

[0143] Table 8 shows the molecular weights of all 212 biomarkers found in pretreatment samples of visceral leishmaniasis patients after the 2nd pass analysis. Highlighted molecular weights indicate proteins that were additionally detected in the BPS analysis (for decision tree building).

F1CL	F1CH	F3CL	F3CH	F4CL	F4CH	F2IL	F5IL	F5IH	F6IL	F6IH
2898	10406	3266	15211	3371		2502	4746	12299	3168	10959
2960	10432	3539	28474		14708	2726		12333		11053
3248	10456			4139	14870	3538			3582	11074
3372	10488	4088		4531	16626	3549	13917	12678	3599	11128
3518	10607	4156		6018	16736	3582	33646	12735	4261	11161
	10536	4302		13901	16827	3743	66992	14586	4293	11730
3893	11797	4847		16431	17339	3749	71918	14703	4363	12238
4301	12324	4870			17482	4286		15675	4474	12307
4388	12437	5585			17591	4303		26333	4644	12393
4525	12586	5755		51774	19605	4617		49192	4631	
4593	12793	5912		52093	26371	4852		49982	4745	12584
4806	15914			81850	26535			50581	5073	12625
4826	16022				29387				6200	12666
4851	16139				50020			51555	6406	12786
5176	16626				51367			52076	7285	12851
5192	16850				56942			52721	7629	12931
5264					80097			54017	7950	13004
6394	29246				81845			66719	11551	13076
15152	32326				117781			108685	11740	13210
15890	33582							133227	13785	14711
17762	38039								13907	14883
17894	39879								14094	15358
18584	41774								15178	15558
39926	44534								15370	16626
44341	55196								15905	16767
	68000								16113	16825
	59261								17317	28543
	66549								28184	29326
	79207								29018	50252
	124504								50985	50627
	157018								51355	51218
									53784	59346
									60791	60195
									62383	61361
									91819	89621
										94758
										133020

Table 2: 212 biomarkers detected in pretreatment samples of visceral leishmaniasis patients after 2nd pass analysis. Highlighted MW's indicate markers also found in BPS.

F = fraction

C = CM10

I = IMAC30

L = low energy settings

H = high energy settings

[0144] Altogether 212 preliminary biomarkers (p-value < 0.001) were detected after the 2nd pass analysis using Ciphergen Express. Molecular weights ranged between 2.5 kDa and 157 kDa. The greatest number of biomarkers was found in fraction 6 bound on an IMAC30 array read under high energy settings (see table 8). 15 of the 212 biomarkers were also detected using BPS (indicated with highlighted MW in table 8).

Cluster Plots

[0145] The cluster plots in Figures 7-8 illustrate the ability of candidate biomarkers to distinguish between the positive and negative samples. Figure 1 shows a m/z scatter plot of the 3.3 kDa biomarker found in F6IL of pretreatment sera from VL cases, where each data point represents one serum sample. The scatter plot distinguishes successfully between the positive (pretreatment) and negative (control) samples (ROC value of 0.98). The p-value is very low, and the intensity of the protein is relatively high (the 3.3 kDa biomarker had the highest overall intensity). Fig. 8 shows levels of the 3.3 kDa biomarker as measured by SDS-PAGE, and illustrates the substantial difference in expression levels between the pretreatment and control groups.

[0146] Fig. 9 shows an m/z scatter plot of the biomarker with a MW of 12463 Da found in F6IH, which successfully distinguishes between the positive (pretreatment) and negative (control) samples (ROC value of 0.98) with a very low p-value and a high signal intensity.

Classification Trees (Biomarker Pattern Software BPS)

[0147] Biomarker Patterns™ Software is a Windows-based package for supervised classification of SELDI mass spectral data sets derived from the Ciphergen ProteinChip® platform. It identifies proteins that are relevant to a particular disease and summarizes and displays the results in a clear way. With this program it is possible to discover protein multi-markers and to translate them into assays with high predictive accuracy.

[0148] Figure 4 shows a classification tree that combines three biomarkers to correctly classify all 21 positive sera from the 19 controls. The first biomarker (MW of 51,351 Da) correctly classifies 17 out of 21 positive samples, the second biomarker (MW of 28,238 Da) correctly classifies an additional 3 positive samples (out of the remaining 4), and finally the third biomarker (MW of 3379 Da) correctly classifies the remaining positive sample. Thus, by combining the 3 biomarkers it is possible to successfully distinguish between all 21 positive and 19 negative samples.

[0149] Figure 5 shows a decision tree for the 12.4 kDa biomarker. If the intensity of the 12.4 kDa biomarker is ≤ 0.155 , all 18 control sera are correctly classified as negative, and if the protein intensity is > 0.155 all 20 sera from visceral leishmaniasis patients are correctly classified positive. Thus, it is possible using only the 12.4 kDa biomarker to correctly classify all positive samples from the negative controls.

Correlation between Peak Intensity and Clinical Data

[0150] No correlation between the clinical data (duration of fever, cough, hepatomegaly, splenomegaly, BM aspirate, albumin, bilirubin, Hb) and the peak intensities of any biomarker was detectable.

Identification

[0151] The 15 potential biomarkers detected in the 2nd pass analysis (using CiphergenExpress) and BPS were isolated and purified using SDS-PAGE and identified with LC-MS-MS. Four biomarkers are visible on the gels shown in Figures 6 and 7 (2 down-regulated proteins: 28.2 kDa and 14.5 kDa, and 2 up-regulated proteins: 51.1 kDa and 12.4 kDa, see Table 2). The gel of Fig. 6 shows 5 targeted biomarkers (MW: 27,603 Da, 51,327 Da, 28,238 Da, 51,351 Da and 14,580 Da) with their associated controls (C). 2 biomarkers (indicated by boxes on the gel) could be visualized. The gel of Fig. 7 shows 5 targeted biomarkers (9,309 Da, 9,478 Da, 12,610 Da, 51,111 Da and 12,463 Da) with their associated controls (C). As in Fig. 6, 2 biomarkers (indicated with boxes and upregulated in the positive sera) were detected on the gel. The band in the 12.4 kDa range was faint but clearly visible on the original gel. Sequencing of the 51.1 kDa candidate biomarker revealed that it likely represents kininogen 1 precursor. The paired samples used to visualize biomarkers in Figure 6 and Figure 7 also show several additional differences in expression levels.

Pretreatment Sera Visceral Leishmaniasis										
F1		F3		F4		F2	F5		F6	
CL	CH	CL	CH	CL	CH	IL	IL	IH	IL	IH
3534	27603	3562	51327	3379	14580	5103	9309	12610	3375	12463
				28238			9478	51111		
				51351						

Table 3: Targeted and visible biomarkers on gels (the numbers represent the MWs of the proteins; highlighted/boldface numbers, indicate the visible markers).

Validation of the biomarkers

[0152] To validate the 15 candidate biomarkers (found in sample set 1 using 2nd pass analysis/BPS) we tested the remaining 24 pretreatment samples (under the same settings as set 1) and obtained substantially identical MW measurements across all fractions (Dalton shift max +/- 3% when the second set was run on a different day). 14 from 15 preliminary biomarkers in set 1 were also detected in sample set 2. Only one (down-regulated) protein

with a MW of 9478 Da (found in set 1 in F5IL) was undetectable in the 2nd sample set.. To validate the 15 candidate biomarkers (found in sample set 1 using 2nd pass analysis/BPS) we tested the remaining 24 pretreatment samples (under the same settings as set 1) and got across all fractions a maximum Dalton shift of $\pm 1\%$ when the second set was run on a different day (see Table 4 below).

F1CL		F1CH		F3CL		F3CH		F4CL		F4CH	
1	2	1	2	1	2	1	2	1	2	1	2
3534	3538	27603	28068	3562	3564	51327	51572	3379	3375	14580	14589
								28238	28235		
								51351	51549		

F2IL		F5IL		F5IH		F6IL		F6IH	
1	2	1	2	1	2	1	2	1	2
5103	5097	9309	8970	12610	12612	3375	3374	12463	12476
		9478	n.d.	51111	51616				

Table 4: Molecular weights of the biomarkers detected in sample set 1 and 2 (validation).

1: MW of biomarkers found in sample set 1

2: MW of biomarkers found in sample set 2 (validation)

n.d.: not detected

Table 5
Identification of Certain Exemplary Biomarkers

Molecular Weight m/z Avg (kDa)	Fraction	Protein Identity
9.3	F5ISL	unnamed protein product
9.4	F5ISL	apolipoprotein AI, apo AI [human, spleen, Peptide Mutant, 88 aa]
12.4	F6ISH	NADH dehydrogenase (ubiquinone) 1 beta subcomplex
12.6	F5ISH	Serum amyloid A protein precursor (SAA) [Contains: Amyloid protein A (Amyloid fibril protein AA);
14.5	F4CSH	Chain B, Alpha-Ferrous-Carbonmonoxy, Beta-Cobaltous-Deoxy Hemoglobin
27.6	F1CSH	alpha-1-acid glycoprotein 1 precursor
28.2	F4CSL	lipoprotein Gln I
51.1	F5ISH	Kininogen-1 precursor
51.3	F4CSH	antithrombin III

Significant hits:

gi1911815 antitubulin IgG1 kappa VL chain (N-terminal) [human, serum, immunocytic sarcom patient PER isolate,
gi4389275 Chain , Human Serum Albumin In A Complex With Myristic Acid And Tri-Iodobenzoic Acid
gi106635 Ig lambda chain - human
gi3169770 immunoglobulin kappa light chain [Homo sapiens]
gi223373 complex-forming glycoprotein HC
gi197102 (V-J). Ig heavy chain V-region
gi229536 Ig A L
gi841148 immunoglobulin kappa light chain

2. **gi1911815** Mass: 24028 Score: 203 Queries matched: 3
 antitubulin IgG1 kappa VL chain (N-terminal) [human, serum, immunocytic sarcom patient PER isolate,
 Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide

✓ 47 652.20 1302.39 1302.61 -0.22 0 87 7.9e-07 1 R.FSGSGSGCTDFTLK.I
 ✓ 54 751.75 1501.49 1501.75 -0.26 0 77 8.4e-06 1 K.DSTYSLSSTLTLSK.A
 ✓ 54 973.34 1944.66 1945.02 -0.36 0 39 0.037 1 R.TVAAPSVFIFPPSDEQLK.S

gi223373 Mass: 20592 Score: 102 Queries matched: 2
 complex-forming glycoprotein HC

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide
 ✓ 33 511.17 1020.33 1020.52 -0.19 0 55 0.0016 1 R.ETLLQDFR.V
 ✓ 68 1010.83 2019.64 2019.99 -0.36 0 47 0.0054 1 R.VVAQGVGIPEDSIFTMADR.G + Oxidation (M)

Significant hits:

gi178775 proapolipoprotein
gi229479 lipoprotein Gln I
gi21669317 Immunoglobulin kappa light chain VLJ region
gi4389275 Chain , Human Serum Albumin In A Complex With Myristic Acid And Tri-Iodobenzoic Acid

gi|3212347 Chain L, Diels Alder Catalytic Antibody Germline Precursor
 gi|2533362 apolipoprotein AI, apo AI [human, spleen, Peptide Mutant, 88 aa]
 gi|3169770 immunoglobulin kappa light chain
 gi|229526 protein Rei,Bence-Jones
 gi|229528 protein Len,Bence-Jones
 gi|223373 complex-forming glycoprotein HC
 gi|3915607 Apolipoprotein A-I precursor (Apo-AI) (ApoA-I)
 gi|619383 apolipoprotein D, apoD [human, plasma, Peptide, 246 aa]
 gi|87890 Ig lambda chain precursor - human
 gi|106635 Ig lambda chain - human
 gi|229536 Ig A L
 gi|28317 unnamed protein product
 gi|21619848 IGL@ protein
 gi|197102 (V-J). Ig heavy chain V-region
 gi|841148 immunoglobulin kappa light chain
 gi|553734 putative [Homo sapiens]

gi|178775 Mass: 28944 Score: 765 Queries matched: 22

proapolipoprotein

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
42	506.70	1011.39	1011.57	-0.18	0	73	2.2e-05	1	K.AKPALEDLR.Q
48	516.17	1030.33	1030.51	-0.18	0	(55)	0.0016	1	K.LSPLGEEMR.D
54	524.16	1046.31	1046.51	-0.19	0	56	0.0012	1	K.LSPLGEEMR.D + Oxidation (M)
55	524.17	1046.33	1046.51	-0.18	0	(42)	0.028	1	K.LSPLGEEMR.D + Oxidation (M)
73	615.74	1229.46	1229.70	-0.24	0	(16)	11	1	R.QGLLPVLESFK.V
74	615.74	1229.46	1229.70	-0.24	0	(18)	7.2	1	R.QGLLPVLESFK.V
75	615.74	1229.47	1229.70	-0.23	0	(22)	2.6	1	R.QGLLPVLESFK.V
76	615.74	1229.47	1229.70	-0.23	0	58	0.00075	1	R.QGLLPVLESFK.V
78	618.23	1234.45	1234.68	-0.23	0	53	0.0023	1	K.DLATVYVDVLK.D
81	626.70	1251.38	1251.61	-0.23	0	15	11	1	K.VQPYLDDFQK.K
84	434.47	1300.40	1300.64	-0.24	0	63	0.00018	1	R.THLAPYSDEL.R.Q
85	651.21	1300.40	1300.64	-0.24	0	(51)	0.0031	1	R.THLAPYSDEL.R.Q
96	693.73	1385.44	1385.71	-0.27	0	(27)	0.69	1	K.VSFLSALEEYTK.K
97	693.73	1385.45	1385.71	-0.26	0	(58)	0.00052	1	K.VSFLSALEEYTK.K
98	693.74	1385.46	1385.71	-0.25	0	(78)	6e-06	1	K.VSFLSALEEYTK.K
99	693.74	1385.46	1385.71	-0.25	0	101	3.1e-08	1	K.VSFLSALEEYTK.K
100	700.71	1399.40	1399.66	-0.27	0	78	6.8e-06	1	R.DYVSQFEGSALGK.Q
101	700.72	1399.43	1399.66	-0.24	0	(50)	0.0043	1	R.DYVSQFEGSALGK.Q
107	488.20	1461.56	1461.84	-0.28	1	63	0.00019	1	R.VKDLATVYVDVLK.D
112	806.75	1611.49	1611.78	-0.29	0	104	1.3e-08	1	K.LLDNWDVSTSTFSK.L
132	966.80	1931.58	1931.93	-0.35	0	51	0.0022	1	R.EQLGPVTQEFWDNLEK.E
146	734.57	2200.69	2201.11	-0.43	1	51	0.0021	1	K.LREQLGPVTQEFWDNLEK.E

2. gi|229479 Mass: 28329 Score: 527 Queries matched: 18

lipoprotein Gln I

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
42	506.70	1011.39	1011.57	-0.18	0	73	2.2e-05	1	K.AKPALEDLR.Q
48	516.17	1030.33	1030.51	-0.18	0	(55)	0.0016	1	K.LSPLGEEMR.D
54	524.16	1046.31	1046.51	-0.19	0	56	0.0012	1	K.LSPLGEEMR.D + Oxidation (M)
55	524.17	1046.33	1046.51	-0.18	0	(42)	0.028	1	K.LSPLGEEMR.D + Oxidation (M)
68	613.67	1225.32	1225.54	-0.22	0	48	0.0074	1	-DEFFQSPWDR.V

