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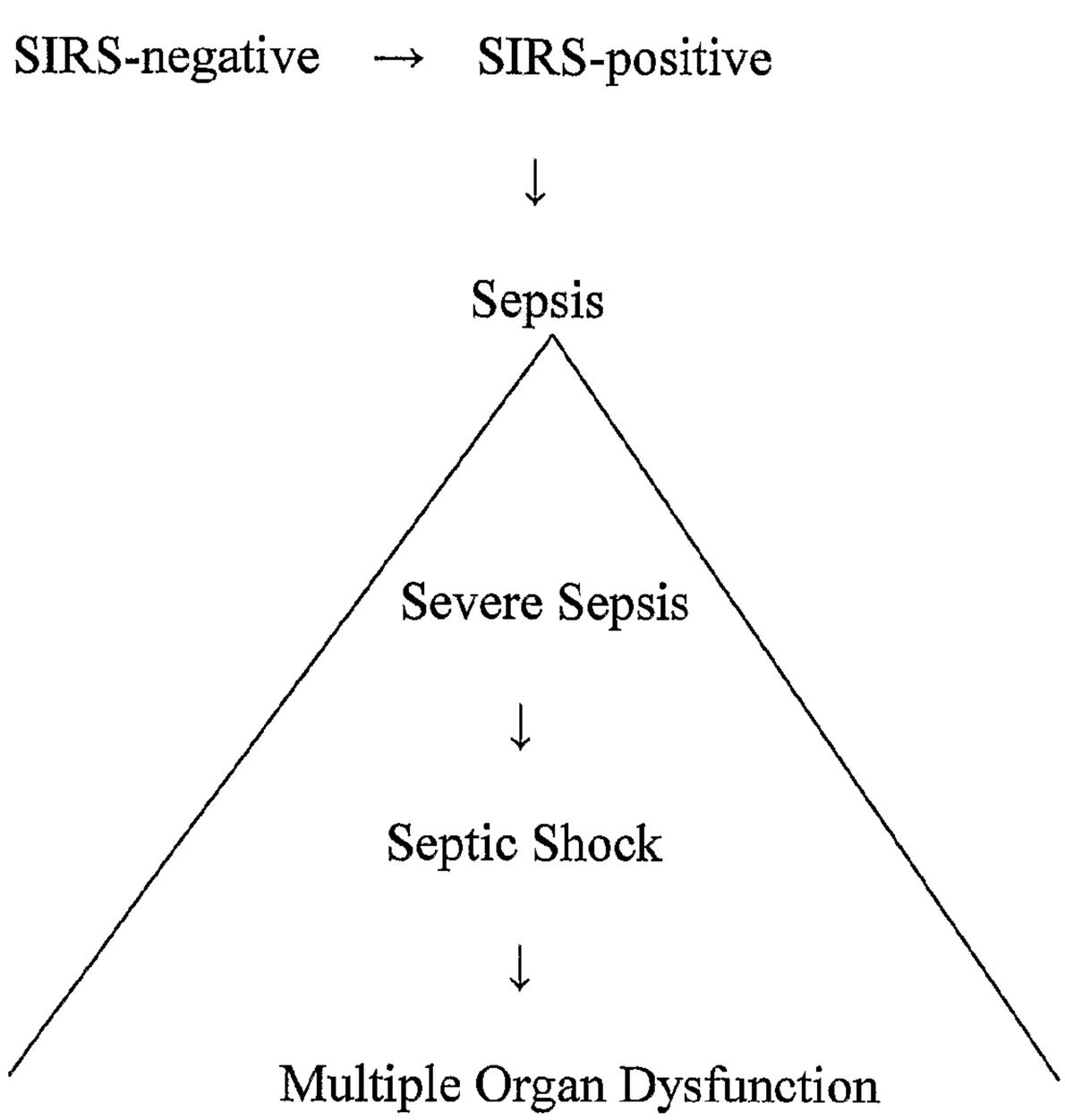
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(54) Title: DIAGNOSIS OF SEPSIS OR SIRS USING BIOMARKER PROFILES



(57) Abrégé/Abstract:
The early prediction or diagnosis of sepsis advantageously allows for clinical intervention before the disease rapidly progresses beyond initial stages to the more severe stages, such as severe sepsis or septic shock, which are associated with high mortality. Early prediction or diagnosis is accomplished by comparing an individual's profile of biomarker expression to profiles obtained from one or more control, or reference, populations, which may include a population that develops sepsis. Recognition of features in the individual's biomarker profile that are characteristic of the onset of sepsis allows a clinician to diagnose the onset of sepsis from a bodily fluid isolated from the individual at a single point in time. The necessity of monitoring the patient over a period of time is, therefore, avoided, advantageously allowing clinical intervention before the onset of serious symptoms of sepsis.

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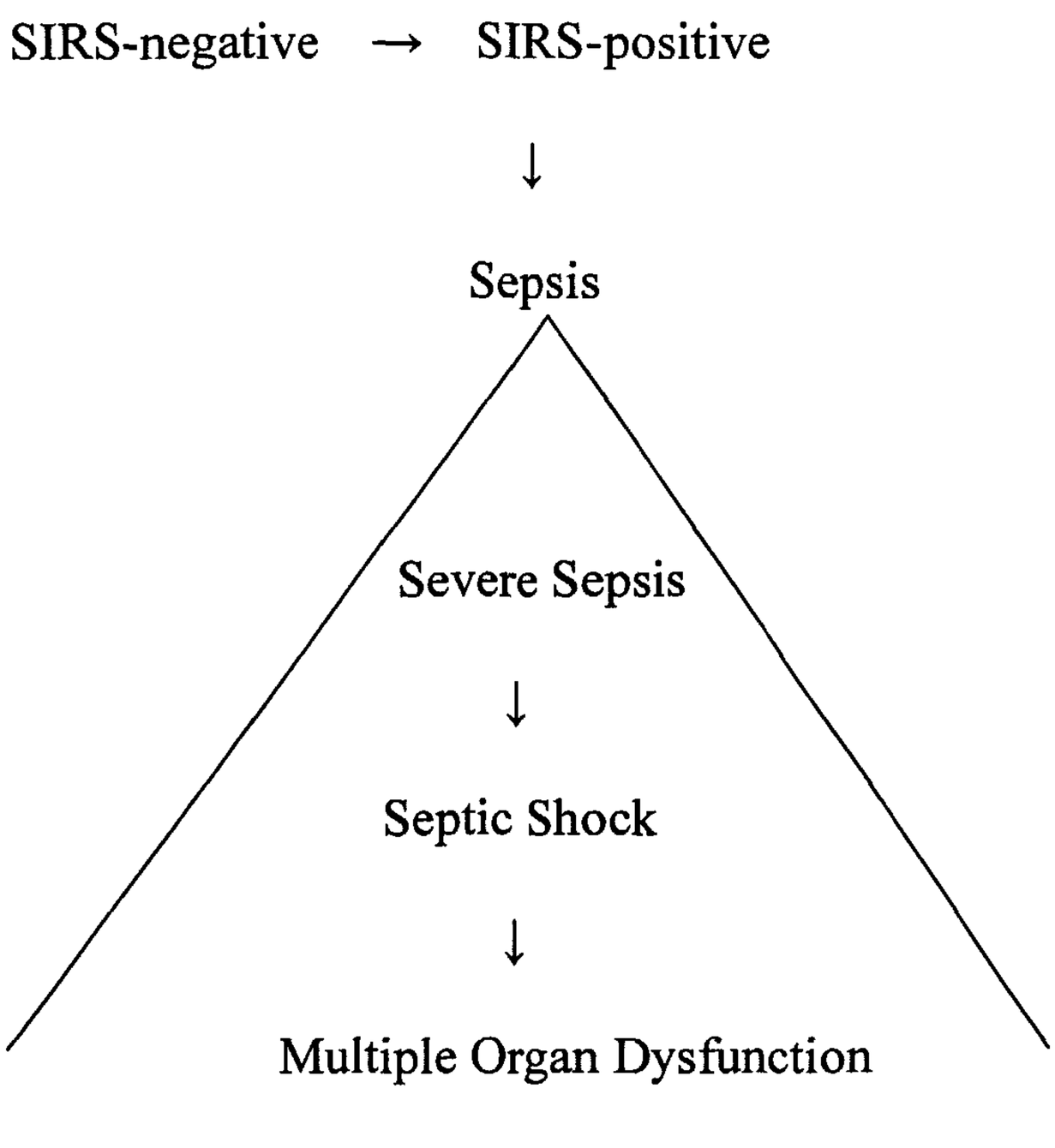
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(54) Title: DIAGNOSIS OF SEPSIS OR SIRS USING BIOMARKER PROFILES



(57) Abstract: The early prediction or diagnosis of sepsis advantageously allows for clinical intervention before the disease rapidly progresses beyond initial stages to the more severe stages, such as severe sepsis or septic shock, which are associated with high mortality. Early prediction or diagnosis is accomplished by comparing an individual's profile of biomarker expression to profiles obtained from one or more control, or reference, populations, which may include a population that develops sepsis. Recognition of features in the individual's biomarker profile that are characteristic of the onset of sepsis allows a clinician to diagnose the onset of sepsis from a bodily fluid isolated from the individual at a single point in time. The necessity of monitoring the patient over a period of time is, therefore, avoided, advantageously allowing clinical intervention before the onset of serious symptoms of sepsis.

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DIAGNOSIS OF SEPSIS OR SIRS USING BIOMARKER PROFILES

[0001] The present application claims priority to United States Provisional Patent Application Serial No. 60/425,322, filed November 12, 2002, and to United States Provisional Patent Application Serial No. 60/511,644, filed October 17, 2003, both of which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to methods of diagnosing or predicting sepsis or its stages of progression in an individual. The present invention also relates to methods of diagnosing systemic inflammatory response syndrome in an individual.

BACKGROUND OF THE INVENTION

[0003] Early detection of a disease condition typically allows for a more effective therapeutic treatment with a correspondingly more favorable clinical outcome. In many cases, however, early detection of disease symptoms is problematic; hence, a disease may become relatively advanced before diagnosis is possible. Systemic inflammatory conditions represent one such class of diseases. These conditions, particularly sepsis, typically result from an interaction between a pathogenic microorganism and the host's defense system that triggers an excessive and dysregulated inflammatory response in the host. The complexity of the host's response during the systemic inflammatory response has complicated efforts towards understanding disease pathogenesis. (Reviewed in Healy, *Annul. Pharmacother.* 36: 648-54 (2002).) An incomplete understanding of the disease pathogenesis, in turn, contributes to the difficulty in finding diagnostic biomarkers. Early and reliable diagnosis is imperative, however, because of the remarkably rapid progression of sepsis into a life-threatening condition.

[0004] Sepsis follows a well-described time course, progressing from systemic inflammatory response syndrome ("SIRS")-negative to SIRS-positive to sepsis, which may then progress to severe sepsis, septic shock, multiple organ dysfunction ("MOD"), and ultimately death. Sepsis also may arise in an infected individual when the individual subsequently develops SIRS. "SIRS" is commonly defined as the presence of two or more of the following parameters: body temperature greater than 38°C or less than 36°C; heart rate greater than 90 beats per minute; respiratory rate greater than 20 breaths per minute; P_{CO2} less than 32 mm Hg; and a white blood cell count either less than $4.0x10^9$ cells/L or greater than

$12.0x10^9$ cells/L, or having greater than 10% immature band forms. "Sepsis" is commonly defined as SIRS with a confirmed infectious process. "Severe sepsis" is associated with MOD, hypotension, disseminated intravascular coagulation ("DIC") or hypoperfusion abnormalities, including lactic acidosis, oliguria, and changes in mental status. "Septic shock" is commonly defined as sepsis-induced hypotension that is resistant to fluid resuscitation with the additional presence of hypoperfusion abnormalities.

[0005] Documenting the presence of the pathogenic microorganisms clinically significant to sepsis has proven difficult. Causative microorganisms typically are detected by culturing a patient's blood, sputum, urine, wound secretion, in-dwelling line catheter surfaces, etc. Causative microorganisms, however, may reside only in certain body microenvironments such that the particular material that is cultured may not contain the contaminating microorganisms. Detection may be complicated further by low numbers of microorganisms at the site of infection. Low numbers of pathogens in blood present a particular problem for diagnosing sepsis by culturing blood. In one study, for example, positive culture results were obtained in only 17% of patients presenting clinical manifestations of sepsis. (Rangel-Frausto *et al.*, *JAMA* 273: 117-23 (1995).) Diagnosis can be further complicated by contamination of samples by non-pathogenic microorganisms. For example, only 12.4% of detected microorganisms were clinically significant in a study of 707 patients with septicemia. (Weinstein *et al.*, *Clinical Infectious Diseases* 24: 584-602 (1997).)

[0006] The difficulty in early diagnosis of sepsis is reflected by the high morbidity and mortality associated with the disease. Sepsis currently is the tenth leading cause of death in the United States and is especially prevalent among hospitalized patients in non-coronary intensive care units (ICUs), where it is the most common cause of death. The overall rate of mortality is as high as 35%, with an estimated 750,000 cases per year occurring in the United States alone. The annual cost to treat sepsis in the United States alone is in the order of billions of dollars.

[0007] A need, therefore, exists for a method of diagnosing sepsis sufficiently early to allow effective intervention and prevention. Most existing sepsis scoring systems or predictive models predict only the risk of late-stage complications, including death, in patients who already are considered septic. Such systems and models, however, do not predict the development of sepsis itself. What is particularly needed is a way to categorize

those patients with SIRS who will or will not develop sepsis. Currently, researchers will typically define a single biomarker that is expressed at a different level in a group of septic patients versus a normal (*i.e.*, non-septic) control group of patients. United States Patent Application Serial No. 10/400,275, filed March 26, 2003, the entire contents of which are hereby incorporated by reference, discloses a method of indicating early sepsis by analyzing time-dependent changes in the expression level of various biomarkers. Accordingly, optimal methods of diagnosing early sepsis currently require both measuring a plurality of biomarkers and monitoring the expression of these biomarkers over a period of time.

[0008] There is a continuing urgent need in the art to diagnose sepsis with specificity and sensitivity, without the need for monitoring a patient over time. Ideally, diagnosis would be made by a technique that accurately, rapidly, and simultaneously measures a plurality of biomarkers at a single point in time, thereby minimizing disease progression during the time required for diagnosis.

SUMMARY OF THE INVENTION

[0009] The present invention allows for accurate, rapid, and sensitive prediction and diagnosis of sepsis through a measurement of more than one biomarker taken from a biological sample at a single point in time. This is accomplished by obtaining a biomarker profile at a single point in time from an individual, particularly an individual at risk of developing sepsis, having sepsis, or suspected of having sepsis, and comparing the biomarker profile from the individual to a reference biomarker profile. The reference biomarker profile may be obtained from a population of individuals (a "reference population") who are, for example, afflicted with sepsis or who are suffering from either the onset of sepsis or a particular stage in the progression of sepsis. If the biomarker profile from the individual contains appropriately characteristic features of the biomarker profile from the reference population, then the individual is diagnosed as having a more likely chance of becoming septic, as being afflicted with sepsis or as being at the particular stage in the progression of sepsis as the reference population. The reference biomarker profile may also be obtained from various populations of individuals including those who are suffering from SIRS or those who are suffering from an infection but who are not suffering from SIRS. Accordingly, the present invention allows the clinician to determine, *inter alia*, those patients who do not have SIRS, who have SIRS but are not likely to develop sepsis within the time frame of the investigation, who have sepsis, or who are at risk of eventually becoming septic.

[0010] Although the methods of the present invention are particularly useful for detecting or predicting the onset of sepsis in SIRS patients, one of ordinary skill in the art will understand that the present methods may be used for any patient including, but not limited to, patients suspected of having SIRS or of being at any stage of sepsis. For example, a biological sample could be taken from a patient, and a profile of biomarkers in the sample could be compared to several different reference biomarker profiles, each profile derived from individuals such as, for example, those having SIRS or being at a particular stage of sepsis. Classification of the patient's biomarker profile as corresponding to the profile derived from a particular reference population is predictive that the patient falls within the reference population. Based on the diagnosis resulting from the methods of the present invention, an appropriate treatment regimen could then be initiated.

[0011] Existing methods for the diagnosis or prediction of SIRS, sepsis or a stage in the progression of sepsis are based on clinical signs and symptoms that are nonspecific; therefore, the resulting diagnosis often has limited clinical utility. Because the methods of the present invention accurately detect various stages of sepsis, they can be used to identify those individuals who might appropriately be enrolled in a therapeutic study. Because sepsis may be predicted or diagnosed from a "snapshot" of biomarker expression in a biological sample obtained at a single point in time, this therapeutic study may be initiated before the onset of serious clinical symptoms. Because the biological sample is assayed for its biomarker profile, identification of the particular biomarkers is unnecessary. Nevertheless, the present invention provides methods to identify specific biomarkers of the profiles that are characteristic of sepsis or of a particular stage in the progression of sepsis. Such biomarkers themselves will be useful tools in predicting or diagnosing sepsis.

[0012] Accordingly, the present invention provides, *inter alia*, methods of predicting the onset of sepsis in an individual. The methods comprise obtaining a biomarker profile at a single point in time from the individual and comparing the individual's biomarker profile to a reference biomarker profile. Comparison of the biomarker profiles can predict the onset of sepsis in the individual with an accuracy of at least about 60%. This method may be repeated again at any time prior to the onset of sepsis.

[0013] The present invention also provides a method of diagnosing sepsis in an individual having or suspected of having sepsis comprising obtaining a biomarker profile at a single

point in time from the individual and comparing the individual's biomarker profile to a reference biomarker profile. Comparison of the biomarker profiles can diagnose sepsis in the individual with an accuracy of at least about 60%. This method may be repeated on the individual at any time.

[0014] The present invention further provides a method of determining the progression (*i.e.*, the stage) of sepsis in an individual having or suspected of having sepsis. This method comprises obtaining a biomarker profile at a single point in time from the individual and comparing the individual's biomarker profile to a reference biomarker profile. Comparison of the biomarker profiles can determine the progression of sepsis in the individual with an accuracy of at least about 60%. This method may also be repeated on the individual at any time.

[0015] Additionally, the present invention provides a method of diagnosing SIRS in an individual having or suspected of having SIRS. This method comprises obtaining a biomarker profile at a single point in time from the individual and comparing the individual's biomarker profile to a reference biomarker profile. Comparison of the biomarker profiles can diagnose SIRS in the individual with an accuracy of at least about 60%. This method may also be repeated on the individual at any time.

[0016] In another embodiment, the invention provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual comprising applying a decision rule. The decision rule comprises comparing (i) a biomarker profile generated from a biological sample taken from the individual at a single point in time with (ii) a biomarker profile generated from a reference population. Application of the decision rule determines the status of sepsis or diagnoses SIRS in the individual. The method may be repeated on the individual at one or more separate, single points in time.

[0017] The present invention further provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual comprising obtaining a biomarker profile from a biological sample taken from the individual and comparing the individual's biomarker profile to a reference biomarker profile. A single such comparison is capable of classifying the individual as having membership in the reference population. Comparison of the biomarker profile determines the status of sepsis or diagnoses SIRS in the individual.

[0018] The invention further provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual comprising obtaining a biomarker profile from a biological sample taken from the individual and comparing the individual's biomarker profile to a reference biomarker profile obtained from biological samples from a reference population. The reference population may be selected from the group consisting of a normal reference population, a SIRS-positive reference population, an infected/SIRS-negative reference population, a sepsis-positive reference population, a reference population at a particular stage in the progression of sepsis, a SIRS-positive reference population that will be confirmed as having sepsis by conventional techniques after about 0-36 hours, a SIRS-positive reference population that will be confirmed as having sepsis by conventional techniques after about 36-60 hours, and a SIRS-positive reference population that will be confirmed as having sepsis by conventional techniques after about 60-84 hours. A single such comparison is capable of classifying the individual as having membership in the reference population, and the comparison determines the status of sepsis or diagnoses SIRS in the individual.