73	615.74	1229.46	1229.70	-0.24	0	(16)	11	1	R.QGLLPVLESFK.V
74	615.74	1229.46	1229.70	-0.24	0	(18)	7.2	1	R.QGLLPVLESFK.V
75	615.74	1229.47	1229.70	-0.23	0	(22)	2.6	1	R.QGLLPVLESFK.V
76	615.74	1229.47	1229.70	-0.23	0	58	0.00075	1	R.QGLLPVLESFK.V
78	618.23	1234.45	1234.68	-0.23	0	53	0.0023	1	K.DLATVYVDVLK.D
81	626.70	1251.38	1251.61	-0.23	0	15	11	1	K.VQPYLDDFQK.K
84	434.47	1300.40	1300.64	-0.24	0	63	0.00018	1	R.THLAPYSDEL.R.Q
85	651.21	1300.40	1300.64	-0.24	0	(51)	0.0031	1	R.THLAPYSDEL.R.Q
96	693.73	1385.44	1385.71	-0.27	0	(27)	0.69	1	K.VSFLSALEEYTK.L
97	693.73	1385.45	1385.71	-0.26	0	(58)	0.00052	1	K.VSFLSALEEYTK.L
98	693.74	1385.46	1385.71	-0.25	0	(78)	6e-06	1	K.VSFLSALEEYTK.L
99	693.74	1385.46	1385.71	-0.25	0	101	3.1e-08	1	K.VSFLSALEEYTK.L
107	488.20	1461.56	1461.84	-0.28	1	63	0.00019	1	R.VKDLATVYVDVLK.D

gi3212347 Mass: 23894 Score: 349 Queries matched: 7

Chain L, Diels Alder Catalytic Antibody Germline Precursor

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
87	652.19	1302.37	1302.61	-0.24	0	61	0.00031	1	R.FSGSGSGTDFTLK.I
109	751.75	1501.48	1501.75	-0.27	0	74	1.7e-05	1	K.DSTYLSSTLTLSK.A
125	899.29	1796.56	1796.89	-0.33	0	79	4e-06	1	K.SGTASVVCLLNFFPR.E
129	625.87	1874.58	1874.92	-0.34	0	44	0.012	1	K.VYACEVTHQGLSSPVT.K.S
136	973.34	1944.66	1945.02	-0.36	0	42	0.019	1	R.TVAAPSVFIFPPSDEQLK.S
137	973.34	1944.67	1945.02	-0.35	0	(13)	15	1	R.TVAAPSVFIFPPSDEQLK.S
144	1068.30	2134.59	2134.96	-0.37	0	48	0.0037	1	K.VDNALQSGNSQESVTEQDSK.D

7. gi253362 Mass: 10155 Score: 319 Queries matched: 5

apolipoprotein AI, apo AI [human, spleen, Peptide Mutant, 88 aa]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
68	613.67	1225.32	1225.54	-0.22	0	48	0.0074	1	-DEPPQSPWDR.V
78	618.23	1234.45	1234.68	-0.23	0	53	0.0023	1	K.DLATVYVDVLK.D
107	488.20	1461.56	1461.84	-0.28	1	63	0.00019	1	R.VKDLATVYVDVLK.D
112	806.75	1611.49	1611.78	-0.29	0	104	1.3e-08	1	K.LLDNWDVSTSTFSK.R
132	966.80	1931.58	1931.93	-0.35	0	51	0.0022	1	R.EQLGPVTQEFWDNLEK.E

gi229526 Mass: 23779 Score: 240 Queries matched: 6

protein Rei, Bence-Jones

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
109	751.75	1501.48	1501.75	-0.27	0	74	1.7e-05	1	K.DSTYLSSTLTLSK.A
125	899.29	1796.56	1796.89	-0.33	0	79	4e-06	1	K.SGTASVVCLLNFFPR.E
130	630.54	1888.58	1888.94	-0.35	0	47	0.0059	1	K.VYACEVTHQGLSSPVT.K.S
135	649.22	1944.63	1945.02	-0.38	0	3	1.4e+02	2	R.TVAAPSVFIFPPSDQELK.S
136	973.34	1944.66	1945.02	-0.36	0	42	0.019	1	R.TVAAPSVFIFPPSDEQLK.S
137	973.34	1944.67	1945.02	-0.35	0	(13)	15	1	R.TVAAPSVFIFPPSDEQLK.S

12. gi229528 Mass: 24499 Score: 235 Queries matched: 6

protein Len, Bence-Jones

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
109	751.75	1501.48	1501.75	-0.27	0	74	1.7e-05	1	K.DSTYLSSTLTLSK.A
125	899.29	1796.56	1796.89	-0.33	0	79	4e-06	1	K.SGTASVVCLLNFFPR.E
129	625.87	1874.58	1874.92	-0.34	0	44	0.012	1	K.VYACEVTHQGLSSPVT.K.S
135	649.22	1944.63	1945.02	-0.38	0	3	1.4e+02	2	R.TVAAPSVFIFPPSDQELK.S
136	973.34	1944.66	1945.02	-0.36	0	42	0.019	1	R.TVAAPSVFIFPPSDEQLK.S
137	973.34	1944.67	1945.02	-0.35	0	(13)	15	1	R.TVAAPSVFIFPPSDEQLK.S

Significant hits:

- gi29446 unnamed protein product [Homo sapiens]
- gi229752 Chain B, Alpha-Ferrous-Carbonmonoxy, Beta-Cobaltous-Deoxy Hemoglobin (T State)
- gi179409 beta-globin
- gi62901559 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
- gi17942890 Chain A, An Engineered Transferrin Monomer That Is Non-Amyloidogenic - Unless Partially Denatured
- gi62901575 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
- gi443296 Chain A, Transferrin (Formerly Prealbumin)
- gi62901563 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
- gi230651 Chain A, Prealbumin (Human Plasma)
- gi229172 hemoglobin delta
- gi122427 Hemoglobin subunit alpha-A/Q/R/T (Hemoglobin alpha-A/Q/R/T chain) (Alpha-A/Q/R/T-globin)
- gi122711 Hemoglobin subunit delta (Hemoglobin delta chain) (Delta-globin)
- gi122710 Hemoglobin subunit delta (Hemoglobin delta chain) (Delta-globin)

gi122671 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
 gi122647 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
 gi122568 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
 gi229594 hemoglobin alpha
 gi229751 Chain A, Alpha-Ferrous-Carbonmonoxy, Beta-Cobaltous-Deoxy Hemoglobin (T State)
 gi122676 Hemoglobin subunit beta-1/2 (Hemoglobin beta-1/2 chain) (Beta-1/2-globin)
 gi122484 Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)
 gi28549 alpha globin [Homo sapiens]
 gi122472 Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)
 gi122450 Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)
 gi56122358 transthyretin
 gi122413 Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)
 gi227298 medullasin inhibitor 2
 gi122621 Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)
 gi75040810 Transthyretin precursor (Prealbumin)
 gi33694204 hemoglobin beta chain
 gi122725 Hemoglobin subunit epsilon (Hemoglobin epsilon chain) (Epsilon-globin)
 gi6685052 beta-globin [Callithrix jacchus]
 gi121032 Ig gamma-1 chain C region
 gi543794 Serum albumin precursor (Allergen Equ c 3)
 gi553734 putative [Homo sapiens]

1.	gi29446	Mass: 16070	Score: 733	Queries matched: 43						
	unnamed protein product [Homo sapiens]									
	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒	23	466.67	931.32	931.51	-0.19	0	45	0.018	1	K.SAVTAIWCK.V
☒	52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFRL
☒	53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFRL
☒	54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
☒	55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
☒	56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
☒	66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
☐	67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
☒	68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
☒	69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
☒	70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF
☒	71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQRF
☒	72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQRF
☒	73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
☒	74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
☒	75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
☒	81	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
☒	82	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
☒	83	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
☒	84	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
☒	96	689.71	1377.41	1377.69	-0.28	0	54	0.0018	1	K.EFTPPVQAAYQK.V
☒	106	493.46	1477.37	1477.69	-0.32	0	(72)	2.2e-05	1	K.GTFATLSELHCDK.L
☒	107	739.70	1477.39	1477.69	-0.30	0	83	2e-06	1	K.GTFATLSELHCDK.L
☒	121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDGLAHLNLIK.G

✓	122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLHLNLK.G
✓	123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLHLNLK.G
✓	124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLHLNLK.G
✓	125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLHLNLK.G
✓	126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLHLNLK.G
✓	130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
✓	131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
✓	136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLHLNLK.G
✓	137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLHLNLK.G
✓	146	1029.77	2057.52	2057.94	-0.42	0	(39)	0.032	1	R.FFESFGDLSTPDVAVMGPNK.V
✓	147	692.18	2073.51	2073.94	-0.43	0	(44)	0.01	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	148	1037.76	2073.51	2073.94	-0.43	0	(48)	0.0042	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	149	1037.76	2073.51	2073.94	-0.43	0	74	1.2e-05	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	150	1037.77	2073.52	2073.94	-0.42	0	(22)	1.5	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	151	1037.77	2073.52	2073.94	-0.42	0	(57)	0.00051	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	152	1037.77	2073.52	2073.94	-0.42	0	(56)	0.00073	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	153	1037.77	2073.52	2073.94	-0.42	0	(54)	0.0011	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
✓	154	1037.77	2073.53	2073.94	-0.41	0	(48)	0.0039	1	R.FFESFGDLSTPDVAVMGPNK.V + Oxidation (M)
	161	743.91	2228.71	2229.10	-0.39	1	2	1.5e+02	5	K.SAVTADWGKYNVDEVGGEALGR.L

2. [gi229752](#) Mass: 15971 Score: 733 Queries matched: 42
Chain B, Alpha-Ferrous-Carbonmonoxy, Beta-Cobaltous-Deoxy Hemoglobin (T State)

✓ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
23	466.67	931.32	931.51	-0.19	0	45	0.018	1	K.SAVTALWGK.V
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
81	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
96	689.71	1377.41	1377.69	-0.28	0	54	0.0018	1	K.EFTPPVQAAYQK.V
106	493.46	1477.37	1477.69	-0.32	0	(72)	2.2e-05	1	K.GTFATLSELHCDK.L
107	739.70	1477.39	1477.69	-0.30	0	83	2e-06	1	K.GTFATLSELHCDK.L
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLHLNLK.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLHLNLK.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLHLNLK.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLHLNLK.G

125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNLIK.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNLIK.G
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNLIK.G
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLGLAHLNLIK.G
146	1029.77	2057.52	2057.94	-0.42	0	(39)	0.032	1	R.FFESFGDLSTPDVAVMGNPK.V
147	692.18	2073.51	2073.94	-0.43	0	(44)	0.01	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
148	1037.76	2073.51	2073.94	-0.43	0	(48)	0.0042	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
149	1037.76	2073.51	2073.94	-0.43	0	74	1.2e-05	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
150	1037.77	2073.52	2073.94	-0.42	0	(22)	1.5	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
151	1037.77	2073.52	2073.94	-0.42	0	(57)	0.00051	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
152	1037.77	2073.52	2073.94	-0.42	0	(56)	0.00073	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
153	1037.77	2073.52	2073.94	-0.42	0	(54)	0.0011	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)
154	1037.77	2073.53	2073.94	-0.41	0	(48)	0.0039	1	R.FFESFGDLSTPDVAVMGNPK.V + Oxidation (M)

3. gil179402 Mass: 15984 Score: 560 Queries matched: 20

beta-globin

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
23	466.67	931.32	931.51	-0.19	0	45	0.018	1	K.SAVTALWGK.V
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
96	689.71	1377.41	1377.73	-0.32	1	54	0.0018	1	K.EFTPPVKAAYQK.V
97	690.21	1378.41	1378.68	-0.27	0	29	0.53	1	K.EFTPPVKAAYQK.V
106	493.46	1477.37	1477.69	-0.32	0	(72)	2.2e-05	1	K.GTFATLSELHCDK.L
107	739.70	1477.39	1477.69	-0.30	0	83	2e-06	1	K.GTFATLSELHCDK.L
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNLIK.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNLIK.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNLIK.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNLIK.G
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNLIK.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNLIK.G
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNLIK.G
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLGLAHLNLIK.G

4. gil62901559 Mass: 16160 Score: 547 Queries matched: 34

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
81	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
88	664.70	1327.39	1327.67	-0.28	0	61	0.00032	1	K.VNVDEVGGEALGR.L
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNLIK.S
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNLIK.S
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNLIK.S
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNLIK.S
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNLIK.S
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNLIK.S
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNLIK.S

137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDGLAHLNLIK.S
147	692.18	2073.51	2073.94	-0.43	0	(2)	1.8e+02	6	R.FFESFGDLSSPDVMSNP.K.V
151	1037.77	2073.52	2073.94	-0.42	0	8	45	3	R.FFESFGDLSSPDVMSNP.K.V
152	1037.77	2073.52	2073.94	-0.42	0	(2)	1.6e+02	4	R.FFESFGDLSSPDVMSNP.K.V
154	1037.77	2073.53	2073.94	-0.41	0	(5)	84	5	R.FFESFGDLSSPDVMSNP.K.V

5. gi17942890 Mass: 13812 Score: 489 Queries matched: 11

Chain A, An Engineered Transthyretin Monomer That Is Non-Amyloidogenic - Unless Partially Denatured

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide

✓	23	456.16	1365.46	1365.75	-0.29	0	(50)	0.0036	1	R.GSPAINVAVHVFR.K
✓	24	683.74	1365.47	1365.75	-0.28	0	98	5.9e-08	1	R.GSPAINVAVHVFR.K
✓	99	697.67	1393.33	1393.61	-0.29	0	(47)	0.0078	1	K.AADDTWEPFASGK.T
✓	100	697.68	1393.34	1393.61	-0.28	0	62	0.00025	1	K.AADDTWEPFASGK.T
✓	108	498.85	1493.53	1493.85	-0.31	1	56	0.00095	1	R.GSPAINVAVHVFR.K.A
✓	110	508.14	1521.40	1521.71	-0.31	1	(42)	0.024	1	R.KAADDTWEPFASGK.T
✓	111	761.71	1521.40	1521.71	-0.31	1	107	6.4e-09	1	R.KAADDTWEPFASGK.T
✓	173	613.42	2449.67	2450.16	-0.50	0	(38)	0.032	1	K.ALGISPMHEHAEEVFTANDSGPR.R + Oxidation (M)
✓	174	817.57	2449.68	2450.16	-0.48	0	88	3.2e-07	1	K.ALGISPMHEHAEEVFTANDSGPR.R + Oxidation (M)
✓	178	819.89	2453.65	2454.14	-0.50	0	(70)	2.2e-05	1	K.TSESSELHGLTTEEFVEGIYK.V
✓	179	1227.84	2453.66	2454.14	-0.48	0	77	3.8e-06	1	K.TSESSELHGLTTEEFVEGIYK.V

6. gi62901575 Mass: 16147 Score: 462 Queries matched: 29

Homoglobin subunit beta (Homoglobin beta chain) (Beta-globin)

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide

52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
81	657.68	1313.35	1313.66	-0.31	0	(47)	0.0079	3	K.VNVEDVGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(35)	0.12	3	K.VNVEDVGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(36)	0.11	3	K.VNVEDVGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	49	0.0049	3	K.VNVEDVGGEALGR.L
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNLIK.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNLIK.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNLIK.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNLIK.G
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNLIK.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNLIK.G
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDGLAHLNLIK.Q
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDGLAHLNLIK.G

7. gi443295 Mass: 13810 Score: 451 Queries matched: 10

Chain A, Transthyretin (Formerly Prealbumin)

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide

23	456.16	1365.46	1365.75	-0.29	0	(50)	0.0036	1	R.GSPAINVAVHVFR.K
24	683.74	1365.47	1365.75	-0.28	0	98	5.9e-08	1	R.GSPAINVAVHVFR.K
99	697.67	1393.33	1393.61	-0.29	0	(47)	0.0078	1	K.AADDTWEPFASGK.T
100	697.68	1393.34	1393.61	-0.28	0	62	0.00025	1	K.AADDTWEPFASGK.T