[0019] In yet another embodiment, the present invention provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual. The method comprises comparing a measurable characteristic of at least one biomarker between a biomarker profile obtained from a biological sample from the individual and a biomarker profile obtained from biological samples from a reference population. Based on this comparison, the individual is classified as belonging to or not belonging to the reference population. The comparison, therefore, determines the status of sepsis or diagnoses SIRS in the individual. The biomarkers, in one embodiment, are selected from the group of biomarkers shown in any one of TABLES 15 – 23 and 26 – 50.

[0020] In a further embodiment, the present invention provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual comprising selecting at least two features from a set of biomarkers in a profile generated from a biological sample of an individual. These features are compared to a set of the same biomarkers in a profile generated from biological samples from a reference population. A single such comparison is capable of classifying the individual as having membership in the reference population with an accuracy of at least about 60%, and the comparison determines the status of sepsis or diagnoses SIRS in the individual.

[0021] The present invention also provides, *inter alia*, a method of determining the status of sepsis or diagnosing SIRS in an individual comprising determining the changes in the abundance of at least two biomarkers contained in a biological sample of an individual and comparing the abundance of these biomarkers in the individual's sample to the abundance of these biomarkers in biological samples from a reference population. The comparison is capable of classifying the individual as having membership in the reference population, and the comparison determines the status of sepsis or diagnoses SIRS in the individual.

[0022] In another embodiment, the invention provides, *inter alia*, a method of determining the status of sepsis in an individual, comprising determining changes in the abundance of at least one, two, three, four, five, 10 or 20 biomarkers as compared to changes in the abundance of the at least one, two, three, four, five, 10 or 20 biomarkers for biological samples from a reference population that contracted sepsis and one that did not. The biomarkers are selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50. Alternatively, the abundance of the at least one, two, three, four, five, 10 or 20 biomarkers may be compared to the abundance of the at least one, two, three, four, five, 10 or 20 biomarkers.

[0023] The present invention further provides, *inter alia*, a method of isolating a biomarker, the presence of which in a biological sample is diagnostic or predictive of sepsis. This method comprises obtaining a reference biomarker profile from a population of individuals and identifying a feature of the reference biomarker profile that is predictive or diagnostic of sepsis or one of the stages in the progression of sepsis. This method further comprises identifying a biomarker that corresponds with the feature and then isolating the biomarker.

[0024] In another embodiment, the present invention provides a kit comprising at least one, two, three, four, five, 10 or all of the biomarkers selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

[0025] In another embodiment, the reference biomarker profile may comprise a combination of at least two features, preferably five, 10, or 20 or more, where the features are characteristics of biomarkers in the sample. In this embodiment, the features will contribute to the prediction of the inclusion of an individual in a particular reference population. The relative contribution of the features in predicting inclusion may be determined by a data

analysis algorithm that predicts class inclusion with an accuracy of at least about 60%, at least about 70%, at least about 80%, at least about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%. In one embodiment, the combination of features allows the prediction of the onset of sepsis about 24, about 48, or about 72 hours prior to the actual onset of sepsis, as determined using conventional techniques.

[0026] In yet another embodiment, the reference biomarker profile may comprise at least two features, at least one of which is characteristic of the corresponding biomarker and where the feature will allow the prediction of inclusion of an individual in a sepsis-positive or SIRS-positive population. In this embodiment, the feature is assigned a p-value, which is obtained from a nonparametric test, such as a Wilcoxon Signed Rank Test, that is directly related to the degree of certainty with which the feature can classify an individual as belonging to a sepsis-positive or SIRS-positive population. In another embodiment, the feature classifies an individual as belonging to a sepsis-positive or SIRS-positive population with an accuracy of at least about 60%, about 70%, about 80%, or about 90%. In still another embodiment, the feature allows the prediction of the onset of sepsis about 24, about 48, or about 72 hours prior to the actual onset of sepsis, as determined using conventional techniques.

[0027] In yet another embodiment, the present invention provides an array of particles, with capture molecules attached to the surface of the particles that can bind specifically to at least one, two, three, four, five, 10 or all of the biomarkers selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIGURE 1 illustrates the progression of SIRS to sepsis. The condition of sepsis consists of at least three stages, with a septic patient progressing from severe sepsis to septic shock to multiple organ dysfunction.

[0029] FIGURE 2 shows the relationship between sepsis and SIRS. The various sets shown in the Venn diagram correspond to populations of individuals having the indicated condition.

[0030] FIGURE 3 shows the natural log of the ratio in average normalized peak intensities for about 400 ions for a sepsis-positive population versus a SIRS-positive population.

[0031] FIGURE 4 shows the intensity of an ion having an *m/z* of 437.2 Da and a retention time on a C_{18} reverse phase column of 1.42 min in an ESI-mass spectrometer profile. FIGURE 4A shows changes in the presence in the ion in various populations of individuals who developed sepsis. Clinical suspicion of sepsis in the sepsis group occurred at "time 0," as measured by conventional techniques. "Time –24 hours" and "time –48 hours" represent samples taken about 24 hours and about 48 hours, respectively, preceding the clinical suspicion of the onset of sepsis in the sepsis group. Individuals entered the study at "Day 1." FIGURE 4B shows the presence of the same ion in samples taken from populations of individuals who did not develop sepsis at time 0.

[0032] FIGURE 5 is a classification tree fitted to data from time 0 in 10 sepsis patients and 10 SIRS patients, showing three biomarkers identified by electrospray mass spectrometry that are involved in distinguishing sepsis from SIRS.

[0033] FIGURE 6 shows representative LC/MS and LC/MS/MS spectra obtained on plasma samples, using the configuration described in the examples.

[0034] FIGURES 7A and 7B show proteins that are regulated at higher levels in plasma up to 48 hours before conversion to sepsis.

[0035] FIGURES 8A and 8B show proteins that are regulated at lower levels in plasma up to 48 hours before conversion to sepsis.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0036] The present invention allows for the rapid, sensitive, and accurate diagnosis or prediction of sepsis using one or more biological samples obtained from an individual at a single time point ("snapshot") or during the course of disease progression. Advantageously, sepsis may be diagnosed or predicted prior to the onset of clinical symptoms, thereby allowing for more effective therapeutic intervention.

[0037] "Systemic inflammatory response syndrome," or "SIRS," refers to a clinical response to a variety of severe clinical insults, as manifested by two or more of the following conditions within a 24-hour period:

- body temperature greater than 38°C (100.4°F) or less than 36°C (96.8°F);
- heart rate (HR) greater than 90 beats/minute;

- respiratory rate (RR) greater than 20 breaths/minute, or P_{CO2} less than 32 mm Hg, or requiring mechanical ventilation; and
- white blood cell count (WBC) either greater than 12.0 x 10^9/L or less than 4.0 x 10^9/L or having greater than 10% immature forms (bands).

[0038] These symptoms of SIRS represent a consensus definition of SIRS that may be modified or supplanted by an improved definition in the future. The present definition is used to clarify current clinical practice and does not represent a critical aspect of the invention.

[0039] A patient with SIRS has a clinical presentation that is classified as SIRS, as defined above, but is not clinically deemed to be septic. Individuals who are at risk of developing sepsis include patients in an ICU and those who have otherwise suffered from a physiological trauma, such as a burn or other insult. "Sepsis" refers to a SIRS-positive condition that is associated with a confirmed infectious process. Clinical suspicion of sepsis arises from the suspicion that the SIRS-positive condition of a SIRS patient is a result of an infectious process. As used herein, "sepsis" includes all stages of sepsis including, but not limited to, the onset of sepsis, severe sepsis and MOD associated with the end stages of sepsis.

[0040] The "onset of sepsis" refers to an early stage of sepsis, *i.e.*, prior to a stage when the clinical manifestations are sufficient to support a clinical suspicion of sepsis. Because the methods of the present invention are used to detect sepsis prior to a time that sepsis would be suspected using conventional techniques, the patient's disease status at early sepsis can only be confirmed retrospectively, when the manifestation of sepsis is more clinically obvious. The exact mechanism by which a patient becomes septic is not a critical aspect of the invention. The methods of the present invention can detect changes in the biomarker profile independent of the origin of the infectious process. Regardless of how sepsis arises, the methods of the present invention allow for determining the status of a patient having, or suspected of having, sepsis or SIRS, as classified by previously used criteria.

[0041] "Severe sepsis" refers to sepsis associated with organ dysfunction, hypoperfusion abnormalities, or sepsis-induced hypotension. Hypoperfusion abnormalities include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. "Septic shock" refers to sepsis-induced hypotension that is not responsive to adequate intravenous fluid

challenge and with manifestations of peripheral hypoperfusion. A "converter patient" refers to a SIRS-positive patient who progresses to clinical suspicion of sepsis during the period the patient is monitored, typically during an ICU stay. A "non-converter patient" refers to a SIRS-positive patient who does not progress to clinical suspicion of sepsis during the period the patient is monitored, typically during an ICU stay.

[0042] A "biomarker" is virtually any biological compound, such as a protein and a fragment thereof, a peptide, a polypeptide, a proteoglycan, a glycoprotein, a lipoprotein, a carbohydrate, a lipid, a nucleic acid, an organic or inorganic chemical, a natural polymer, and a small molecule that are present in the biological sample and that may be isolated from, or measured in, the biological sample. Furthermore, a biomarker can be the entire intact molecule, or it can be a portion thereof that may be partially functional or recognized, for example, by an antibody or other specific binding protein. A biomarker is considered to be informative if a measurable aspect of the biomarker is associated with a given state of the patient, such as a particular stage of sepsis. Such a measurable aspect may include, for example, the presence, absence, or concentration of the biomarker in the biological sample from the individual and/or its presence as part of a profile of biomarkers. Such a measurable aspect of a biomarker is defined herein as a "feature." A feature may also be a ratio of two or more measurable aspects of biomarkers, which biomarkers may or may not be of known identity, for example. A "biomarker profile" comprises at least two such features, where the features can correspond to the same or different classes of biomarkers such as, for example, a nucleic acid and a carbohydrate. A biomarker profile may also comprise at least three, four, five, 10, 20, 30 or more features. In one embodiment, a biomarker profile comprises hundreds, or even thousands, of features. In another embodiment, the biomarker profile comprises at least one measurable aspect of at least one internal standard.