<u>108</u>	498.85	1493.53	1493.85	-0.31	1	56	0.00095	1	R.GSPAINVAVHVFRK.A
<u>110</u>	508.14	1521.40	1521.71	-0.31	1	(42)	0.024	1	R.KAADDTWEPFASGK.T
<u>111</u>	761.71	1521.40	1521.71	-0.31	1	107	6.4e-09	1	R.KAADDTWEPFASGK.T
<u>170</u>	1180.38	2358.75	2359.23	-0.48	0	51	0.0018	1	R.YTIAALLSPYSYSTTAVVTNPK.E
<u>178</u>	818.89	2453.65	2454.14	-0.50	0	(70)	2.2e-05	1	K.TSESGELHGLTTEEFVEGIYK.V
<u>179</u>	1227.84	2453.66	2454.14	-0.48	0	77	3.8e-06	1	K.TSESGELHGLTTEEFVEGIYK.V

8. gi62901565 Mass: 16161 Score: 432 Queries matched: 28

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>52</u>	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
<u>53</u>	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
<u>54</u>	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
<u>55</u>	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
<u>56</u>	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
<u>66</u>	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
<u>67</u>	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
<u>68</u>	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
<u>69</u>	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
<u>70</u>	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
<u>71</u>	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
<u>72</u>	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
<u>73</u>	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
<u>74</u>	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
<u>75</u>	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
<u>81</u>	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
<u>82</u>	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
<u>83</u>	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
<u>84</u>	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
<u>121</u>	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDGLAHLNKLK.G
<u>122</u>	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDGLAHLNKLK.G
<u>123</u>	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDGLAHLNKLK.G
<u>124</u>	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDGLAHLNKLK.G
<u>125</u>	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDGLAHLNKLK.G
<u>126</u>	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDGLAHLNKLK.G
<u>128</u>	884.30	1766.58	1766.99	-0.41	0	16	7.9	1	R.LLGNVLVCVLAQHFGK.E
<u>136</u>	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDGLAHLNKLK.G
<u>137</u>	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDGLAHLNKLK.G

9. gi230651 Mass: 13809 Score: 420 Queries matched: 12

Chain A, Prealbumin (Human Plasma)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>93</u>	456.16	1365.46	1365.75	-0.29	0	(50)	0.0036	1	R.GSPAINVAVHVFRK.K
<u>94</u>	683.74	1365.47	1365.75	-0.28	0	98	5.9e-08	1	R.GSPAINVAVHVFRK.K
<u>99</u>	697.67	1393.33	1393.61	-0.29	0	(47)	0.0078	1	K.AADDTWEPFASGK.T
<u>100</u>	697.68	1393.34	1393.61	-0.28	0	62	0.00025	1	K.AADDTWEPFASGK.T
<u>108</u>	498.85	1493.53	1493.85	-0.31	1	56	0.00095	1	R.GSPAINVAVHVFRK.A
<u>110</u>	508.14	1521.40	1521.71	-0.31	1	(42)	0.024	1	R.KAADDTWEPFASGK.T
<u>111</u>	761.71	1521.40	1521.71	-0.31	1	107	6.4e-09	1	R.KAADDTWEPFASGK.T
<u>170</u>	1180.38	2358.75	2359.23	-0.48	0	51	0.0018	1	R.YTIAALLSPYSYSTTAVVTNPK.E
<u>175</u>	818.87	2453.60	2453.16	0.44	0	(25)	0.61	1	K.TSESGELHGLTTEEQFVEGIYK.V
<u>176</u>	818.88	2453.63	2453.16	0.47	0	46	0.0056	1	K.TSESGELHGLTTEEQFVEGIYK.V
<u>177</u>	818.88	2453.63	2453.16	0.47	0	(34)	0.073	1	K.TSESGELHGLTTEEQFVEGIYK.V

10. gi229172 Mass: 16011 Score: 392 Queries matched: 24

hemoglobin delta

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>52</u>	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
<u>53</u>	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
<u>54</u>	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
<u>55</u>	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
<u>56</u>	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
<u>66</u>	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
<u>67</u>	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
<u>68</u>	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
<u>69</u>	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
<u>70</u>	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
<u>71</u>	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
<u>72</u>	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F

73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
87	663.76	1325.51	1325.79	-0.27	0	41	0.03	1	R.LLGNVLVCVLAR.N
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNLK.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNLK.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNLK.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNLK.G
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNLK.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNLK.G
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNLK.G
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLGLAHLNLK.G

11. gi122427 Mass: 15159 Score: 379 Queries matched: 18

Hemoglobin subunit alpha-A/Q/R/T (Hemoglobin alpha-A/Q/R/T chain) (Alpha-A/Q/R/T-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFKL
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFKL
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFKL
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSLY
141	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPHFDLSHGSAQVK.G
142	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPHFDLSHGSAQVK.G
190	753.71	3010.82	3010.52	0.30	0	55	0.00052	1	K.VADALTAVGHVDDMPQALSALSDLHAHKL + Oxidation (M)
191	785.73	3138.89	3138.61	0.27	1	81	1e-06	2	K.KVADALTAVGHVDDMPQALSALSDLHAHKL + Oxidation (M)

Proteins matching the same set of peptides:

12. gi122711 Mass: 16037 Score: 376 Queries matched: 25

Hemoglobin subunit delta (Hemoglobin delta chain) (Delta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQRF
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQRF
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
81	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
87	663.76	1325.51	1325.79	-0.27	0	41	0.03	1	R.LLGNVLVCVLAR.N

121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNKL.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNKL.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNKL.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNKL.G
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNKL.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNKL.G
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNKL.G
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLGLAHLNKL.G

Hemoglobin subunit delta (Hemoglobin delta chain) (Delta-globin)

13. gil122710 Mass: 15867 Score: 371 Queries matched: 22

Hemoglobin subunit delta (Hemoglobin delta chain) (Delta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQRF
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQRF
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
87	663.76	1325.51	1325.79	-0.27	0	41	0.03	1	R.LLGNVLCVLAARN
88	664.70	1327.39	1327.67	-0.28	0	61	0.00032	1	K.VNVEEVGGEALGR.L
121	557.18	1668.52	1668.88	-0.37	0	(60)	0.00031	1	K.VLGAFSDDLGLAHLNKL.G
122	557.18	1668.53	1668.88	-0.36	0	83	1.5e-06	1	K.VLGAFSDDLGLAHLNKL.G
123	557.18	1668.53	1668.88	-0.35	0	(38)	0.052	1	K.VLGAFSDDLGLAHLNKL.G
124	557.18	1668.53	1668.88	-0.35	0	(61)	0.00027	1	K.VLGAFSDDLGLAHLNKL.G
125	835.27	1668.53	1668.88	-0.35	0	(78)	5.1e-06	1	K.VLGAFSDDLGLAHLNKL.G
126	557.19	1668.54	1668.88	-0.34	0	(59)	0.00043	1	K.VLGAFSDDLGLAHLNKL.G
136	599.87	1796.60	1796.98	-0.38	1	86	9.2e-07	1	K.KVLGAFSDDLGLAHLNKL.G
137	450.16	1796.60	1796.98	-0.38	1	(65)	0.00011	1	K.KVLGAFSDDLGLAHLNKL.G

14. gil122671 Mass: 16111 Score: 358 Queries matched: 19

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQRF
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQRF
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
88	664.70	1327.39	1327.67	-0.28	0	61	0.00032	1	K.VNVEEVGGEALGR.L
96	689.71	1377.41	1377.69	-0.28	0	54	0.0018	1	K.EFTPPVQAAYQK.V
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLCVLAHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLCVLAHFGK.E

15. gil122647 Mass: 16181 Score: 340 Queries matched: 30

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF

71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
81	657.68	1313.35	1313.66	-0.31	0	66	0.0001	1	K.VNVDEVGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(53)	0.0019	1	K.VNVDEVGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(47)	0.0077	1	K.VNVDEVGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	(66)	0.00011	1	K.VNVDEVGGEALGR.L
130	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	R.LLGNVLVCVLAHHFGK.E
131	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	R.LLGNVLVCVLAHHFGK.E
146	1029.77	2057.52	2057.94	-0.42	0	(14)	11	2	R.FFESFGDLSSPDAIMGNPK.V
147	692.18	2073.51	2073.94	-0.43	0	(15)	8.1	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
148	1037.76	2073.51	2073.94	-0.43	0	(20)	2.5	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
149	1037.76	2073.51	2073.94	-0.43	0	30	0.27	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
150	1037.77	2073.52	2073.94	-0.42	0	(13)	12	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
151	1037.77	2073.52	2073.94	-0.42	0	(26)	0.61	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
152	1037.77	2073.52	2073.94	-0.42	0	(15)	7.7	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
153	1037.77	2073.52	2073.94	-0.42	0	(20)	2.5	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)
154	1037.77	2073.53	2073.94	-0.41	0	(27)	0.59	2	R.FFESFGDLSSPDAIMGNPK.V + Oxidation (M)

16. gi122568 Mass: 16109 Score: 325 Queries matched: 18

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1 K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1 K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3 K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1 K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1 K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1 R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1 R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1 R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1 R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1 R.LLVVYPWTQRF
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1 R.LLVVYPWTQRF
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1 R.LLVVYPWTQRF
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1 R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1 R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1 R.LLVVYPWTQRF
88	664.70	1327.39	1327.67	-0.28	0	61	0.00032	1 K.VNVEEVGGEALGR.L
106	493.46	1477.37	1477.69	-0.32	0	(72)	2.2e-05	1 K.GTFATLSELHCDK.L
107	739.70	1477.39	1477.69	-0.30	0	83	2e-06	1 K.GTFATLSELHCDK.L

17. gi229594 Mass: 15117 Score: 321 Queries matched: 18

hemoglobin alpha

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
141	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPHFDLSHGSAQVK.G
142	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPHFDLSHGSAQVK.G
190	753.71	3010.82	3010.52	0.30	0	33	0.075	3	K.VADALTVAVAHVDDMPNSLTALSDLHAHK.L
191	785.73	3138.89	3138.61	0.27	1	45	0.0043	3	K.KVADALTVAVAHVDDMPNSLTALSDLHAHK.L

18. gi229751 Mass: 15174 Score: 317 Queries matched: 18

Chain A, Alpha-Ferrous-Carbonmonoxy, Beta-Cobaltous-Deoxy Hemoglobin (T State)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1 R.VDPVNFK.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1 R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1 R.MFLSFPTTK.T + Oxidation (M)

37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDVPVNF.K.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDVPVNF.K.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLT.S.K.Y
✓ 112	765.22	1528.42	1528.73	-0.31	0	75	1.1e-05	1	K.VGAHAGEYGAEALER.M
✓ 113	510.48	1528.42	1528.73	-0.31	0	(72)	2.5e-05	1	K.VGAHAGEYGAEALER.M
141	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPHFDLSHGSAQVK.G
142	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPHFDLSHGSAQVK.G

19. gill6006423 Mass: 15148 Score: 296 Queries matched: 18

hemoglobin alpha chain [Bos taurus]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNF.K.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDVPVNF.K.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDVPVNF.K.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLT.S.K.Y
112	765.22	1528.42	1528.73	-0.31	0	(39)	0.045	6	K.VGGHAAEYGAELER.M
113	510.48	1528.42	1528.73	-0.31	0	54	0.0015	6	K.VGGHAAEYGAELER.M
141	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPHFDLSHGSAQVK.G
142	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPHFDLSHGSAQVK.G

20. gill22676 Mass: 16179 Score: 295 Queries matched: 21

Hemoglobin subunit beta-1/2 (Hemoglobin beta-1/2 chain) (Beta-1/2-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
23	466.67	931.32	931.51	-0.19	0	45	0.018	1	K.SAVTALWGR.V
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
88	664.70	1327.39	1327.67	-0.28	0	61	0.00032	1	K.VNVEEVGGELGR.L
147	692.18	2073.51	2073.95	-0.44	0	(2)	1.7e+02	5	R.FFESFGDLSSANAVMNNPK.V
151	1037.77	2073.52	2073.95	-0.43	0	8	45	3	R.FFESFGDLSSANAVMNNPK.V
152	1037.77	2073.52	2073.95	-0.43	0	(2)	1.8e+02	5	R.FFESFGDLSSANAVMNNPK.V
154	1037.77	2073.53	2073.95	-0.42	0	(6)	69	3	R.FFESFGDLSSANAVMNNPK.V

21. gill36429 Mass: 25078 Score: 291 Queries matched: 12

Trypsin precursor

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 5	421.67	841.32	841.50	-0.19	0	(53)	0.0021	1	R.VATVSLPR.S
✓ 7	421.67	841.32	841.50	-0.18	0	(32)	0.26	1	R.VATVSLPR.S

☒	8	421.67	841.32	841.50	-0.18	0	(50)	0.0041	1	R.VATVSLPRS
☒	9	421.67	841.33	841.50	-0.17	0	53	0.002	1	R.VATVSLPRS
☒	29	523.18	1044.35	1044.56	-0.21	0	(42)	0.032	1	K.LSSPATLNSR.V
☒	30	523.18	1044.35	1044.56	-0.21	0	77	9.5e-06	1	K.LSSPATLNSR.V
☒	31	523.18	1044.35	1044.56	-0.20	0	(50)	0.0044	1	K.LSSPATLNSR.V
☒	158	737.55	2209.63	2210.10	-0.46	0	81	1.9e-06	1	R.LGEHNIDVLEGNEQFINAAKI
☒	159	1105.84	2209.66	2210.10	-0.43	0	(75)	7.8e-06	1	R.LGEHNIDVLEGNEQFINAAKI
☒	164	761.57	2281.70	2282.17	-0.48	0	(72)	1.6e-05	1	K.IITHPNFNGNTLDNDIMLIK.L
☒	165	766.90	2297.67	2298.17	-0.50	0	(64)	8.6e-05	1	K.IITHPNFNGNTLDNDIMLIK.L + Oxidation (M)
☒	166	766.90	2297.69	2298.17	-0.48	0	80	2.2e-06	1	K.IITHPNFNGNTLDNDIMLIK.L + Oxidation (M)

22. gi122484 Mass: 15297 Score: 266 Queries matched: 16

Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
112	765.22	1528.42	1528.73	-0.31	0	(55)	0.0012	5	K.IGGHGAEGYGAELER.M
113	510.48	1528.42	1528.73	-0.31	0	57	0.00066	5	K.IGGHGAEGYGAELER.M

23. gi186592 Mass: 15624 Score: 259 Queries matched: 15

hemoglobin alpha-B chain - thick-tailed bush baby

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Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
120	753.71	3010.82	3010.52	0.30	0	55	0.00052	1	K.VADALTNAVLHVDDMPALSALSSDLHAHK.L
121	785.73	3138.89	3138.61	0.27	1	87	2.7e-07	1	K.KVADALTNAVLHVDDMPALSALSSDLHAHK.L

24. gi28549 Mass: 13631 Score: 251 Queries matched: 8

alpha globin [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
36	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
86	442.46	1324.37	1324.65	-0.28	0	10	38	1	R.HFDLSHGSAQVK.G

112 765.22 1528.42 1528.73 -0.31 0 75 1.1e-05 1 K.VGAHAGEYGAEALER.H
 113 510.48 1528.42 1528.73 -0.31 0 (72) 2.5e-05 1 K.VGAHAGEYGAEALER.H

25. gi122672 Mass: 16041 Score: 250 Queries matched: 17

Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
32	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQRF
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQRF
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQRF
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQRF
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQRF
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQRF
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQRF
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQRF
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQRF
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQRF
87	663.76	1325.51	1325.79	-0.27	0	41	0.03	1	R.LLGNVLVCVLAR.H
88	664.70	1327.39	1327.71	-0.32	1	28	0.7	4	K.VKVDEVGGEALGR.L

26. gi122472 Mass: 15221 Score: 240 Queries matched: 16

Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
35	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
36	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
37	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
38	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
39	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
40	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
41	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
42	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
43	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
44	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
116	515.15	1542.42	1542.74	-0.32	0	29	0.46	2	K.VGGHVGGEYGAEALER.M
141	611.84	1832.50	1832.88	-0.38	0	3	1.7e+02	6	K.TYFPHDLAHGSSQVKA