[0043] A "phenotypic change" is a detectable change in a parameter associated with a given state of the patient. For instance, a phenotypic change may include an increase or decrease of a biomarker in a bodily fluid, where the change is associated with sepsis or the onset of sepsis. A phenotypic change may further include a change in a detectable aspect of a given state of the patient that is not a change in a measurable aspect of a biomarker. For example, a change in phenotype may include a detectable change in body temperature, respiration rate, pulse, blood pressure, or other physiological parameter. Such changes can be determined via clinical observation and measurement using conventional techniques that are

well-known to the skilled artisan. As used herein, "conventional techniques" are those techniques that classify an individual based on phenotypic changes without obtaining a biomarker profile according to the present invention.

[0044] A "decision rule" is a method used to classify patients. This rule can take on one or more forms that are known in the art, as exemplified in Hastie *et al.*, in "The Elements of Statistical Learning," Springer-Verlag (Springer, New York (2001)), herein incorporated by reference in its entirety. Analysis of biomarkers in the complex mixture of molecules within the sample generates features in a data set. A decision rule may be used to act on a data set of features to, *inter alia*, predict the onset of sepsis, to determine the progression of sepsis, to diagnose sepsis, or to diagnose SIRS.

[0045] The application of the decision rule does not require perfect classification. A classification may be made with at least about 90% certainty, or even more, in one embodiment. In other embodiments, the certainty is at least about 80%, at least about 70%, or at least about 60%. The useful degree of certainty may vary, depending on the particular method of the present invention. "Certainty" is defined as the total number of accurately classified individuals divided by the total number of individuals subjected to classification. As used herein, "certainty" means "accuracy." Classification may also be characterized by its "sensitivity." The "sensitivity" of classification relates to the percentage of sepsis patients who were correctly identified as having sepsis. "Sensitivity" is defined in the art as the number of true positives divided by the sum of true positives and false negatives. In contrast, the "specificity" of the method is defined as the percentage of patients who were correctly identified as not having sepsis. That is, "specificity" relates to the number of true negatives divided by the sum of true negatives and false positives. In one embodiment, the sensitivity and/or specificity is at least 90%, at least 80%, at least 70% or at least 60%. The number of features that may be used to classify an individual with adequate certainty is typically about four. Depending on the degree of certainty sought, however, the number of features may be more or less, but in all cases is at least one. In one embodiment, the number of features that may be used to classify an individual is optimized to allow a classification of an individual with high certainty.

[0046] "Determining the status" of sepsis or SIRS in a patient encompasses classification of a patient's biomarker profile to (1) detect the presence of sepsis or SIRS in the patient, (2)

predict the onset of sepsis or SIRS in the patient, or (3) measure the progression of sepsis in a patient. "Diagnosing" sepsis or SIRS means to identify or detect sepsis or SIRS in the patient. Because of the greater sensitivity of the present invention to detect sepsis before an overtly observable clinical manifestation, the identification or detection of sepsis includes the detection of the onset of sepsis, as defined above. That is, "predicting the onset of sepsis" means to classify the patient's biomarker profile as corresponding to the profile derived from individuals who are progressing from a particular stage of SIRS to sepsis or from a state of being infected to sepsis (*i.e.*, from infection to infection with concomitant SIRS). "Detecting the progression" or "determining the progression" of sepsis or SIRS means to classify the biomarker profile of a patient who is already diagnosed as having sepsis or SIRS. For instance, classifying the biomarker profile of a patient who has been diagnosed as having sepsis can encompass detecting or determining the progression of the patient from sepsis to severe sepsis or to sepsis with MOD.

[0047] According to the present invention, sepsis may be diagnosed or predicted by obtaining a profile of biomarkers from a sample obtained from an individual. As used herein, "obtain" means "to come into possession of." The present invention is particularly useful in predicting and diagnosing sepsis in an individual who has an infection, or even sepsis, but who has not yet been diagnosed as having sepsis, who is suspected of having sepsis, or who is at risk of developing sepsis. In the same manner, the present invention may be used to detect and diagnose SIRS in an individual. That is, the present invention may be used to confirm a clinical suspicion of SIRS. The present invention also may be used to detect various stages of the sepsis process such as infection, bacteremia, sepsis, severe sepsis, septic shock and the like.

[0048] The profile of biomarkers obtained from an individual, *i.e.,* the test biomarker profile, is compared to a reference biomarker profile. The reference biomarker profile can be generated from one individual or a population of two or more individuals. The population, for example, may comprise three, four, five, ten, 15, 20, 30, 40, 50 or more individuals. Furthermore, the reference biomarker profile and the individual's (test) biomarker profile that are compared in the methods of the present invention may be generated from the same individual, provided that the test and reference profiles are generated from biological samples taken at different time points and compared to one another. For example, a sample may be obtained from an individual at the start of a study period. A reference biomarker profile

taken from that sample may then be compared to biomarker profiles generated from subsequent samples from the same individual. Such a comparison may be used, for example, to determine the status of sepsis in the individual by repeated classifications over time.

[0049] The reference populations may be chosen from individuals who do not have SIRS ("SIRS-negative"), from individuals who do not have SIRS but who are suffering from an infectious process, from individuals who are suffering from SIRS without the presence of sepsis ("SIRS-positive"), from individuals who are suffering from the onset of sepsis, from individuals who are sepsis-positive and suffering from one of the stages in the progression of sepsis, or from individuals with a physiological trauma that increases the risk of developing sepsis. Furthermore, the reference populations may be SIRS-positive and are then subsequently diagnosed with sepsis using conventional techniques. For example, a population of SIRS-positive patients used to generate the reference profile may be diagnosed with sepsis about 24, 48, 72, 96 or more hours after biological samples were taken from them for the purposes of generating a reference profile. In one embodiment, the population of SIRS-positive individuals is diagnosed with sepsis using conventional techniques about 0-36 hours, about 36-60 hours, about 60-84 hours, or about 84-108 hours after the biological samples were taken. If the biomarker profile is indicative of sepsis or one of its stages of progression, a clinician may begin treatment prior to the manifestation of clinical symptoms of sepsis. Treatment typically will involve examining the patient to determine the source of the infection. Once locating the source, the clinician typically will obtain cultures from the site of the infection, preferably before beginning relevant empirical antimicrobial therapy and perhaps additional adjunctive therapeutic measures, such as draining an abscess or removing an infected catheter. Therapies for sepsis are reviewed in Healy, *supra*.

[0050] The methods of the present invention comprise comparing an individual's biomarker profile with a reference biomarker profile. As used herein, "comparison" includes any means to discern at least one difference in the individual's and the reference biomarker profiles. Thus, a comparison may include a visual inspection of chromatographic spectra, and a comparison may include arithmetical or statistical comparisons of values assigned to the features of the profiles. Such statistical comparisons include, but are not limited to, applying a decision rule. If the biomarker profiles comprise at least one internal standard, the comparison to discern a difference in the biomarker profiles may also include features of these internal standards, such that features of the biomarker are correlated to features of the

internal standards. The comparison can predict, *inter alia*, the chances of acquiring sepsis or SIRS; or the comparison can confirm the presence or absence of sepsis or SIRS; or the comparison can indicate the stage of sepsis at which an individual may be.

[0051] The present invention, therefore, obviates the need to conduct time-intensive assays over a monitoring period, as well as the need to identify each biomarker. Although the invention does not require a monitoring period to classify an individual, it will be understood that repeated classifications of the individual, *i.e.*, repeated snapshots, may be taken over time until the individual is no longer at risk. Alternatively, a profile of biomarkers obtained from the individual may be compared to one or more profiles of biomarkers obtained from the same individual at different points in time. The artisan will appreciate that each comparison made in the process of repeated classifications is capable of classifying the individual as having membership in the reference population.

[0052] Individuals having a variety of physiological conditions corresponding to the various stages in the progression of sepsis, from the absence of sepsis to MOD, may be distinguished by a characteristic biomarker profile. As used herein, an "individual" is an animal, preferably a mammal, more preferably a human or non-human primate. The terms "individual," "subject" and "patient" are used interchangeably herein. The individual can be normal, suspected of having SIRS or sepsis, at risk of developing SIRS or sepsis, or confirmed as having SIRS or sepsis. While there are many known biomarkers that have been implicated in the progression of sepsis, not all of these markers appear in the initial, pre-clinical stages. The subset of biomarkers characteristic of early-stage sepsis may, in fact, be determined only by a retrospective analysis of samples obtained from individuals who ultimately manifest clinical symptoms of sepsis. Without being bound by theory, even an initial pathologic infection that results in sepsis may provoke physiological changes that are reflected in particular changes in biomarker expression. Once the characteristic biomarker profile of a stage of sepsis, for example, is determined, the profile of biomarkers from a biological sample obtained from an individual may be compared to this reference profile to determine whether the test subject is also at that particular stage of sepsis.

[0053] The progression of a population from one stage of sepsis to another, or from normalcy (*i.e.*, a condition characterized by not having sepsis or SIRS) to sepsis or SIRS and *vice versa*, will be characterized by changes in biomarker profiles, as certain biomarkers are

expressed at increasingly higher levels and the expression of other biomarkers becomes down-regulated. These changes in biomarker profiles may reflect the progressive establishment of a physiological response in the reference population to infection and/or inflammation, for example. The skilled artisan will appreciate that the biomarker profile of the reference population also will change as a physiological response subsides. As stated above, one of the advantages of the present invention is the capability of classifying an individual with a biomarker profile from a single biological sample as having membership in a particular population. The artisan will appreciate, however, that the determination of whether a particular physiological response is becoming established or is subsiding may be facilitated by a subsequent classification of the individual. To this end, the present invention provides numerous biomarkers that both increase and decrease in level of expression as a physiological response to sepsis or SIRS is established or subsides. For example, an investigator can select a feature of an individual's biomarker profile that is known to change in intensity as a physiological response to sepsis becomes established. A comparison of the same feature in a profile from a subsequent biological sample from the individual can establish whether the individual is progressing toward more severe sepsis or is progressing toward normalcy.

[0054] The molecular identity of biomarkers is not essential to the invention. Indeed, the present invention should not be limited to biomarkers that have previously been identified. (*See, e.g.*, U.S. Patent Application Serial No. 10/400,275, filed March 26, 2003.) It is, therefore, expected that novel biomarkers will be identified that are characteristic of a given population of individuals, especially a population in one of the early stages of sepsis. In one embodiment of the present invention, a biomarker is identified and isolated. It then may be used to raise a specifically-binding antibody, which can facilitate biomarker detection in a variety of diagnostic assays. For this purpose, any immunoassay may use any antibodies, antibody fragment or derivative capable of binding the biomarker molecules (*e.g.*, Fab, Fv, or scFv fragments). Such immunoassays are well-known in the art. If the biomarker is a protein, it may be sequenced and its encoding gene may be cloned using well-established techniques.

[0055] The methods of the present invention may be employed to screen, for example, patients admitted to an ICU. A biological sample such as, for example, blood, is taken immediately upon admission. The complex mixture of proteins and other molecules within

the blood is resolved as a profile of biomarkers. This may be accomplished through the use of any technique or combination of techniques that reproducibly distinguishes these molecules on the basis of some physical or chemical property. In one embodiment, the molecules are immobilized on a matrix and then are separated and distinguished by laser desorption/ionization time-of-flight mass spectrometry. A spectrum is created by the characteristic desorption pattern that reflects the mass/charge ratio of each molecule or its fragments. In another embodiment, biomarkers are selected from the various mRNA species obtained from a cellular extract, and a profile is obtained by hybridizing the individual's mRNA species to an array of cDNAs. The diagnostic use of cDNA arrays is well known in the art. (*See, e.g.*, Zou, *et. al.*, *Oncogene* 21: 4855-4862 (2002).) In yet another embodiment, a profile may be obtained using a combination of protein and nucleic acid separation methods.

[0056] The invention also provides kits that are useful in determining the status of sepsis or diagnosing SIRS in an individual. The kits of the present invention comprise at least one biomarker. Specific biomarkers that are useful in the present invention are set forth herein. The biomarkers of the kit can be used to generate biomarker profiles according to the present invention. Examples of classes of compounds of the kit include, but are not limited to, proteins, and fragments thereof, peptides, polypeptides, proteoglycans, glycoproteins, lipoproteins, carbohydrates, lipids, nucleic acids, organic and inorganic chemicals, and natural and synthetic polymers. The biomarker(s) may be part of an array, or the biomarker(s) may be packaged separately and/or individually. The kit may also comprise at least one internal standard to be used in generating the biomarker profiles of the present invention. Likewise, the internal standards can be any of the classes of compounds described above. The kits of the present invention also may contain reagents that can be used to detectably label biomarkers contained in the biological samples from which the biomarker profiles are generated. For this purpose, the kit may comprise a set of antibodies or functional fragments thereof that specifically bind at least two, three, four, five, 10, 20 or more of the biomarkers set forth in any one of the following TABLES that list biomarkers. The antibodies themselves may be detectably labeled. The kit also may comprise a specific biomarker binding component, such as an aptamer. If the biomarkers comprise a nucleic acid, the kit may provide an oligonucleotide probe that is capable of forming a duplex with the biomarker or with a complementary strand of a biomarker. The oligonucleotide probe may be detectably labeled.

[0057] The kits of the present invention may also include pharmaceutical excipients, diluents and/or adjuvants when the biomarker is to be used to raise an antibody. Examples of pharmaceutical adjuvants include, but are not limited to, preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of an injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

Generation of Biomarker Profiles

[0058] According to one embodiment, the methods of the present invention comprise obtaining a profile of biomarkers from a biological sample taken from an individual. The biological sample may be blood, plasma, serum, saliva, sputum, urine, cerebral spinal fluid, cells, a cellular extract, a tissue sample, a tissue biopsy, a stool sample and the like. The reference biomarker profile may be obtained, for example, from a population of individuals selected from the group consisting of SIRS-negative individuals, SIRS-positive individuals, individuals who are suffering from the onset of sepsis and individuals who already have sepsis. The reference biomarker profile from individuals who already have sepsis may be obtained at any stage in the progression of sepsis, such as infection, bacteremia, severe sepsis, septic shock or MOD.

[0059] In one embodiment, a separation method may be used to create a profile of biomarkers, such that only a subset of biomarkers within the sample is analyzed. For example, the biomarkers that are analyzed in a sample may consist of mRNA species from a cellular extract, which has been fractionated to obtain only the nucleic acid biomarkers within the sample, or the biomarkers may consist of a fraction of the total complement of proteins within the sample, which have been fractionated by chromatographic techniques.

Alternatively, a profile of biomarkers may be created without employing a separation method. For example, a biological sample may be interrogated with a labeled compound that forms a specific complex with a biomarker in the sample, where the intensity of the label in the specific complex is a measurable characteristic of the biomarker. A suitable compound for forming such a specific complex is a labeled antibody. In one embodiment, a biomarker is measured using an antibody with an amplifiable nucleic acid as a label. In yet another

embodiment, the nucleic acid label becomes amplifiable when two antibodies, each conjugated to one strand of a nucleic acid label, interact with the biomarker, such that the two nucleic acid strands form an amplifiable nucleic acid.

[0060] In another embodiment, the biomarker profile may be derived from an assay, such as an array, of nucleic acids, where the biomarkers are the nucleic acids or complements thereof. For example, the biomarkers may be ribonucleic acids. The biomarker profile also may be obtained using a method selected from the group consisting of nuclear magnetic resonance, nucleic acid arrays, dot blotting, slot blotting, reverse transcription amplification and Northern analysis. In another embodiment, the biomarker profile is detected immunologically by reacting antibodies, or functional fragments thereof, specific to the biomarkers. A functional fragment of an antibody is a portion of an antibody that retains at least some ability to bind to the antigen to which the complete antibody binds. The fragments, which include, but are not limited to, scFv fragments, Fab fragments and $F(ab)_2$ fragments, can be recombinantly produced or enzymatically produced. In another embodiment, specific binding molecules other than antibodies, such as aptamers, may be used to bind the biomarkers. In yet another embodiment, the biomarker profile may comprise a measurable aspect of an infectious agent or a component thereof. In yet another embodiment, the biomarker profile may comprise measurable aspects of small molecules, which may include fragments of proteins or nucleic acids, or which may include metabolites.

[0061] Biomarker profiles may be generated by the use of one or more separation methods. For example, suitable separation methods may include a mass spectrometry method, such as electrospray ionization mass spectrometry (ESI-MS), ESI-MS/MS, ESI-MS/$(MS)^n$ (n is an integer greater than zero), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), desorption/ionization on silicon (DIOS), secondary ion mass spectrometry (SIMS), quadrupole time-of-flight (Q-TOF), atmospheric pressure chemical ionization mass spectrometry (APCI-MS), APCI-MS/MS, APCI-$(MS)^n$, atmospheric pressure photoionization mass spectrometry (APPI-MS), APPI-MS/MS, and APPI-$(MS)^n$. Other mass spectrometry methods may include, *inter alia*, quadrupole, fourier transform mass spectrometry (FTMS) and ion trap. Other suitable separation methods may include chemical extraction partitioning, column chromatography, ion exchange chromatography, hydrophobic (reverse phase) liquid

chromatography, isoelectric focusing, one-dimensional polyacrylamide gel electrophoresis (PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) or other chromatography, such as thin-layer, gas or liquid chromatography, or any combination thereof. In one embodiment, the biological sample may be fractionated prior to application of the separation method.

[0062] Biomarker profiles also may be generated by methods that do not require physical separation of the biomarkers themselves. For example, nuclear magnetic resonance (NMR) spectroscopy may be used to resolve a profile of biomarkers from a complex mixture of molecules. An analogous use of NMR to classify tumors is disclosed in Hagberg, *NMR Biomed.* 11: 148-56 (1998), for example. Additional procedures include nucleic acid amplification technologies, which may be used to generate a profile of biomarkers without physical separation of individual biomarkers. (*See* Stordeur *et al.*, *J. Immunol. Methods* 259: 55-64 (2002) and Tan *et al.*, *Proc. Nat'l Acad. Sci. USA* 99: 11387-11392 (2002), for example.)

[0063] In one embodiment, laser desorption/ionization time-of-flight mass spectrometry is used to create a profile of biomarkers where the biomarkers are proteins or protein fragments that have been ionized and vaporized off an immobilizing support by incident laser radiation. A profile is then created by the characteristic time-of-flight for each protein, which depends on its mass-to-charge ("*m/z*") ratio. A variety of laser desorption/ionization techniques are known in the art. (*See, e.g.*, Guttman *et al.*, *Anal. Chem.* 73: 1252-62 (2001) and Wei *et al.*, *Nature* 399: 243-46 (1999).)

[0064] Laser desorption/ionization time-of-flight mass spectrometry allows the generation of large amounts of information in a relatively short period of time. A biological sample is applied to one of several varieties of a support that binds all of the biomarkers, or a subset thereof, in the sample. Cell lysates or samples are directly applied to these surfaces in volumes as small as 0.5 μL, with or without prior purification or fractionation. The lysates or sample can be concentrated or diluted prior to application onto the support surface. Laser desorption/ionization is then used to generate mass spectra of the sample, or samples, in as little as three hours.