27. gi12846963 Mass: 15160 Score: 223 Queries matched: 7

unnamed protein product [Mus musculus]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
36	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
112	765.22	1528.42	1528.73	-0.31	0	(55)	0.0012	5	K.IGGHGAEGYGAELER.M
113	510.48	1528.42	1528.73	-0.31	0	57	0.00066	5	K.IGGHGAEGYGAELER.M

28. gi122450 Mass: 15204 Score: 201 Queries matched: 6

Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
36	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
63	626.73	1251.45	1251.71	-0.26	0	91	3e-07	1	K.FLASVSTVLTSK.Y
116	515.15	1542.42	1542.74	-0.32	0	36	0.092	1	K.IGGHAAEGYGAELER.M

29. gi56122358 Mass: 13196 Score: 198 Queries matched: 8

transferrin [Pan troglodytes]

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Peptide

✓	102	704.68	1407.35	1407.63	-0.28	0	29	0.47	1	K.AAETWEFFASGK.T
✓	169	787.25	2358.72	2358.27	0.45	0	30	0.23	1	R.YTIAALLSPYSYSTTAVVTIPK.E
	170	1180.38	2358.75	2358.27	0.48	0	(7)	42	2	R.YTIAALLSPYSYSTTAVVTIPK.E
	178	818.89	2453.65	2454.14	-0.50	0	(70)	2.2e-05	1	K.TSESGELHGLTTEEFVEGIYK.V
	179	1227.84	2453.66	2454.14	-0.48	0	77	3.8e-06	1	K.TSESGELHGLTTEEFVEGIYK.V
✓	183	830.26	2487.74	2487.31	0.43	1	(15)	6.1	1	R.YTIAALLSPYSYSTTAVVTIPK.E-
✓	184	1244.89	2487.77	2487.31	0.46	1	37	0.04	1	R.YTIAALLSPYSYSTTAVVTIPK.E-
✓	186	839.27	2514.79	2514.37	0.42	1	25	0.81	1	R.RYTIAALLSPYSYSTTAVVTIPK.E

Proteins matching the same set of peptides:

30. [gi122413](#) Mass: 15152 Score: 173 Queries matched: 8
Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

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Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
26	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
46	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
47	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
112	765.22	1528.42	1528.73	-0.31	0	(59)	0.00046	4	K.IGGHAADYGAEALER.M
113	510.48	1528.42	1528.73	-0.31	0	65	0.00013	4	K.IGGHAADYGAEALER.M
141	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPFDLSHGSAQVK.A
142	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPFDLSHGSAQVK.A

31. [gi227298](#) Mass: 3957 Score: 171 Queries matched: 5
medullasin inhibitor 2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
57	584.19	1166.36	1166.60	-0.24	0	37	0.089	1	-VCVLAHHFGKE
96	689.71	1377.41	1377.69	-0.28	0	54	0.0018	1	K.EFTPPVQAAYQK.V

32. [gi122621](#) Mass: 15948 Score: 166 Queries matched: 14
Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
54	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
55	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
56	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
88	664.70	1327.39	1327.71	-0.32	1	28	0.7	4	K.VDVEKVGGEALGR.L

33. [gi75040810](#) Mass: 15908 Score: 157 Queries matched: 4
Transferrin precursor (Prealbumin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
102	704.68	1407.35	1407.63	-0.28	0	29	0.47	1	R.AAETWEFFASGK.T
170	1180.38	2358.75	2359.23	-0.48	0	51	0.0018	1	R.YTIAALLSPYSYSTTAVVTNPK.E
178	818.89	2453.65	2454.14	-0.50	0	(70)	2.2e-05	1	K.TSESGELHGLTTEEFVEGIYK.V
179	1227.84	2453.66	2454.14	-0.48	0	77	3.8e-06	1	K.TSESGELHGLTTEEFVEGIYK.V

34. [gi33694204](#) Mass: 4567 Score: 155 Queries matched: 6
hemoglobin beta chain [Homo sapiens]

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>54</u>	575.20	1148.38	1148.67	-0.29	0	(11)	40	3	K.VVAGVANALAHK.Y
<u>55</u>	575.22	1148.42	1148.67	-0.25	0	81	3.4e-06	1	K.VVAGVANALAHK.Y
<u>56</u>	383.81	1148.42	1148.67	-0.24	0	(74)	1.7e-05	1	K.VVAGVANALAHK.Y
<u>97</u>	690.21	1378.41	1378.68	-0.27	0	12	28	2	K.EFTPPVQAAYEK.V
<u>130</u>	444.91	1775.61	1775.99	-0.38	0	(37)	0.072	1	-LLGNVLVCVLAHFGK.E
<u>131</u>	592.88	1775.61	1775.99	-0.37	0	62	0.00019	1	-LLGNVLVCVLAHFGK.E

35. gil122440 Mass: 15296 Score: 150 Queries matched: 6
Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>3</u>	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
<u>36</u>	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
<u>46</u>	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
<u>47</u>	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
<u>112</u>	765.22	1528.42	1528.73	-0.31	0	75	1.1e-05	1	K.IGGHAGEYGAEALER.M
<u>113</u>	510.48	1528.42	1528.73	-0.31	0	(72)	2.5e-05	1	K.IGGHAGEYGAEALER.M

36. gil52000974 Mass: 15220 Score: 144 Queries matched: 7
Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>1</u>	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
<u>36</u>	544.16	1086.30	1086.62	-0.32	1	(8)	98	7	K.LRVDPVNFK.L
<u>46</u>	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	K.LRVDPVNFK.L
<u>47</u>	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	K.LRVDPVNFK.L
<u>116</u>	515.15	1542.42	1542.74	-0.32	0	36	0.092	1	K.IGGHAAEYGAELER.M
<u>141</u>	611.84	1832.50	1832.88	-0.38	0	34	0.13	1	K.TYFPFHDLSHGSAQVK.A
<u>142</u>	459.13	1832.50	1832.88	-0.38	0	(4)	1.2e+02	2	K.TYFPFHDLSHGSAQVK.A

37. gil122500 Mass: 15694 Score: 142 Queries matched: 14
Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>1</u>	409.64	817.27	817.43	-0.16	0	21	5.1	1	R.VDPVNFK.L
<u>35</u>	544.15	1086.29	1086.54	-0.25	0	(23)	3	1	R.MFLSFPTTK.T + Oxidation (M)
<u>36</u>	544.16	1086.30	1086.54	-0.24	0	(27)	1.3	1	R.MFLSFPTTK.T + Oxidation (M)
<u>37</u>	544.16	1086.30	1086.54	-0.24	0	(23)	2.6	1	R.MFLSFPTTK.T + Oxidation (M)
<u>38</u>	544.16	1086.31	1086.54	-0.23	0	(26)	1.5	1	R.MFLSFPTTK.T + Oxidation (M)
<u>39</u>	544.16	1086.31	1086.54	-0.23	0	(20)	5.6	1	R.MFLSFPTTK.T + Oxidation (M)
<u>40</u>	544.16	1086.31	1086.54	-0.23	0	46	0.015	1	R.MFLSFPTTK.T + Oxidation (M)
<u>41</u>	544.16	1086.31	1086.54	-0.23	0	(36)	0.15	1	R.MFLSFPTTK.T + Oxidation (M)
<u>42</u>	544.17	1086.32	1086.54	-0.22	0	(28)	0.86	1	R.MFLSFPTTK.T + Oxidation (M)
<u>43</u>	544.17	1086.32	1086.54	-0.22	0	(18)	10	1	R.MFLSFPTTK.T + Oxidation (M)
<u>44</u>	544.17	1086.32	1086.54	-0.22	0	(32)	0.33	1	R.MFLSFPTTK.T + Oxidation (M)
<u>46</u>	544.20	1086.39	1086.62	-0.23	1	(44)	0.021	1	R.LRVDPVNFK.L
<u>47</u>	363.14	1086.40	1086.62	-0.22	1	53	0.0026	1	R.LRVDPVNFK.L
<u>141</u>	611.84	1832.50	1832.88	-0.38	0	24	1.1	4	K.TYFPFHDLSHGSGQIK.A

38. gil122687 Mass: 15831 Score: 116 Queries matched: 11
Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>66</u>	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
<u>67</u>	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
<u>68</u>	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
<u>69</u>	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F

70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
88	664.70	1327.39	1327.67	-0.28	0	59	0.00055	3	K.VNVDEIGGEALGR.L

39. gi122695 Mass: 15779 Score: 111 Queries matched: 11
Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.P
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
96	689.71	1377.41	1377.69	-0.28	0	54	0.0018	1	K.EFTPPVQSAFQK.V

40. gi122581 Mass: 16025 Score: 100 Queries matched: 13
Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
77	642.69	1283.37	1283.68	-0.31	0	0	3.7e+02	4	K.VNVGEIGAEALGR.L

41. gi122725 Mass: 16227 Score: 71 Queries matched: 14
Hemoglobin subunit epsilon (Hemoglobin epsilon chain) (Epsilon-globin)

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
66	637.72	1273.43	1273.72	-0.28	0	(15)	13	1	R.LLVVYPWTQR.F
67	637.73	1273.44	1273.72	-0.28	0	(34)	0.16	1	R.LLVVYPWTQR.F
68	637.73	1273.44	1273.72	-0.27	0	57	0.00083	1	R.LLVVYPWTQR.F
69	637.73	1273.45	1273.72	-0.27	0	(42)	0.025	1	R.LLVVYPWTQR.F
70	637.73	1273.45	1273.72	-0.27	0	(54)	0.0016	1	R.LLVVYPWTQR.F
71	637.73	1273.45	1273.72	-0.27	0	(46)	0.011	1	R.LLVVYPWTQR.F
72	637.73	1273.45	1273.72	-0.27	0	(32)	0.26	1	R.LLVVYPWTQR.F
73	637.73	1273.46	1273.72	-0.26	0	(44)	0.019	1	R.LLVVYPWTQR.F
74	637.74	1273.46	1273.72	-0.26	0	(50)	0.0039	1	R.LLVVYPWTQR.F
75	637.74	1273.46	1273.72	-0.25	0	(45)	0.013	1	R.LLVVYPWTQR.F
81	657.68	1313.35	1313.66	-0.31	0	(12)	27	6	K.VNIEEAGGEALGR.L
82	657.70	1313.38	1313.66	-0.28	0	(8)	62	6	K.VNIEEAGGEALGR.L
83	657.70	1313.38	1313.66	-0.27	0	(6)	1e+02	7	K.VNIEEAGGEALGR.L
84	657.70	1313.39	1313.66	-0.27	0	14	16	4	K.VNIEEAGGEALGR.L

42. gi16685052 Mass: 1251 Score: 71 Queries matched: 1
beta-globin [*Callithrix jacchus*]

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
24	476.19	950.36	950.53	-0.17	0	71	3.5e-05	1	-AGVANALAHK.Y

43. [gi|62460516](#) Mass: 16560 Score: 50 Queries matched: 3
hemoglobin, beta, epsilon 4 [Bos taurus]

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
52	563.67	1125.32	1125.56	-0.23	0	(31)	0.36	1	K.LHVDPENFR.L
53	376.11	1125.32	1125.56	-0.23	0	43	0.022	1	K.LHVDPENFR.L
88	664.70	1327.39	1327.71	-0.32	0	7	83	9	K.VNVEVVGESLAR.L

Proteins matching the same set of peptides:

[gi|62460522](#) Mass: 16638 Score: 50 Queries matched: 3
globin, beta, epsilon 2 [Bos taurus]

44. [gi|121039](#) Mass: 36596 Score: 49 Queries matched: 1
Ig gamma-1 chain C region

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
60	593.70	1185.39	1185.64	-0.25	0	49	0.0057	1	K.GPSVFPLAPSSK.S

Significant hits:

[gi|4389275](#) Chain , Human Serum Albumin In A Complex With Myristic Acid And Tri-Iodobenzoic Acid
[gi|15990507](#) Serpin peptidase inhibitor, clade A (alpha-1 antitrypsin), member 1 [Homo sapiens]
[gi|28332](#) unnamed protein product [Homo sapiens]
[gi|34526063](#) unnamed protein product [Homo sapiens]
[gi|229601](#) Ig G1 H Nle
[gi|34527698](#) unnamed protein product [Homo sapiens]
[gi|16741036](#) IGHA1 protein [Homo sapiens]
[gi|34526220](#) unnamed protein product [Homo sapiens]
[gi|223099](#) Ig A alpha1 Bur
[gi|125507](#) Kininogen-1 precursor (Alpha-2-thiol proteinase inhibitor) [Contains: Kininogen-1 heavy chain; Brad
[gi|10334611](#) Immunoglobulin heavy chain [Homo sapiens]
[gi|28317](#) unnamed protein product [Homo sapiens]
[gi|230581](#) Chain H, Immunoglobulin G1
[gi|1212947](#) haptoglobin [Homo sapiens]
[gi|14277816](#) Chain A, Crystal Structure Of A Human Fcγ Receptor In Complex With An Fc Fragment Of Igγ1
[gi|306889](#) preprohaptoglobin
[gi|179161](#) antithrombin III
[gi|36573](#) unnamed protein product [Homo sapiens]
[gi|139641](#) Vitamin D-binding protein precursor (DBP) (Group-specific component) (Gc-globulin) (VDB)
[gi|47523844](#) similar to serine (or cysteine) proteinase inhibitor, clade A (alpha-1antitrypsin, antitrypsin).

1. [gi|4389275](#) Mass: 67988 Score: 825 Queries matched: 16

Chain , Human Serum Albumin In A Complex With Myristic Acid And Tri-Iodobenzoic Acid

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
25	464.15	926.28	926.49	-0.21	0	29	0.55	1	K.YLYEIAR.R
27	467.16	932.30	932.51	-0.21	0	43	0.025	1	K.LCTVATLRE
31	480.68	959.34	959.56	-0.22	0	46	0.012	1	K.FQNALLVR.Y
71	575.18	1148.35	1148.61	-0.26	0	60	0.00043	1	K.LVNEVTEFAK.T
96	656.23	1310.44	1310.73	-0.30	0	58	0.00072	1	R.HPDYSVVLRL.L
101	671.67	1341.34	1341.63	-0.29	0	(64)	0.00018	1	K.AVMDDFAAFVEK.C
104	679.67	1357.33	1357.62	-0.30	0	94	1.5e-07	1	K.AVMDDFAAFVEK.C + Oxidation (M)
116	722.17	1442.33	1442.63	-0.30	0	32	0.24	1	K.YICENQDSISSK.L