[0065] In another embodiment, the total mRNA from a cellular extract of the individual is assayed, and the various mRNA species that are obtained from the biological sample are used

as biomarkers. Profiles may be obtained, for example, by hybridizing these mRNAs to an array of probes, which may comprise oligonucleotides or cDNAs, using standard methods known in the art. Alternatively, the mRNAs may be subjected to gel electrophoresis or blotting methods such as dot blots, slot blots or Northern analysis, all of which are known in the art. (*See, e.g.*, Sambrook *et al.* in "Molecular Cloning, 3rd ed.," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2001).) mRNA profiles also may be obtained by reverse transcription followed by amplification and detection of the resulting cDNAs, as disclosed by Stordeur *et al.*, *supra*, for example. In another embodiment, the profile may be obtained by using a combination of methods, such as a nucleic acid array combined with mass spectroscopy.

Use of a Data Analysis Algorithm

[0066] In one embodiment, comparison of the individual's biomarker profile to a reference biomarker profile comprises applying a decision rule. The decision rule can comprise a data analysis algorithm, such as a computer pattern recognition algorithm. Other suitable algorithms include, but are not limited to, logistic regression or a nonparametric algorithm that detects differences in the distribution of feature values (*e.g.*, a Wilcoxon Signed Rank Test). The decision rule may be based upon one, two, three, four, five, 10, 20 or more features. In one embodiment, the decision rule is based on hundreds or more of features. Applying the decision rule may also comprise using a classification tree algorithm. For example, the reference biomarker profile may comprise at least three features, where the features are predictors in a classification tree algorithm. The data analysis algorithm predicts membership within a population (or class) with an accuracy of at least about 60%, at least about 70%, at least about 80% and at least about 90%.

[0067] Suitable algorithms are known in the art, some of which are reviewed in Hastie *et al.*, *supra*. Such algorithms classify complex spectra from biological materials, such as a blood sample, to distinguish individuals as normal or as possessing biomarker expression levels characteristic of a particular disease state. While such algorithms may be used to increase the speed and efficiency of the application of the decision rule and to avoid investigator bias, one of ordinary skill in the art will realize that computer-based algorithms are not required to carry out the methods of the present invention.

[0068] Algorithms may be applied to the comparison of biomarker profiles, regardless of the method that was used to generate the biomarker profile. For example, suitable algorithms can be applied to biomarker profiles generated using gas chromatography, as discussed in Harper, "Pyrolysis and GC in Polymer Analysis," Dekker, New York (1985). Further, Wagner *et al.*, *Anal. Chem.* 74: 1824-35 (2002) disclose an algorithm that improves the ability to classify individuals based on spectra obtained by static time-of-flight secondary ion mass spectrometry (TOF-SIMS). Additionally, Bright *et al.*, *J. Microbiol. Methods* 48: 127-38 (2002) disclose a method of distinguishing between bacterial strains with high certainty (79-89% correct classification rates) by analysis of MALDI-TOF-MS spectra. Dalluge, *Fresenius J. Anal. Chem.* 366: 701-11 (2000) discusses the use of MALDI-TOF-MS and liquid chromatography-electrospray ionization mass spectrometry (LC/ESI-MS) to classify profiles of biomarkers in complex biological samples.

Biomarkers

[0069] The methods of the present invention can be carried out by generation of a biomarker profile that is diagnostic or predictive of sepsis or SIRS. Because profile generation is sufficient to carry out the invention, the biomarkers that constitute the profile need not be known or subsequently identified.

[0070] Biomarkers that can be used to generate the biomarker profiles of the present invention may include those known to be informative of the state of the immune system in response to infection; however, not all of these biomarkers may be equally informative. These biomarkers can include hormones, autoantibodies, soluble and insoluble receptors, growth factors, transcription factors, cell surface markers and soluble markers from the host or from the pathogen itself, such as coat proteins, lipopolysaccharides (endotoxin), lipoteichoic acids, etc. Other biomarkers include, but are not limited to, cell-surface proteins such as CD64 proteins; CD11b proteins; HLA Class II molecules, including HLA-DR proteins and HLA-DQ proteins; CD54 proteins; CD71 proteins; CD86 proteins; surface-bound tumor necrosis factor receptor (TNF-R); pattern-recognition receptors such as Toll-like receptors; soluble markers such as interleukins IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, and IL-18; tumor necrosis factor alpha (TNF-α); neopterin; C-reactive protein (CRP); procalcitonin (PCT); 6-keto Flα; thromboxane B_2; leukotrienes B4, C3, C4, C5, D4 and E4; interferon gamma (IFNγ); interferon alpha/beta (IFN α/β); lymphotoxin alpha (LTα); complement components (C'); platelet activating factor (PAF); bradykinin; nitric

oxide (NO); granulocyte macrophage-colony stimulating factor (GM-CSF); macrophage inhibitory factor (MIF); interleukin-1 receptor antagonist (IL-1ra); soluble tumor necrosis factor receptor (sTNFr); soluble interleukin receptors sIL-lr and sIL-2r; transforming growth factor beta (TGFβ); prostaglandin E_2 (PGE_2); granulocyte-colony stimulating factor (G-CSF); and other inflammatory mediators. (Reviewed in Oberholzer *et al.*, *Shock* 16: 83-96 (2001) and Vincent *et al.* in "The Sepsis Text," Carlet *et al.*, eds. (Kluwer Academic Publishers, 2002). Biomarkers commonly and clinically associated with bacteremia are also candidates for biomarkers useful for the present invention, given the common and frequent occurrence of such biomarkers in biological samples. Biomarkers can include low molecular weight compounds, which can be fragments of proteins or nucleic acids, or they may include metabolites. The presence or concentration of the low molecular weight compounds, such as metabolites, may reflect a phenotypic change that is associated with sepsis and/or SIRS. In particular, changes in the concentration of small molecule biomarkers may be associated with changes in cellular metabolism that result from any of the physiological changes in response to SIRS and/or sepsis, such as hypothermia or hyperthermia, increased heart rate or rate of respiration, tissue hypoxia, metabolic acidosis or MOD. Biomarkers may also include RNA and DNA molecules that encode protein biomarkers.

[0071] Biomarkers can also include at least one molecule involved in leukocyte modulation, such as neutrophil activation or monocyte deactivation. Increased expression of CD64 and CD11b is recognized as a sign of neutrophil and monocyte activation. (Reviewed in Oberholzer *et al.*, *supra* and Vincent *et al.*, *supra*.) Among those biomarkers that can be useful in the present invention are those that are associated with macrophage lysis products, as well as markers of changes in cytokine metabolism. (*See* Gagnon *et al.*, *Cell* 110*:* 119-31 (2002); Oberholzer, *et. al.*, *supra*; Vincent, *et. al.*, *supra*.)

[0072] Biomarkers can also include signaling factors known to be involved or discovered to be involved in the inflammatory process. Signaling factors may initiate an intracellular cascade of events, including receptor binding, receptor activation, activation of intracellular kinases, activation of transcription factors, changes in the level of gene transcription and/or translation, and changes in metabolic processes, etc. The signaling molecules and the processes activated by these molecules collectively are defined for the purposes of the present invention as "biomolecules involved in the sepsis pathway." The relevant predictive biomarkers can include biomolecules involved in the sepsis pathway.

[0073] Accordingly, while the methods of the present invention may use an unbiased approach to identifying predictive biomarkers, it will be clear to the artisan that specific groups of biomarkers associated with physiological responses or with various signaling pathways may be the subject of particular attention. This is particularly the case where biomarkers from a biological sample are contacted with an array that can be used to measure the amount of various biomarkers through direct and specific interaction with the biomarkers (*e.g.*, an antibody array or a nucleic acid array). In this case, the choice of the components of the array may be based on a suggestion that a particular pathway is relevant to the determination of the status of sepsis or SIRS in an individual. The indication that a particular biomolecule has a feature that is predictive or diagnostic of sepsis or SIRS may give rise to an expectation that other biomolecules that are physiologically regulated in a concerted fashion likewise may provide a predictive or diagnostic feature. The artisan will appreciate, however, that such an expectation may not be realized because of the complexity of biological systems. For example, if the amount of a specific mRNA biomarker were a predictive feature, a concerted change in mRNA expression of another biomarker might not be measurable, if the expression of the other biomarker was regulated at a post-translational level. Further, the mRNA expression level of a biomarker may be affected by multiple converging pathways that may or may not be involved in a physiological response to sepsis.

[0074] Biomarkers can be obtained from any biological sample, which can be, by way of example and not of limitation, blood, plasma, saliva, serum, urine, cerebral spinal fluid, sputum, stool, cells and cellular extracts, or other biological fluid sample, tissue sample or tissue biopsy from a host or patient. The precise biological sample that is taken from the individual may vary, but the sampling preferably is minimally invasive and is easily performed by conventional techniques.

[0075] Measurement of a phenotypic change may be carried out by any conventional technique. Measurement of body temperature, respiration rate, pulse, blood pressure, or other physiological parameters can be achieved via clinical observation and measurement. Measurements of biomarker molecules may include, for example, measurements that indicate the presence, concentration, expression level, or any other value associated with a biomarker molecule. The form of detection of biomarker molecules typically depends on the method used to form a profile of these biomarkers from a biological sample. For instance,

biomarkers separated by 2D-PAGE are detected by Coomassie Blue staining or by silver staining, which are well-established in the art.

Isolation of Useful Biomarkers

[0076] It is expected that useful biomarkers will include biomarkers that have not yet been identified or associated with a relevant physiological state. In one aspect of the invention, useful biomarkers are identified as components of a biomarker profile from a biological sample. Such an identification may be made by any well-known procedure in the art, including immunoassay or automated microsequencing.

[0077] Once a useful biomarker has been identified, the biomarker may be isolated by one of many well-known isolation procedures. The invention accordingly provides a method of isolating a biomarker that is diagnostic or predictive of sepsis comprising obtaining a reference biomarker profile obtained from a population of individuals, identifying a feature of the reference biomarker profile that is predictive or diagnostic of sepsis or one of the stages in the progression of sepsis, identifying a biomarker that corresponds with that feature, and isolating the biomarker. Once isolated, the biomarker may be used to raise antibodies that bind the biomarker if it is a protein, or it may be used to develop a specific oligonucleotide probe, if it is a nucleic acid, for example.

[0078] The skilled artisan will readily appreciate that useful features can be further characterized to determine the molecular structure of the biomarker. Methods for characterizing biomolecules in this fashion are well-known in the art and include high-resolution mass spectrometry, infrared spectrometry, ultraviolet spectrometry and nuclear magnetic resonance. Methods for determining the nucleotide sequence of nucleic acid biomarkers, the amino acid sequence of polypeptide biomarkers, and the composition and sequence of carbohydrate biomarkers also are well-known in the art.

Application of the Present Invention to SIRS Patients

[0079] In one embodiment, the presently described methods are used to screen SIRS patients who are particularly at risk for developing sepsis. A biological sample is taken from a SIRS-positive patient, and a profile of biomarkers in the sample is compared to a reference profile from SIRS-positive individuals who eventually progressed to sepsis. Classification of the patient's biomarker profile as corresponding to the reference profile of a SIRS-positive population that progressed to sepsis is diagnostic that the SIRS-positive patient will likewise

progress to sepsis. A treatment regimen may then be initiated to forestall or prevent the progression of sepsis.

[0080] In another embodiment, the presently described methods are used to confirm a clinical suspicion that a patient has SIRS. In this case, a profile of biomarkers in a sample is compared to reference populations of individuals who have SIRS or who do not have SIRS. Classification of the patient's biomarker profile as corresponding to one population or the other then can be used to diagnose the individual as having SIRS or not having SIRS.

Examples

[0081] The following examples are representative of the embodiments encompassed by the present invention and in no way limit the subject embraced by the present invention.

Example 1: Identification of small molecule biomarkers using quantitative liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS)

1.1. Samples Received and Analyzed

[0082] Reference biomarker profiles were established for two populations of patients. The first population ("the SIRS group") represented 20 patients who developed SIRS and who entered into the present study at "Day 1," but who did not progress to sepsis during their hospital stay. The second population ("the sepsis group") represented 20 patients who likewise developed SIRS and entered into the present study at Day 1, but who progressed to sepsis at least several days after entering the study. Blood samples were taken approximately every 24 hours from each study group. Clinical suspicion of sepsis in the sepsis group occurred at "time 0," as measured by conventional techniques. "Time –24 hours" and "time –48 hours" represent samples taken about 24 hours and about 48 hours, respectively, preceding the clinical suspicion of the onset of sepsis in the sepsis group. That is, the samples from the sepsis group included those taken on the day of entry into the study (Day 1), about 48 hours prior to clinical suspicion of sepsis (time –48 hours), about 24 hours prior to clinical suspicion of sepsis (time –24 hours), and on the day of clinical suspicion of the onset of sepsis (time 0). In total, 160 blood samples were analyzed: 80 samples from the 20 patients in the sepsis group and 80 samples from the 20 patients in the SIRS group.

1.2. Sample Preparation

[0083] In plasma, a significant number of small molecules may be bound to proteins, which may reduce the number of small molecules that are detected by a pattern-generating method. Accordingly, most of the protein was removed from the plasma samples following the release of small molecules that may be bound to the proteins. Appropriate methods to remove proteins include, but are not limited to, extraction of the plasma with ice-cold methanol, acetonitrile (ACN), butanol, or trichloroacetic acid (TCA), or heat denaturation and acid hydrolysis. In this example, plasma was extracted with ice-cold methanol. Methanol extraction was preferred because it resulted in the detection of the highest number of small molecules. 50 μL from each plasma sample were mixed with 100 μL ice-cold 100% methanol, giving a final volume percent of methanol of 67%. The solution was vortexed for 60 seconds. The samples were then incubated at 4°C for 20 minutes, and proteins were precipitated by centrifugation at 12,000 rpm for 10 minutes. The supernatant was removed, dried, and resuspended in 50 μL water. Prior to LC/MS analysis, two low molecular weight molecules, sulfachloropyridazine and octadecylamine, were added to the extracted plasma samples. These molecules served as internal standards to normalize ion intensities and retention times. Sulfachloropyridazine has a *m/z* of 285.0 Da, determined by MS, and elutes at 44% ACN, determined by LC; octadecylamine has a *m/z* of 270.3 Da and elutes at 89% ACN.

1.3. LC/ESI-MS Analysis

[0084] 10 μL of the resuspended supernatant was injected onto a 2.1 x 100 mm C_{18} Waters Symmetry LC column (particle size = 3.5 μm; interior bore diameter = 100 Å). The column was then eluted at 300 μL/minute at a temperature of 25°C with a three-step linear gradient of ACN in 0.1% formic acid. For t = 0 – 0.5 minutes, the ACN concentration was 9.75% to 24%; for t = 0.5 – 20 minutes, the ACN concentration was 24% to 90.5%; and for t = 20 – 27 minutes, the ACN concentration was 90.5% to 92.4%. The aforementioned experimental conditions are herein referred to as "LC experimental conditions." Under LC experimental conditions, sulfachloropyridazine eluted at 44% ACN with a retention time of 6.4 minutes, and octadecylamine eluted at 89% ACN with a retention time of 14.5 minutes. Samples that were fractionated by LC were then subjected to ESI-MS using an Agilent MSD 1100 quadrupole mass spectrometer that was connected in tandem to the LC column (LC/ESI-MS). Mass spectral data were acquired for ions with a mass/charge ratio (*m/z*)

ranging from 100 or 150 – 1000 Da in positive ion mode with a capillary voltage of 4000 V. The LC/ESI-MS analyses were performed three times for each sample. The data may be expressed as the *m/z* in Daltons and retention time in minutes (as "*m/z*, retention time") of each ion, where the retention time of an ion is the time required for elution from a reverse phase column in a linear ACN gradient. To account for slight variations in the retention time for run to run, however, the data also may be represented as the *m/z* and the percentage of ACN at which the ion elutes from a C_{18} column, which represent inherent properties of the ions that will not be affected greatly by experimental variability. The relationship between retention time and the percent ACN at elution is expressed by the following equations:

$$\% \text{ ACN} = 28.5\,t + 9.75 \qquad \text{for } 0 < t < 0.5;$$

$$\% \text{ ACN} = 3.4103\,(t - 0.5) + 24 \qquad \text{for } 0.5 < t < 20; \text{ and}$$

$$\% \text{ ACN} = 0.27143\,(t - 20) + 90.5 \qquad \text{for } 20 < t < 27.$$

[0085] The values for these parameters nevertheless should be understood to be approximations and may vary slightly between experiments; however, ions can be recognized reproducibly, especially if the samples are prepared with one or more internal standards. In the data shown below, the *m/z* values were determined to within ± 0.4 *m/z*, while the percent ACN at which the ions elute is determined to within ± 10%.

1.4. Data Analysis and Results

[0086] Several hundred spectral features were analyzed from each plasma sample. Similar features were aligned between spectra. The choice of alignment algorithm is not crucial to the present invention, and the skilled artisan is aware of various alignment algorithms that can be used for this purpose. In total, 4930 spectral features were analyzed. For the purpose of this Example, a "feature" is used interchangeably with a "peak" that corresponds to a particular ion. Representative peaks from samples obtained from five different individuals are shown in TABLE 1. The first column lists in parentheses the *m/z* and percentage of ACN at elution for each ion, respectively. The remaining columns are normalized intensities of the corresponding ions from each patient, which were determined by normalizing the intensities to those of the two internal standards. Over 400 peaks had an average normalized intensity higher than 0.1.

TABLE 1

presence of representative ions in various patients

Ion (*m/z*, %ACN)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
(293.2, 26.8)	43.39	42.44	53.81	45.86	23.24
(496.5, 39.0)	37.43	39.88	33.74	36.32	31.81
(520.5, 37.8)	9.067	9.309	7.512	6.086	6.241
(522.5, 37.8)	8.568	8.601	7.234	5.520	5.228
(524.5, 42.2)	11.60	12.73	8.941	7.309	6.810
(275.3, 32.0)	6.966	7.000	8.911	5.896	5.590
(544.5, 37.8)	3.545	3.915	3.182	2.365	2.342
(393.3, 26.4)	1.517	2.092	2.418	2.439	2.498
(132.3, 24.3)	2.317	2.417	3.953	4.786	2.982
(437.4, 27.4)	1.769	1.997	2.418	2.706	2.166
(518.5, 39.0)	3.731	3.792	6.758	3.058	2.605
(349.3, 25.6)	1.249	1.663	1.910	1.806	1.660
(203.2, 24.1)	3.722	3.485	4.900	3.155	2.342
(481.4, 27.7)	1.570	1.259	1.987	2.246	1.612

[0087] Various approaches may be used to identify ions that inform a decision rule to distinguish between the SIRS and sepsis groups. In this Example, the methods chosen were (1) comparing average ion intensities between the two groups, and (2) creating classification trees using a data analysis algorithm.

1.4.1. Comparing Average Ion Intensities

[0088] Comparison of averaged ion intensities effectively highlights differences in individual ion intensities between the SIRS and sepsis patients. Over 1800 normalized ion intensities were averaged separately for the sepsis group and the SIRS group. Ions having an average normalized intensity of less than 0.1 in either the sepsis group or the SIRS group were analyzed separately from those ions having a normalized intensity greater than 0.1 in profiles from both groups. The ratios of average normalized intensities for approximately 400 ions having a normalized intensity greater than 0.1 were determined for the sepsis group

versus the SIRS group. A distribution of relative intensity ratios of these ions is shown in FIGURE 3.

[0089] Using this method, 23 ions, listed in TABLE 2, were observed that displayed an intensity at least three-fold higher in samples from patients with sepsis than patients with SIRS (*see* FIGURE 3, where the natural log of the ion intensity ratio is greater than about 1.1) and that were present in at least half of the patients with sepsis and generally in about a third or a quarter of the patients having SIRS. In this context, the "presence" of a biomarker means that the average normalized intensity of the biomarker in a particular patient was at least 25% of the normalized intensity averaged over all the patients. While these ions, or subsets thereof, will be useful for carrying out the methods of the present invention, additional ions or other sets of ions will be useful as well.