✓	117	489.85	1466.52	1466.84	-0.32	1	72	2.1e-05	1 R.RHPDYSVVLRL
✓	120	756.26	1510.51	1510.84	-0.33	0	92	2e-07	1 K.VPQVSTPTLVEVSR.N
✓	127	820.22	1638.43	1638.78	-0.34	0	52	0.002	1 K.DVFLGMYFLYERAR.R + Oxidation (M)
✓	128	547.19	1638.56	1638.93	-0.37	1	108	5.5e-09	1 K.KVPQVSTPTLVEVSR.N
✓	134	829.20	1656.39	1656.75	-0.35	0	56	0.00078	1 K.QNCELFEQLGEYK.F
✓	142	581.51	1741.50	1741.89	-0.39	0	(42)	0.021	1 R.HPYFYAPELLFFAK.R
✓	143	871.76	1741.51	1741.89	-0.38	0	48	0.0049	1 R.HPYFYAPELLFFAK.R
✓	184	682.22	2043.64	2044.09	-0.45	0	38	0.039	1 K.VFDEFKPLVEEPQNLK.Q

gi15990507 Mass: 46850 Score: 689 Queries matched: 16

Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
5	390.12	778.23	778.40	-0.17	0	15	18	1	K.SPLFMGK.V
14	426.66	851.30	851.49	-0.19	0	10	42	6	R.SASLHLPK.L
21	444.65	887.29	887.50	-0.20	0	44	0.029	1	K.AVLTIDEK.G
43	508.20	1014.38	1014.61	-0.22	0	49	0.0071	1	K.SVLGQLGITK.V
52	538.69	1075.37	1075.61	-0.24	0	(33)	0.25	1	K.LSSWVLLMK.Y
62	546.68	1091.35	1091.60	-0.25	0	47	0.0087	1	K.LSSWVLLMK.Y + Oxidation (M)
65	555.68	1109.34	1109.60	-0.26	0	50	0.0037	1	K.LSITGITVDLK.S
129	821.26	1640.51	1640.86	-0.35	0	73	1.5e-05	1	K.ITPNLAEFAPSLYR.Q
150	601.85	1802.54	1802.95	-0.41	0	(51)	0.0025	1	K.LQHLLENLTHDITK.F
151	451.65	1802.56	1802.95	-0.39	0	71	2.6e-05	1	K.LQHLLENLTHDITK.F
156	917.27	1832.52	1832.92	-0.40	0	119	3.7e-10	1	K.VFSNCAADLSGVTEEAPLK.L
160	619.19	1854.54	1854.97	-0.43	0	(32)	0.17	1	K.FNKPFFVFLMIEQNTK.S
✓	161	621.81	1862.40	1862.82	-0.42	0	33	0.22	1 K.DTEEDDFHVDQATTVK.V
162	624.53	1870.56	1870.97	-0.41	0	63	0.00014	1	K.FNKPFFVFLMIEQNTK.S + Oxidation (M)
189	729.19	2184.55	2185.03	-0.48	1	76	6.8e-06	1	K.LYHSEAFVNFQDTEEAQK.Q
198	1145.82	2289.63	2290.12	-0.49	0	41	0.022	1	K.GTEAAGAMFLEAIPMS(PPEVK.F + 2 Oxidation (M)

gi28332 Mass: 25217 Score: 601 Queries matched: 23

unnamed protein product [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
4	383.61	765.20	765.37	-0.17	0	34	0.16	1	R.DSLEFR.E
30	480.65	959.28	959.50	-0.22	0	70	4.8e-05	1	K.ADLSGITGAR.N
55	531.17	1060.33	1060.58	-0.25	0	(14)	18	1	R.EIGELYLPK.F
56	531.17	1060.33	1060.58	-0.25	0	43	0.021	1	R.EIGELYLPK.F
57	531.18	1060.34	1060.58	-0.24	0	(5)	1.5e+02	8	R.EIGELYLPK.F
58	531.18	1060.35	1060.58	-0.23	0	(2)	3.1e+02	4	R.EIGELYLPK.F
63	547.70	1093.38	1093.62	-0.25	0	(44)	0.015	1	R.NLAVSQVVK.A
64	365.47	1093.38	1093.62	-0.25	0	60	0.00037	1	R.NLAVSQVVK.A
69	571.70	1141.38	1141.65	-0.27	0	7	87	2	M.VLVNVIFFK.A
80	608.22	1214.43	1214.72	-0.30	0	(62)	0.0003	1	K.ITLLSALVETR.T
81	608.22	1214.43	1214.72	-0.29	0	78	6.5e-06	1	K.ITLLSALVETR.T
82	608.23	1214.45	1214.72	-0.28	0	(61)	0.00034	1	K.ITLLSALVETR.T
113	711.19	1420.36	1420.68	-0.31	0	67	6.8e-05	1	R.DBELSCTVVELK.Y
131	824.74	1647.46	1647.81	-0.35	0	84	1.5e-06	1	K.MEEVEAMLLPETLK.R + Oxidation (M)
135	832.73	1663.45	1663.80	-0.36	0	(70)	3.7e-05	1	K.MEEVEAMLLPETLK.R + 2 Oxidation (M)
146	591.79	1772.36	1772.76	-0.40	0	(62)	0.00022	1	K.WEMPFDPQDTHQSR.F
147	597.13	1788.36	1788.75	-0.39	0	69	3.6e-05	1	K.WEMPFDPQDTHQSR.F + Oxidation (M)
171	954.27	1906.53	1906.95	-0.42	0	(75)	8.6e-06	1	K.AVLDFVEEGTEASAATAVK.I
172	954.27	1906.54	1906.95	-0.42	0	(49)	0.0036	1	K.AVLDFVEEGTEASAATAVK.I
173	954.28	1906.54	1906.95	-0.42	0	(72)	1.9e-05	1	K.AVLDFVEEGTEASAATAVK.I
174	954.28	1906.54	1906.95	-0.41	0	89	4e-07	1	K.AVLDFVEEGTEASAATAVK.I
175	954.28	1906.54	1906.95	-0.41	0	(84)	1.2e-06	1	K.AVLDFVEEGTEASAATAVK.I
176	954.28	1906.55	1906.95	-0.41	0	(81)	2.1e-06	1	K.AVLDFVEEGTEASAATAVK.I

7. gi34526063 Mass: 52000 Score: 590 Queries matched: 13

unnamed protein product [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓	10	419.66	837.31	837.50	-0.19	0	23	2.1	1 K.ALPAPIEK.T
✓	74	581.18	1160.35	1160.62	-0.27	0	39	0.052	1 K.NQVSLTCLVK.G

✓	76	593.69	1185.38	1185.64	-0.26	0	53	0.0022	1	K.GPSVFPLAPSSK.S
✓	88	643.70	1285.38	1285.67	-0.29	0	49	0.0058	1	R.EPQVYTLPPSR.D
✓	98	661.20	1320.38	1320.67	-0.30	0	65	0.00013	1	K.STSGGTAALGCLVK.D
✓	136	559.81	1676.42	1676.79	-0.37	0	(51)	0.0025	1	K.FNWWYVDGVEVHNAK.T
✓	137	839.22	1676.42	1676.79	-0.37	0	63	0.00015	1	K.FNWWYVDGVEVHNAK.T
✓	152	603.20	1806.59	1807.00	-0.41	0	72	2.3e-05	1	R.VVSVLTVLHQDWLNGK.E
✓	153	904.31	1806.60	1807.00	-0.40	0	(50)	0.0031	1	R.VVSVLTVLHQDWLNGK.E
✓	163	624.85	1871.54	1871.96	-0.42	1	18	4.5	1	R.EPQVYTLPPSRDELTK.N
✓	164	937.26	1872.51	1872.91	-0.40	0	49	0.0036	1	K.TTPPVLDSDGSFFLYSK.L
✓	185	713.52	2137.55	2138.02	-0.47	0	82	1.6e-06	1	R.TPEVTCVVVDVSHEDPEVK.F
✓	205	711.70	2842.79	2842.50	0.29	1	77	3.9e-06	1	K.THTCPCPCAPKLLGGPSVFLFPPKPK.D

12. gi16741036 Mass: 54327 Score: 448 Queries matched: 6

IGHA1 protein [Homo sapiens]

	Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓	22	448.64	895.26	895.46	-0.20	0	28	0.62	1	K.YLTWASR.Q
	29	475.16	948.31	948.51	-0.21	0	53	0.0031	1	R.VTISVDTSK.N
✓	79	607.18	1212.35	1212.63	-0.27	0	85	1.3e-06	1	R.WLQGSQELPR.E
✓	106	688.16	1374.31	1374.61	-0.30	0	71	2.9e-05	1	K.IFTCTAAYPESK.T
✓	123	770.70	1539.39	1539.72	-0.33	0	88	5.7e-07	1	R.DASGVTFWTWPSSGK.S
✓	157	918.28	1834.54	1834.94	-0.40	0	123	1.7e-10	1	R.QEPSQGTTTFAVTSILR.V

17. gi125507 Mass: 72984 Score: 363 Queries matched: 7

Kininogen-1 precursor (Alpha-2-thiol proteinase inhibitor) [Contains: Kininogen-1 heavy chain; Brad

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 26	466.14	930.28	930.48	-0.21	0	9	65	1	K.DFVQPPTK.I
✓ 41	502.18	1002.34	1002.56	-0.22	0	49	0.0059	1	R.QVVAGLNFR.I
✓ 45	515.65	1029.29	1029.53	-0.24	0	39	0.06	1	K.YFIDFVAR.E
✓ 84	626.16	1250.30	1250.58	-0.28	0	64	0.00016	1	K.TVGSDFYSFK.Y
✓ 108	692.19	1382.36	1382.65	-0.29	0	62	0.00024	1	K.ENFLFLTPDCK.S
✓ 125	800.20	1598.39	1598.74	-0.35	0	85	9.6e-07	1	R.IASFSQNCDIYPGK.D
✓ 186	713.54	2137.60	2138.07	-0.48	0	55	0.00083	1	R.DIPTNSPELEETLTHTITK.L

21. gi230581 Mass: 50444 Score: 322 Queries matched: 8

Chain H, Immunoglobulin G1

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
<u>10</u>	419.66	837.31	837.50	-0.19	0	23	2.1	1	K.ALPAPIEK.T
<u>24</u>	581.18	1160.35	1160.62	-0.27	0	39	0.052	1	K.NQVSLTCLVK.G
<u>76</u>	593.69	1185.38	1185.64	-0.26	0	53	0.0022	1	K.GPSVFPLAPSSK.S
<u>88</u>	643.70	1285.38	1285.67	-0.29	0	49	0.0058	1	R.EPQVYTLPPSR.E
<u>98</u>	661.20	1320.38	1320.67	-0.30	0	65	0.00013	1	K.STSGGTAALGCLVK.D
<u>152</u>	603.20	1806.59	1807.00	-0.41	0	43	0.019	3	R.VVSVLTVLHQNWLDGK.E
<u>153</u>	904.31	1806.60	1807.00	-0.40	0	(33)	0.19	3	R.VVSVLTVLHQNWLDGK.E
<u>164</u>	937.26	1872.51	1872.91	-0.40	0	49	0.0036	1	K.TTPPVLDSDGSFFLYSK.L

gi179161 Mass: 53041 Score: 147 Queries matched: 2

antithrombin III

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓	111	695.22	1388.43	1388.75	-0.32	0	60	0.00044	1 REVLNTHFMGR.V

✓ 114 715.73 1429.45 1429.78 -0.33 0 87 7.1e-07 1 R.VAEGTQVLELPFK.G

gi|139641 Mass: 54526 Score: 113 Queries matched: 3

Vitamin D-binding protein precursor (DBP) (Group-specific component) (Gc-globulin) (VDB)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 6	400.16	798.30	798.49	-0.19	0	28	0.68	1	K.VLEPTLK.S
✓ 24	458.13	914.25	914.45	-0.20	0	30	0.5	1	K.YTFELSR.R
✓ 29	442.82	1325.45	1325.75	-0.29	1	55	0.0014	1	R.RTHLPEVFLSK.V

Significant hits:

gi|1197209 alpha-1-acid glycoprotein 1 precursor

gi|38026 Zn-alpha2-glycoprotein [Homo sapiens]

gi|4505522 orosomucoid 2 [Homo sapiens]

gi|123510 Haptoglobin-related protein precursor

gi|179674 complement component C4A

gi|28317 unnamed protein product

gi|14318424 apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death

gi|1197209 Mass: 23579 Score: 405 Queries matched: 9

alpha-1-acid glycoprotein 1 precursor [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 30	497.63	993.24	993.51	-0.27	0	42	0.026	1	K.TEDTIFLR.E
✓ 41	556.62	1111.23	1111.52	-0.29	0	55	0.0011	1	K.SDVVYTDWK.K
✓ 46	580.65	1159.28	1159.58	-0.30	0	48	0.0075	1	K.WFYIASAF.R.N
✓ 71	723.15	1444.29	1444.65	-0.37	0	82	2.5e-06	1	K.TYMLAFDVNDEK.N
✓ 73	731.15	1460.28	1460.65	-0.37	0	(76)	1e-05	1	K.TYMLAFDVNDEK.N + Oxidation (M)
✓ 83	570.14	1707.41	1707.85	-0.44	0	33	0.18	1	K.NWGLSVYADKPETTK.E
✓ 84	871.69	1741.36	1741.80	-0.43	0	84	1.1e-06	1	K.EQLGEFYEALDCLR.I
✓ 85	438.88	1751.48	1751.95	-0.47	0	(54)	0.0012	1	R.YVGGQEHFAHLLIL.R.D
✓ 86	584.83	1751.48	1751.95	-0.47	0	60	0.00029	1	R.YVGGQEHFAHLLIL.R.D

3. gi|2116653 Mass: 38143 Score: 253 Queries matched: 7

alpha-2-HS glycoprotein [Chlorocbebus aethiops]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 3	407.12	812.23	812.44	-0.22	0	42	0.025	1	K.FSVVYAK.C
✓ 37	540.65	1079.29	1079.59	-0.30	0	(26)	1	1	-APHGPGLIYR.Q
✓ 38	360.77	1079.30	1079.59	-0.29	0	51	0.0031	1	-APHGPGLIYR.Q
✓ 52	598.66	1195.31	1195.62	-0.31	0	66	0.0001	1	K.HTLNQIDEVK.V
✓ 53	399.45	1195.32	1195.62	-0.30	0	(38)	0.056	1	K.HTLNQIDEVK.V
✓ 81	554.11	1659.31	1659.76	-0.44	0	94	1.6e-07	1	K.EHAVEGDCDFQLLK.V
✓ 82	830.67	1659.33	1659.76	-0.42	0	(62)	0.00022	1	K.EHAVEGDCDFQLLK.V

4. gi|38026 Mass: 34942 Score: 230 Queries matched: 4

Zn-alpha2-glycoprotein [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
✓ 42	564.14	1126.27	1126.56	-0.29	0	42	0.027	1	R.AGEVQEPFL.R.G
✓ 70	704.69	1407.37	1407.73	-0.35	0	72	2.4e-05	1	R.YSLTYIYTGSLKH
✓ 72	726.16	1450.30	1450.68	-0.37	0	67	6.4e-05	1	K.AYLEEECPATLR.K

88 891.76 1781.50 1781.94 -0.44 0 49 0.0046 1 K.EIPAWVPFDPAQITK.Q

5. [gi14505529](#) Mass: 23873 Score: 191 Queries matched: 4
orosomucoid 2

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
30	497.63	993.24	993.51	-0.27	0	42	0.026	1	K.TEOTIFLR.E
45	580.61	1159.20	1159.49	-0.29	0	59	0.00058	1	R.SDVMYTDWK.K + Oxidation (M)
46	580.65	1159.28	1159.58	-0.30	0	48	0.0075	1	K.WFYIASAFRN
57	412.13	1233.37	1233.70	-0.32	0	45	0.015	1	R.EHVAHLLFLR.D

[gi179674](#) Mass: 194337 Score: 101 Queries matched: 1

complement component C4A

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
76	771.20	1540.39	1540.81	-0.42	0	101	2.7e-08	1	K.VLSLAQEQVGCSPK.L

15. [gi14318424](#) Score: 42 Queries matched: 1

apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
43	564.14	1126.27	1126.67	-0.40	1	42	0.027	1	R.QEVKEILLR.K

Significant hits:

[gi116596](#) Complement C3 alpha chain

[gi27414491](#) coiled-coil domain containing 28A [Homo sapiens]

13. [gi116596](#) Mass: 82704 Score: 49 Queries matched: 1

Complement C3 alpha chain

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
36	546.75	1091.49	1091.62	-0.14	0	49	0.0061	1	K.NTLIIVLDK.I

14. [gi27414491](#) Mass: 30519 Score: 43 Queries matched: 3

coiled-coil domain containing 28A [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
1	363.17	724.33	724.40	-0.07	1	8	63	3	M.PRAEPRA
13	428.71	855.40	855.44	-0.04	1	(15)	15	5	-MPRAEPRA
14	428.71	855.40	855.44	-0.03	1	37	0.099	1	-MPRAEPRA

Significant hits:

[gi36321](#) SAA precursor polypeptide (119 AA)

[gi134167](#) Serum amyloid A protein precursor (SAA) [Contains: Amyloid protein A (Amyloid fibril protein AA); S

[gi261970](#) serum amyloid A isotype 1, SAA1= major fibril protein (internal fragment) [human, thyroid gland, Pep

[gi134169](#) Serum amyloid A-2 protein precursor

[gi36321](#) Mass: 13184 Score: 237 Queries matched: 4

SAA precursor polypeptide (119 AA) [Homo sapiens]

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
58	728.30	1454.58	1454.73	-0.15	0	82	2e-06	1	R.GPGGAWAAEVISNAR.E
60	775.80	1549.58	1549.72	-0.14	0	78	5.9e-06	1	R.SFFSFLGEAFDGAR.D
64	547.56	1639.66	1639.81	-0.15	1	33	0.17	1	R.DPNHFRPAGLPEKY.-
66	557.54	1669.60	1669.78	-0.18	1	44	0.014	1	R.EANYIGSDKYFHAR.G

4. [gi134167](#) Mass: 13581 Score: 227 Queries matched: 4

Serum amyloid A protein precursor (SAA) [Contains: Amyloid protein A (Amyloid fibril protein AA); S

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
60	775.80	1549.58	1549.72	-0.14	0	78	5.9e-06	1	R.SFFSFLGEAFDGAR.D
64	547.56	1639.66	1639.81	-0.15	1	33	0.17	1	R.DPNHFRPAGLPEKY.-
66	557.54	1669.60	1669.78	-0.18	1	44	0.014	1	R.EANYIGSDKYFHAR.G
77	726.58	2176.72	2176.96	-0.24	0	72	1.6e-05	1	R.FFCHGAEDSLAQANWGR.S

5. [gi|261970](#) Mass: 1584 Score: 171 Queries matched: 2

serum amyloid A isotype 1, SAA1=major fibril protein (internal fragment) [human, thyroid gland, Pep

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☒ 55	714.78	1427.55	1427.68	-0.13	0	96	8.4e-08	1	R.GPGGAWAAEAISDAR-
☒ 61	528.88	1583.60	1583.78	-0.18	1	74	1.3e-05	1	-RCPCGAWAAEAISDAR-

[gi|27414491](#) Mass: 30519 Score: 38 Queries matched: 3

coiled-coil domain containing 28A

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
1	363.16	724.31	724.40	-0.08	1	15	12	4	M.PRAEPRA
☒ 19	428.72	855.42	855.44	-0.02	1	(17)	10	1	-MPRAEPRA
☒ 20	428.72	855.43	855.44	-0.01	1	25	1.4	1	-MPRAEPRA

Significant hits:

[gi|61679690](#) Chain A, T-To-Thigh Quaternary Transitions In Human Hemoglobin: Alpha191a Deoxy Low-Salt[gi|224917](#) lipoprotein CIII[gi|122676](#) Hemoglobin subunit beta-1/2 (Hemoglobin beta-1/2 chain) (Beta-1/2-globin)[gi|61679690](#) Mass: 15164 Score: 131 Queries matched: 4

Chain A, T-To-Thigh Quaternary Transitions In Human Hemoglobin: Alpha191a Deoxy Low-Salt

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.67	817.32	817.43	-0.11	0	15	21	2	R.VDPVNFK.L
☒ 33	544.21	1086.40	1086.54	-0.14	0	44	0.024	1	R.MFLSFPTTK.T + Oxidation (M)
☒ 49	626.78	1251.55	1251.71	-0.16	0	65	0.00013	1	K.FLASVSTVLT\$K.V
78	459.17	1832.66	1832.88	-0.23	0	8	56	2	K.TYFPHFDLSHGSAQVK.G

[gi|61679690](#) Mass: 15164 Score: 131 Queries matched: 4

Chain A, T-To-Thigh Quaternary Transitions In Human Hemoglobin: Alpha191a Deoxy Low-Salt

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
3	409.67	817.32	817.43	-0.11	0	15	21	2	R.VDPVNFK.L
☒ 33	544.21	1086.40	1086.54	-0.14	0	44	0.024	1	R.MFLSFPTTK.T + Oxidation (M)
☒ 49	626.78	1251.55	1251.71	-0.16	0	65	0.00013	1	K.FLASVSTVLT\$K.V
78	459.17	1832.66	1832.88	-0.23	0	8	56	2	K.TYFPHFDLSHGSAQVK.G

22. [gi|122676](#) Mass: 16179 Score: 92 Queries matched: 3

Hemoglobin subunit beta-1/2 (Hemoglobin beta-1/2 chain) (Beta-1/2-globin)

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
25	466.70	931.39	931.51	-0.12	0	15	18	2	K.SAVTALWGK.V
38	383.85	1148.52	1148.67	-0.15	0	68	7.6e-05	1	K.VVAGVANALAHK.Y
81	1037.85	2073.68	2073.95	-0.26	0	10	28	4	R.FFESFGDLSSANAVMNNPK.V

Discussion

[0153] In this study we show that SELDI-TOF-MS can be successfully used to detect candidate biomarkers in visceral VL patients. Using CiphergenExpress on its own, the largest number of biomarkers was detected in fraction 6 bound on IMAC30 arrays, whereas using both software programs the largest number was found in fraction 4 on CM10 arrays. The ROCs in the m/z scatter plots show that the biomarkers were able to distinguish successfully between positive and negative serum samples.

[0154] In the past concerns about the reproducibility of SELDI-data were mentioned by several groups. Generation of reproducible SELDI-TOF spectra requires prompt separation of serum and storage at -20°C or below, and because repeated freeze-thaw cycles result in degradation of spectra (Papadopoulos, M.C. *et al.*, 2004, *Lancet* 363:1358-1363), it is necessary to use a robust cold chain (Agranoff *et al.*, 2005).

[0155] Ransohoff (2005) discusses that lessons can be learned from how the question of reproducibility has been effectively addressed in other “-omics” research Ransohoff, D. , 2005, *Journal of the National Cancer Institute* 97. Rosenwald *et al* (2002) showed in a genomics study that by splitting the samples in a training set (to derive a pattern-recognition model) and an independent validation set, chance or over-fitting does not explain the results. Rosenwald,, A. *et al.*, 2002, *N Engl J Med*, 346:1937-47. They demonstrated reproducibility in different patients in a setting in which technical features were held constant.

[0156] In our study we targeted the reproducibility problem in the same way and successfully validated the biomarkers in a second sample set (consisting of pretreatment sera from VL patients which were compared to the same controls as used for set 1) under the same settings. The intensities of the biomarkers found in the validation set were very similar to the ones in the original sample set. One biomarker with 9478 Da found in sample set 1 (fraction 5 bound on an IMAC30 array) was down-regulated in the positive samples. In the validation set, this protein was not detected as a biomarker at all (positive samples compared to controls). Therefore, its value as a marker can be questioned.

[0157] The small molecular weight differences are most likely caused by the fact that we processed the validation set with the sensitive SELDI technology on a different day using different arrays (even with the same settings on the same SELDI machine in the same

laboratory using the same array type small molecular weight differences can be visible). This problem is discussed by Gretzer *et al.*, 2003, *Reviews in Urology*, 5: 81-89. They mention that one limitation of SELDI is the lot-to-lot reproducibility of the chip surface chemistry. To maintain the reproducibility of mass-produced chips they ask for improved manufacturing methods. Agranoff *et al.*, *Trends in Parasitology*, 21: 154-157 (2005) report that the biological complexity of most disease states means that single biomarkers, with a few notable exceptions such as Troponin I or Troponin T as indicators of myocardial injury, have extremely limited diagnostic sensitivities and specificities. They mention that analyzing combinations of multiple biomarkers offers the possibility of greatly enhanced diagnostic accuracy. But in fact there are already existing examples of highly sensitive and specific single protein 'biomarkers' (e.g., hepatitis B surface antigen, circulating malaria antigens).

[0158] In this study single biomarkers which distinguished correctly between infected patients and controls were detected. Each candidate biomarker shown in Figures 7 and 9, fulfilling that task very well. It could be that because of the high sensitivity of SELDI-TOF-MS it is easier to find single biomarkers which distinguish between positive and negative samples.

[0159] It was possible to successfully visualize at least four biomarkers on the gel. Sequencing of the 51 kDa band detected in pretreatment sera using LC-MS-MS followed by a Mascot search revealed a number of potential proteins. The 51 kDa band can represent kininogen 1 precursor. High and low molecular weight kininogen are strong inhibitors of cysteine proteinases. Three related domains on its heavy chain are responsible for this activity (Higashiyama *et al.*, 1986, *Biochemistry* 25:1669-75).

[0160] Since kininogen-1 precursor was up-regulated in pretreatment samples and *Leishmania* parasites have cysteine proteases, an infection with *Leishmania* could up-regulate cysteine protease inhibitors in the host. Kininogen is not in its active form because the parasite blocks its activation, which would be in favor of *Leishmania*.

[0161] High molecular weight kininogen plays a role in blood coagulation. It is also a precursor of bradykinin; this vasodilator substance is released through positive feedback by kallikrein.

[0162] Kininogen is not in its active form because in that way blood does not coagulate, which could be in favor of the sandfly trying to get a bloodmeal. In this way the chance of the parasite to get transmitted into other people is enhanced as well. There are

reports about coagulation disorders in leishmaniasis patients. According to Font *et al* (Font, A. *et al.*, 1993, *Journal of Small Animal Practice*, 34: 466-470; Font, A. *et al.*, 1994, *Journal of the American Veterinary Medical Association*, 204: 1043-44) and Moreno (Moreno, P., 1999, *Veterinary Record*, 144: 169-71) report that one of the characteristics of established VL are hematological disorders. Dubey *et al.* address about low hemoglobin and a deranged coagulation due to thrombocytopenia (Dubey P. K. *et al.*, 2001, *General Anesthesia*, 6/26: 529-531). Sipahi *et al.* describe a VL case with abnormal clotting time and reduced fibrinogen which can develop due to ongoing disseminated intravascular coagulation and hepatic synthetic dysfunction Sipahi *et al.*, 2005, *The Turkish Journal of Pediatrics*, 47: 191-194.).

[0163] As Agranoff *et al* (2005) discussed, identification of the individual biomarkers will enable the design of immunologically based antigen-detection tests that could be implemented in dipstick or cassette formats. Furthermore, insights into disease pathophysiology afforded by knowledge of changes in circulating proteins could, ultimately, help to direct the search for novel drug targets. Because immunodetection of proteins is quantitatively more precise than MS, diagnostic performance of these downstream applications might surpass that of the original high-tech discovery-phase 'test'.

[0164] From the foregoing description, various modifications and changes in the compositions and methods will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein. Each recited range includes all combinations and sub-combinations of ranges, as well as specific numerals contained therein.

[0165] Each recited range includes all combinations and sub-combinations of ranges, as well as specific numerals contained therein.

[0166] All publications and patent documents cited above are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

[0167] Although the foregoing invention has been described in detail by way of example for purposes of clarity of understanding, it will be apparent to the artisan that certain changes and modifications are comprehended by the disclosure and can be practiced without undue experimentation within the scope of the appended claims, which are presented by way of illustration not limitation.

WHAT IS CLAIMED IS:

1. A method for qualifying Leishmaniasis status in a subject comprising:
 - (a) measuring at least one biomarker in a biological sample from the subject, wherein the at least one biomarker is selected from the biomarkers of Table 1; and
 - (b) correlating the measurement with Leishmaniasis status.
2. The method of claim 1, wherein the at least one biomarker is selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.
3. The method of claim 1, wherein the measuring comprises determining the level of expression of the at least one biomarker.
4. The method of claim 3, wherein the mean level of expression of the at least one biomarker in patients having a first Leishmaniasis status is about twice or more than the mean level of expression in patients having a second Leishmaniasis status.
5. The method of claim 3, wherein the correlating comprises comparing the level of expression of the at least one biomarker with a pre-determined level of expression.
6. The method of claim 5, wherein the pre-determined level of expression distinguishes between a first Leishmaniasis status and a second Leishmaniasis status with a probability of about 0.05 or less.
7. The method of claim 5, wherein the pre-determined level of expression distinguishes between a first Leishmaniasis status and a second Leishmaniasis status with an Receiver Operator Characteristic (ROC) of 0.7 or greater.
8. The method of claim 5, wherein the at least one biomarker is two or more biomarkers and the correlating comprises comparing the level of expression of each biomarker with a pre-determined level of expression.

9. The method of claim 8, wherein the pre-determined level of expression of each biomarker distinguishes between a first Leishmaniasis status and a second Leishmaniasis status with an Receiver Operator Characteristic (ROC) of about 0.3 or less or about 0.7 or greater.

10. The method of any of claims 5-9, wherein the at least one biomarker is a single biomarker.

11. The method of any of claims 5-9, wherein the pre-determined level is less than the detection threshold.

12. The method of any of claims 1-11, wherein the at least one biomarker is measured by capturing the biomarker on an adsorbent surface of a SELDI probe and detecting the captured biomarkers by laser desorption-ionization mass spectrometry.

13. The method of any of claims 1-11, wherein the at least one biomarker is measured by immunoassay.

14. The method of any of claims 1-11, wherein the sample is serum.

15. The method of any of claims 1-11, wherein the correlating is performed by a software classification algorithm.

16. The method of any of claims 1-11, wherein Leishmaniasis status is selected from chronic symptomatic, chronic asymptomatic, acute, and uninfected.

17. The method of any of claims 1-11, wherein Leishmaniasis status is selected from Leishmaniasis versus non-Leishmaniasis.

18. The method of any of claims 1-11, further comprising: (c) managing subject treatment based on the status.

19. The method of claim 12, wherein the adsorbent is a cation exchange adsorbent.

20. The method of claim 12, wherein the adsorbent is a metal chelate adsorbent.

21. The method of claim 12, wherein the adsorbent is a biospecific adsorbent.
22. The method of claim 18, wherein, if the measurement correlates with Leishmaniasis, then managing subject treatment comprises administering sodium stibogluconate.
23. The method of claim 18, further comprising: (d) measuring the at least one biomarker after subject management and correlating the measurement with disease progression.
24. The method of any one of claims 1-23, wherein the subject is a mammal.
25. The method of claim 24, wherein the subject is a human.
26. A method for determining the course of Leishmaniasis comprising:
- (a) measuring, at a first time, at least one biomarker in a biological sample from the subject, wherein the at least one biomarker is selected from the group consisting of the biomarkers of Table 1 and Table 5;
 - (b) measuring, at a second time, the at least one biomarker in a biological sample from the subject; and
 - (c) comparing the first measurement and the second measurement; wherein the comparative measurements determine the course of Leishmaniasis.
27. A method comprising measuring at least one biomarker in a sample from a subject, wherein the at least one biomarker is selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.
28. The method of any one of claims 26-27, wherein the comparative measurements determine the course of Visceral Leishmaniasis.
29. A composition comprising a purified biomolecule selected from the group consisting of the biomarkers of Table 1 and Table 5.

30. A composition comprising a biospecific capture reagent that specifically binds a biomolecule selected from the group consisting of the biomarkers of Table 1 and Table 5.

31. The composition of claim 30, wherein the biospecific capture reagent is an antibody.

32. The composition of claim 30, wherein the biospecific capture reagent is bound to a solid support.

33. A composition comprising a biospecific capture reagent bound to a biomarker of Table 1 or Table 5.

34. A kit comprising:

(a) a solid support comprising at least one capture reagent attached thereto, wherein the capture reagent binds at least one biomarker from a first group consisting of the Biomarkers of Table 1 and Table 5; and

(b) instructions for using the solid support to detect a biomarker of Table 1 or Table 5.

35. The kit of claim 34 comprising instructions for using the solid support to detect at least one biomarker selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

36. The kit of any of claims 34-35, wherein the solid support comprising a capture reagent is a SELDI probe.

37. The kit of any of claims 34-36, wherein the capture reagent is a cation exchange adsorbent.

38. The kit of any of claims 34-37, wherein the adsorbent is a metal chelate adsorbent.

39. The kit of any of claims 34-38, additionally comprising: (c) a container containing at least one of the biomarkers of Table 1 or Table 5.

40. The kit of any of claims of any of claims 34-39, additionally comprising: (c) a is a cation exchange adsorbent.

41. The kit of any of claims of any of claims 35-40, additionally comprising: (c) a is a metal chelate adsorbent.

42. A kit comprising:

(a) a solid support comprising at least one capture reagent attached thereto, wherein the capture reagents bind at least one biomarker selected from the group consisting of the biomarkers of Table 1 and Table 5; and

(b) a container containing at least one of the biomarkers.

43. The kit of claim 42, wherein the container contains at least one biomarker selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa 7.7 kDa, about 51.3 kDa, about 56.6 kDa, about 94.1 kDa, about 101.4 kDa, and about 120.9 kDa.

44. The kit of claim 42-43, wherein the container contains at least one biomarker selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

45. The kit of any of claims 42-44, wherein the solid support comprising a capture reagent is a SELDI probe.

46. The kit of any of claims 42-45, wherein the capture reagent is a cation exchange adsorbent.

47. The kit of any of claims 42-46, wherein the capture reagent is a metal chelate adsorbent.

48. A software product comprising:

(a) code that accesses data attributed to a sample, the data comprising measurement of at least one biomarker in the sample, the biomarker selected from the group consisting of the biomarkers of Table 1 and Table 5; and

(b) code that executes a classification algorithm that classifies the <disease> status of the sample as a function of the measurement.

49. The software product of claim 48, wherein the classification algorithm classifies the Leishmaniasis status of the sample as a function of the measurement of a biomarker selected from the group consisting of biomarkers having molecular weights of: about 3.3 kDa, about 3.5 kDa, about 5 kDa, about 9.3 kDa, about 9.4 kDa, about 12.4 kDa, about 12.6 kDa, about 28.2 kDa, and about 51.3 kDa.

50. A method comprising detecting a biomarker of Table 1 by mass spectrometry or immunoassay.

51. A method comprising communicating to a subject a diagnosis relating to Leishmaniasis status determined from the correlation of biomarkers in a sample from the subject, wherein said biomarkers are selected from the group consisting of Table 1 and Table 5.

52. The method of claim 51, wherein the diagnosis is communicated to the subject via a computer-generated medium.

53. A method for identifying a compound that interacts with a biomarker of Table 1 or Table 5 wherein said method comprises:

(a) contacting a biomarker of Table 1 or Table 5 with a test compound; and

(b) determining whether the test compound interacts with a biomarker of Table 1 or Table 5.

54. A method for qualifying Leishmaniasis status in a subject in comparison to the status of a different parasitic infection, the method comprising:

(a) measuring at least one biomarker in a biological sample from the subject, wherein the at least one biomarker specifically indicates the presence of Leishmaniasis and does not indicate the presence of a different parasitic infection; and

(b) correlating the measuring with Leishmaniasis status in comparison to the status of a different parasitic infection.

55. The method of claim 54 further comprising measuring one or more biomarkers selected from the group of the biomarkers of Table 1 and Table 5.

56. The method of claim 54 further comprising measuring one or more biomarkers selected from the group of the biomarkers of Figures 1-7.

57. The method of claim 54 wherein said parasitic infection comprises a kinetoplastidae infection.

58. The method of claim 54 wherein the parasitic infection is African sleeping sickness, Leishmaniasis, or malaria.

59. A method for monitoring the course of progression of Leishmaniasis in a patient comprising :

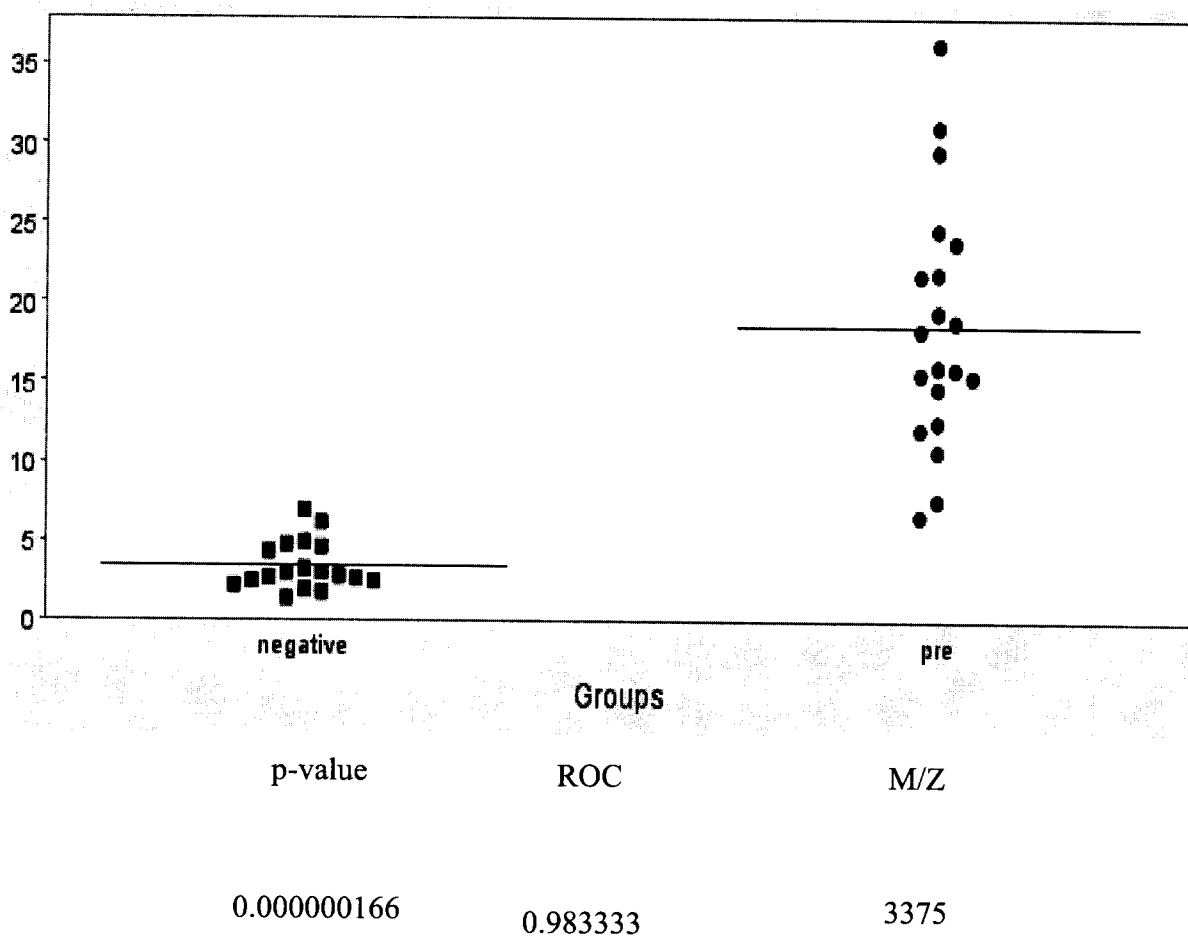
(a) measuring at least one biomarker in a first biological sample from said patient, wherein the at least one biomarker specifically indicates the presence of Leishmaniasis; and

(b) measuring said at least one biomarker in a second biological sample from said subject, wherein said second biological sample was obtained from said subject after said first biological sample; and

(c) correlating said measurements with the progression or regression of Leishmaniasis in said subject.

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Figure 1



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Peak View

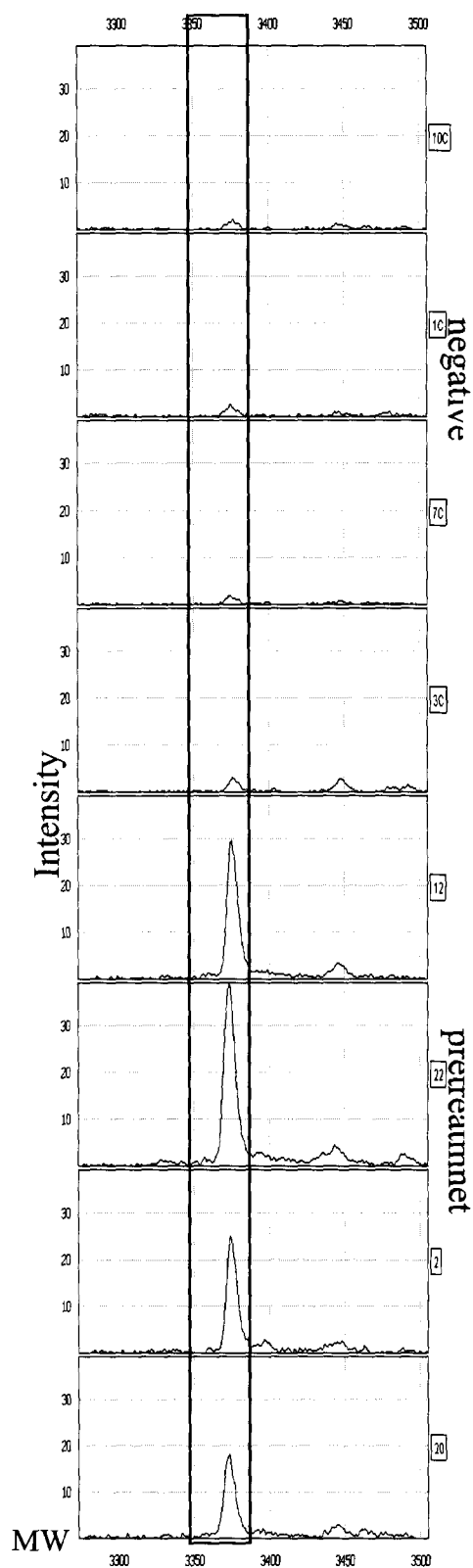
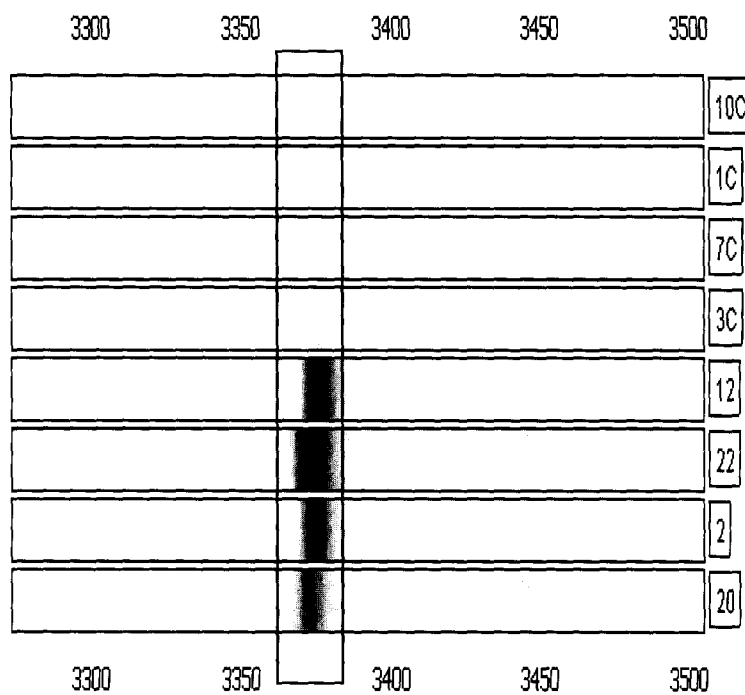


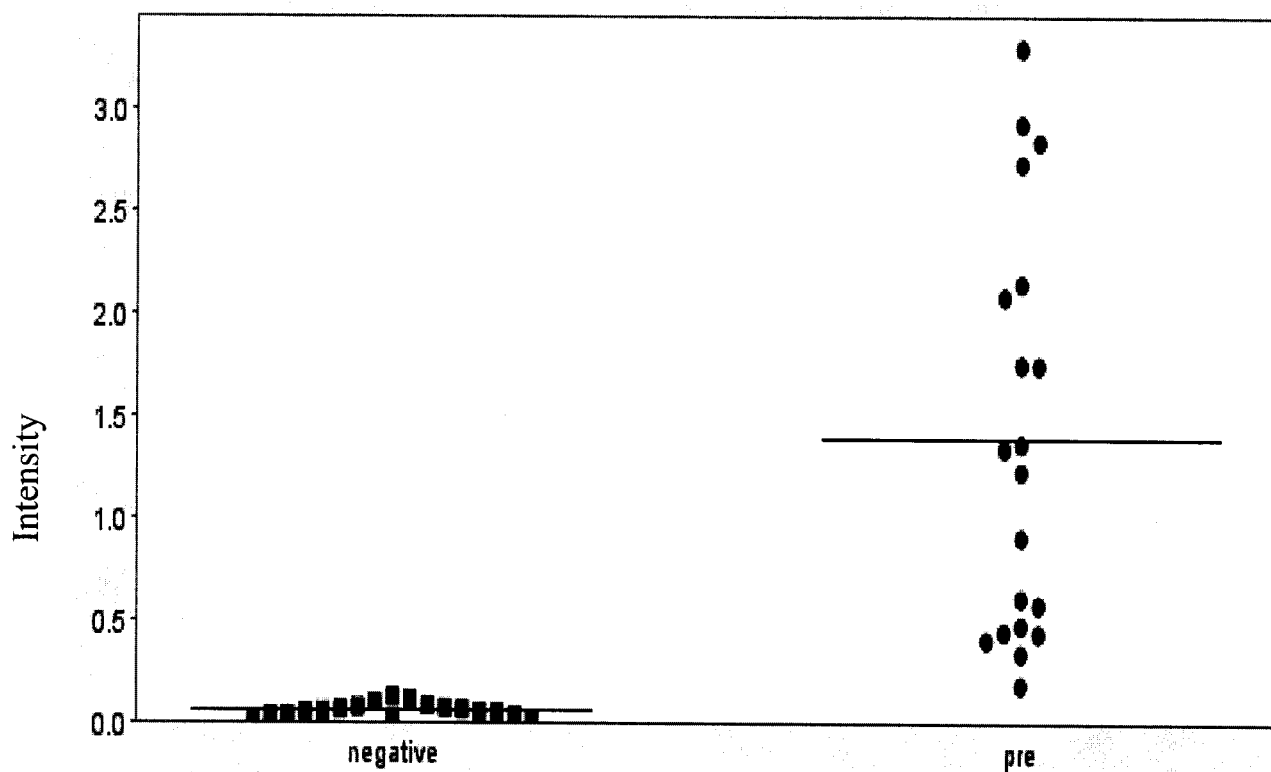
Figure 2

Gel View



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Figure 3



p-value

Groups

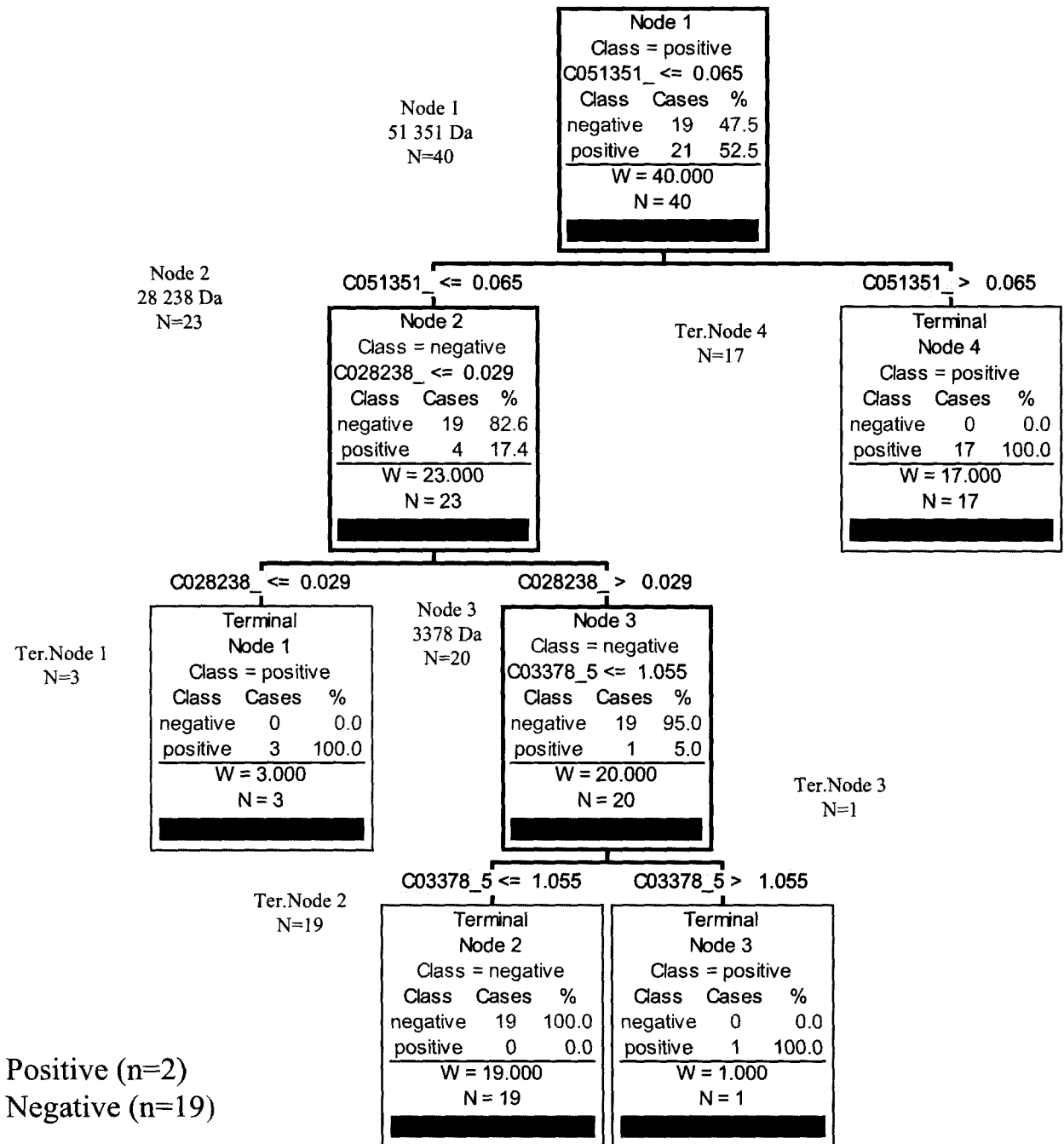
M/Z

0.000000142

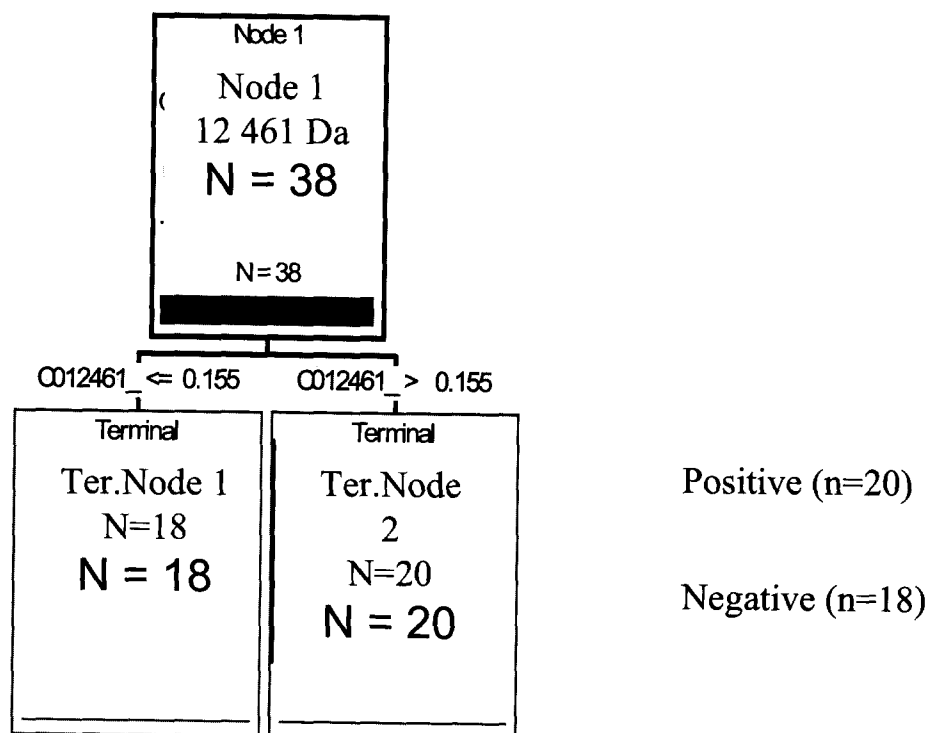
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12463

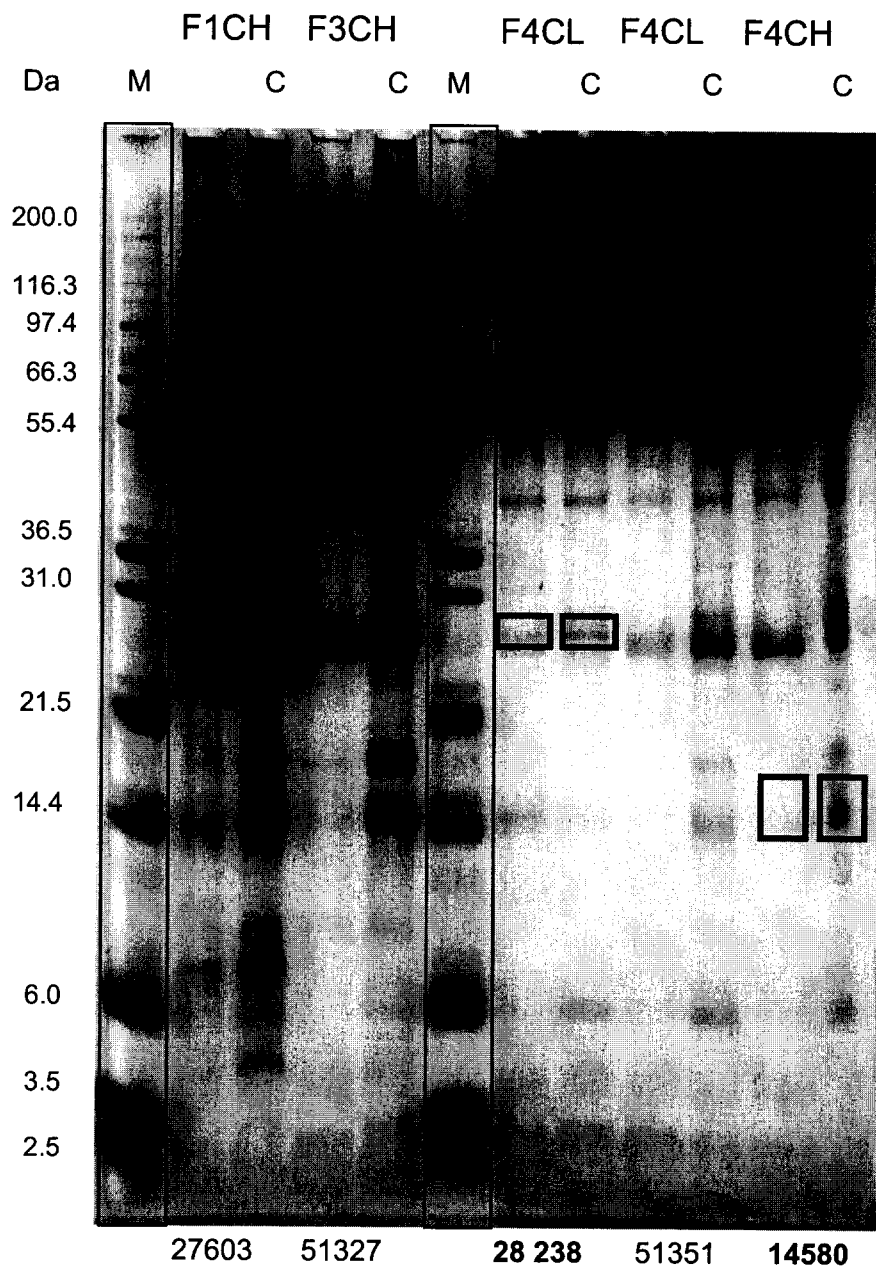
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Figure 4

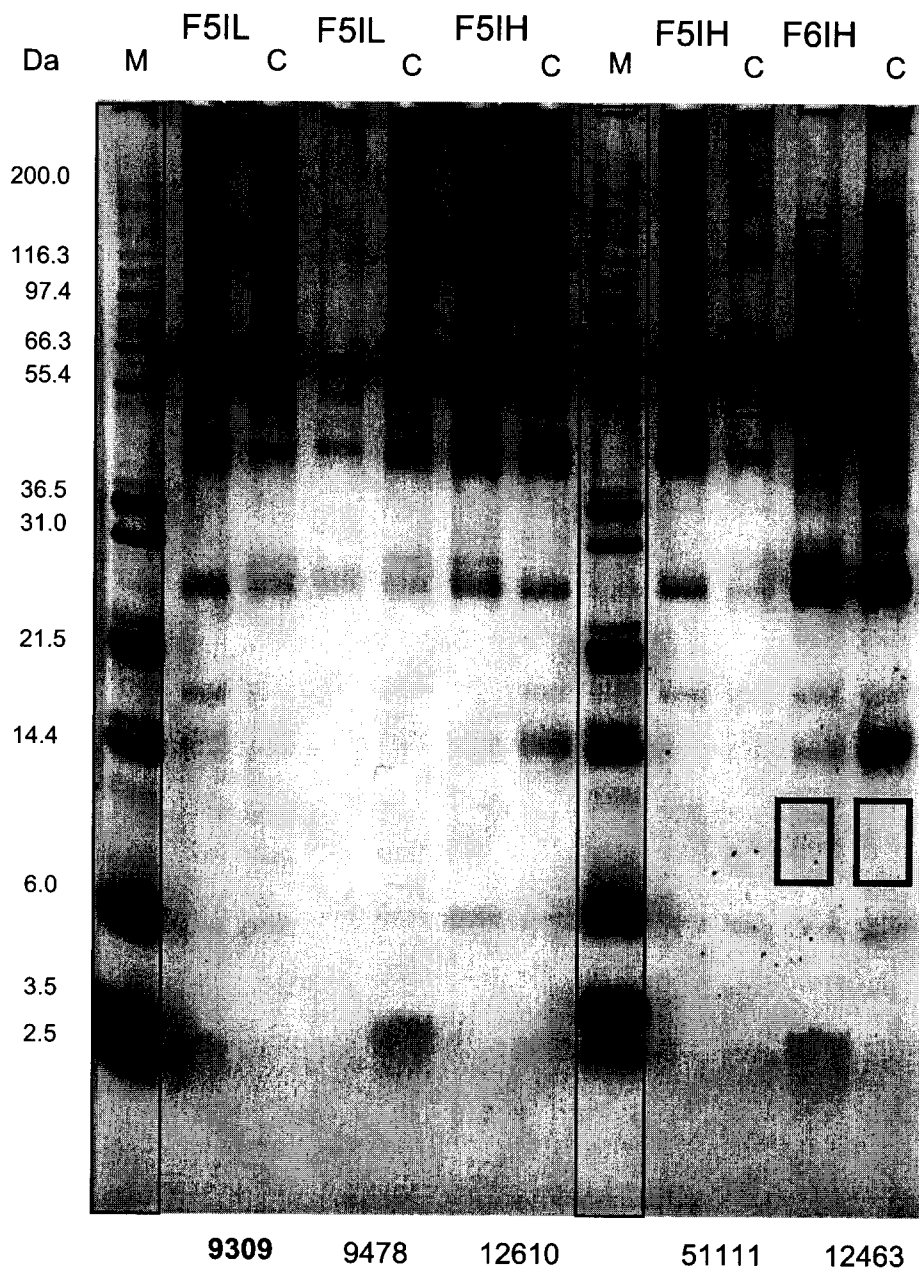
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Figure 5

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Figure 6

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Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2008/001948

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **G01N 33/53** (2006.01) , **A61P 33/02** (2006.01) , **A61K 31/555** (2006.01) , **G01N 27/00** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **G01N 33/53** (2006.01) , **A61P 33/02** (2006.01) , **A61K 31/555** (2006.01) , **G01N 27/00** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

CPD, Derwent, Scopus, Pubmed, Google

search terms: Leishmaniasis Leishmania apolipoprotein SELDI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	BEKAERT, E.D., et al. 'Plasma lipoproteins in infantile visceral Leishmaniasis: deficiency of apolipoproteins A-I and A-II' CLIN. CHIM. ACTA (1989) vol. 184, pages 181-192, see entire document.	1-11, 13-18, 22-26, 55 ----- 12, 19-21
X --- Y	BEKAERT, E.D., et al 'Alterations in lipoprotein density classes in infantile visceral Leishmaniasis: presence of apolipoprotein SAA' EUR. J. CLIN. INVEST. (1992), vol. 22, pages 190-199, see entire document.	1-11, 13-18, 22-26, 55 ----- 12, 19-21
Y	CA 2547861 (CAFFREY, R. et al.) 23 June 2005 (23-06-2005)	12, 19-21

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

27 February 2009 (27-02-2009)

Date of mailing of the international search report

3 March 2009 (03-03-2009)

Name and mailing address of the ISA/CA
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Facsimile No.: 001-819-953-2476

Authorized officer

Kathryn Moore 819- 934-9088

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2008/001948**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

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

1. ☐ Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :
2. ☒ Claim Nos. : 1-26, 55
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

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

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

This International Searching Authority found multiple inventions in this international application, as follows :

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. : 1-26, 55 (partially)

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2008/001948

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA 2547861A1	23-06-2005	BR PI0417231A	17-04-2007
		EP 1718763A2	08-11-2006
		EP 1718763A4	25-02-2009
		US 2005260691A1	24-11-2005
		WO 2005055812A2	23-06-2005
		WO 2005055812A3	15-09-2005

Continuation of Box No. II:

The subject matter of claims 1-26, 55 was not searched for the following reasons: a complete search over the whole scope of these claims is impossible as claims 1, 3-26, 55 define 212 possible biomarkers which are only defined by mass, and so it is not possible to determine what each of these biomarkers actually represents. Further, the nine biomarkers listed in claim 2 are also only defined by mass.

Continuation of Box No. III:

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-26, 55 are directed to a method for qualifying Leishmaniasis status in a subject by measuring a biomarker selected from Table 1.

Group B - Claims 27-28 are directed to a method of measuring a biomarker.

Group C - Claim 29 is directed to a composition comprising a purified biomolecule from Table 1 or Table 5.

Group D - Claims 30-47 are directed to a composition and kits comprising a biospecific capture reagent that specifically binds to a biomolecule from Table 1 or Table 5.

Group E - Claims 48-49 are directed to a software product.

Group F - Claim 50 is directed to a method comprising detecting a biomarker of Table 1 by mass spectrometry or immunoassay.

Group G - Claims 51-52 are directed to a method of communicating to a subject a diagnosis relating to Leishmaniasis status.

Group H - Claim 53 is directed to a method for identifying a compound that interacts with a biomarker of Table 1 or Table 5.

Group I - Claims 54, 56-59 are directed to a method for qualifying or monitoring Leishmaniasis status in a subject by measuring a biomarker that specifically indicates the presence of Leishmaniasis.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT. The common concept linking groups A, C, D, E, F, G, H is a biomarker selected from Table 1. However, the identities of certain biomarkers from Table 1 have been determined, and are shown in Table 5, and include such biomarkers as apolipoprotein-A1 and antithrombin III, which are well known in the art. As such, there exists neither a common concept nor a common or corresponding special technical feature that forms a contribution over the prior art.

Further, each and every biomarker listed in Table 1 or Table 5, or combination thereof, needs to be assessed independently for its contribution to the state of the art. Thus, even within each given group, the claims are directed to a plurality of inventive concepts, each alleged invention being related to a different biomarker. As such, the international search report will be restricted to the first identified biomarker within Table 1, namely apolipoprotein A1 as identified in Table 5.