TABLE 2

percentage of patient samples containing the listed ion

Ion #	(*m/z* [Da], retention time [min])	% ACN at elution	Ion present in % of sepsis patients	Ion present in % of SIRS patients
1	(520.4, 5.12)	39.75	94	35
2	(490.3, 5.12)	39.75	76	35
3	(407.2, 4.72)	38.39	76	25
4	(564.4, 5.28)	40.30	71	35
5	(608.4, 5.39)	40.68	71	30
6	(564.3, 2.14)	29.59	71	25
7	(476.4, 4.96)	39.21	65	30
8	(476.3, 1.86)	28.64	65	35
9	(377.2, 4.61)	38.02	65	15
10	(547.4, 5.28)	40.30	65	20
11	(657.4, 5.53)	41.15	65	30
12	(481.3, 4.96)	39.21	59	25
13	(432.3, 4.80)	38.66	59	30
14	(481.2, 1.86)	28.64	59	20
15	(388.3, 4.58)	37.91	59	20
16	(363.2, 4.40)	37.30	59	20

17	(261.2, 1.26)	26.59	59	40
18	(377.2, 9.32)	54.08	59	15
19	(534.3, 5.30)	40.37	59	30
20	(446.3, 4.94)	39.14	59	25
21	(437.2, 1.42)	27.13	53	25
22	(451.3, 4.94)	39.14	53	15
23	(652.5, 5.51)	41.08	53	20

[0090] Subsets of these biomarkers were present in at least three-fold higher intensities in a majority of the sepsis-positive population. Specifically, at least 12 of these biomarkers were found at elevated levels in over half of the sepsis-positive population, and at least seven biomarkers were present in 85% of the sepsis-positive population, indicating that combinations of these markers will provide useful predictors of the onset of sepsis. All the biomarkers were at elevated levels with respect to the SIRS-positive population, as shown in TABLE 3.

TABLE 3

ion intensity in sepsis group versus SIRS group

Ion	Intensity in sepsis group	Intensity in SIRS group	Ratio of intensities: sepsis/SIRS
(437.2, 1.42)	4.13	0.77	5.36
(520.4, 5.12)	3.65	0.69	5.29
(476.4, 4.96)	3.34	0.78	3.56
(481.3, 4.96)	2.42	0.68	3.56
(564.4, 5.28)	2.39	0.43	5.56
(432.3, 4.80)	2.29	0.59	3.88
(476.3, 1.86)	2.12	0.52	4.08
(481.2, 1.86)	1.88	0.42	4.48
(388.3, 4.58)	1.83	0.51	3.59
(608.4, 5.39)	1.41	0.24	5.88
(363.2, 4.40)	1.35	0.27	5.00
(490.3, 5.12)	1.27	0.25	5.08

(261.2, 1.26)	1.24	0.24	5.17
(407.2, 4.72)	1.05	0.17	6.18
(377.2, 9.32)	1.04	0.27	3.85
(534.3, 5.30)	0.88	0.16	5.50
(446.3, 4.94)	0.88	0.22	4.00
(547.4, 5.28)	0.86	0.16	5.38
(451.3, 4.94)	0.86	0.17	5.06
(377.2, 4.61)	0.84	0.22	3.82
(564.3, 2.14)	0.62	0.14	4.43
(652.5, 5.51)	0.62	0.10	6.20
(657.4, 5.53)	0.39	0.11	3.55

[0091] The two ions listed in TABLE 4 were observed to have an average normalized intensity three-fold higher in the SIRS population than in the sepsis population. (*See* FIGURE 3, where the natural log of the ion intensity ratio is less than about – 1.1.)

TABLE 4

ion intensity in sepsis group versus SIRS group

Ion #	Intensity in sepsis group	Intensity in SIRS group	Ratio of intensities: sepsis/SIRS
(205.0, 0.01)	0.26	0.81	0.32
(205.2, 3.27)	0.29	0.82	0.35

[0092] Thirty-two ions having an average normalized intensity of greater than 0.1 were identified that exhibited at least a three-fold higher intensity in the sepsis group versus the SIRS group. These ions are listed in TABLE 5A. Likewise, 48 ions having an average normalized intensity of less than 0.1 were identified that had a three-fold ratio of intensity higher in the sepsis group versus the SIRS group. These ions are listed in TABLE 5B. (A negative retention time reflects the fact that retention times are normalized against internal standards.)

TABLE 5A

ions having an averaged normalized intensity >0.1

Ion	Intensity in sepsis group	Intensity in SIRS group	Ratio of intensities: sepsis/SIRS	Ln (ratio)
(365.2, 2.69)	1.031828095	0.135995335	7.587231542	2.026467
(305.2, 1.87)	3.070957223	0.481494549	6.377968828	1.85285
(407.2, 4.72)	0.913022768	0.166525859	5.482768698	1.70161
(459.1, 0.83)	0.58484531	0.106723807	5.479989222	1.701103
(652.5, 5.51)	0.528195058	0.102545088	5.150856731	1.639163
(608.4, 5.39)	1.205608851	0.236066662	5.107069514	1.630626
(415.3, 4.80)	2.321268423	0.46651355	4.975779207	1.604582
(319.0, 0.69)	1.034850099	0.209420422	4.941495631	1.597668
(534.3, 5.30)	0.756349296	0.158850924	4.761378001	1.560537
(564.4, 5.28)	2.037002742	0.432651771	4.708180752	1.549302
(437.2, 1.42)	3.536425702	0.770241153	4.591322718	1.524168
(520.4, 5.12)	3.115934457	0.685511116	4.545417838	1.51412
(261.2, 1.26)	1.078475479	0.239640228	4.500394154	1.504165
(363.2, 4.40)	1.159043471	0.265797517	4.360625655	1.472616
(451.3, 4.94)	0.738875795	0.170611107	4.330760214	1.465743
(490.3, 5.12)	1.084054201	0.25339878	4.278056119	1.453499
(409.3, 2.79)	1.172523824	0.281931606	4.158894565	1.425249
(497.3, 4.98)	0.409558491	0.100673382	4.068190437	1.403198
(453.2, 2.97)	0.738638127	0.184100346	4.012149581	1.389327
(481.2, 1.86)	1.609705934	0.418739646	3.844168924	1.346557
(564.3, 2.14)	0.531918507	0.139341563	3.817371482	1.339562
(476.4, 4.96)	2.847539378	0.784495859	3.629769802	1.289169
(446.3, 4.94)	0.752613738	0.216182996	3.481373426	1.247427
(476.3, 1.86)	1.811980008	0.521460142	3.474819762	1.245543
(377.2, 4.61)	0.75347133	0.217838186	3.458857892	1.240938
(344.3, 4.21)	0.560262239	0.164687938	3.401962791	1.224353
(377.2, 9.32)	0.902933137	0.267048623	3.381156311	1.218218
(432.3, 4.80)	1.957941965	0.588612075	3.326370706	1.201882

(595.4, 6.36)	0.41462875	0.125522805	3.303214496	1.194896
(358.3, 4.40)	0.351038883	0.106282278	3.302891964	1.194798
(657.4, 5.53)	0.336357992	0.105101129	3.200327108	1.163253
(388.3, 4.58)	1.561368263	0.510848809	3.056419503	1.117244

TABLE 5B

ions having an averaged normalized intensity <0.1

Ion	Intensity in sepsis group	Intensity in SIRS group	Ratio of intensities: sepsis/SIRS	Ln (ratio)
(282.2, 0.91)	0.16624	0.00024	693.08684	6.54116
(289.2, 6.44)	0.13088	0.00143	91.27187	4.51384
(821.9, 2.49)	0.13670	0.00996	13.72695	2.61936
(385.3, 1.24)	0.32177	0.03201	10.05211	2.30778
(843.9, 2.47)	0.11866	0.01206	9.83497	2.28594
(407.2, 1.17)	0.75611	0.08227	9.19041	2.21816
(350.1, 0.86)	0.10369	0.01174	8.83532	2.17876
(385.3, 4.72)	0.32430	0.03725	8.70689	2.16411
(399.2, 2.99)	0.15303	0.02091	7.31838	1.99039
(152.1, 1.51)	0.28888	0.04167	6.93310	1.93631
(341.0, 0.36)	0.26310	0.03828	6.87289	1.92759
(451.2, 1.42)	0.45398	0.06645	6.83232	1.92166
(231.0, –0.41)	0.19637	0.03362	5.84078	1.76486
(534.2, 2.20)	0.45796	0.08650	5.29427	1.66663
(820.5, 7.02)	0.12838	0.02439	5.26324	1.66075
(578.4, 5.46)	0.45661	0.08861	5.15298	1.63957
(355.1, 2.85)	0.16920	0.03334	5.07491	1.62431
(358.0, 2.13)	0.27655	0.05565	4.96946	1.60331
(696.5, 5.65)	0.20458	0.04223	4.84500	1.57795
(622.4, 5.61)	0.20034	0.04179	4.79410	1.56739
(460.3, 4.02)	0.18099	0.03950	4.58160	1.52205
(718.0, 7.02)	0.11733	0.02564	4.57688	1.52102
(305.3, 6.11)	0.10194	0.02324	4.38703	1.47865

(283.2, 1.85)	0.41312	0.09709	4.25497	1.44809
(701.4, 5.63)	0.18369	0.04321	4.25111	1.44718
(541.2, 1.71)	0.11482	0.02739	4.19217	1.43322
(657.3, 2.49)	0.17904	0.04280	4.18327	1.43109
(239.2, 1.04)	0.10637	0.02553	4.16574	1.42689
(608.3, 2.35)	0.39410	0.09670	4.07556	1.40501
(465.0, 1.19)	0.10817	0.02718	3.98030	1.38136
(333.1, 2.00)	0.35105	0.08919	3.93582	1.37012
(497.3, 0.88)	0.36172	0.09212	3.92666	1.36779
(541.3, 5.12)	0.13883	0.03559	3.90124	1.36129
(627.3, 5.75)	0.16498	0.04259	3.87347	1.35415
(652.1, 5.87)	0.17554	0.04558	3.85130	1.34841
(402.2, 1.19)	0.25423	0.06860	3.70596	1.30994
(553.3, 5.38)	0.16633	0.04578	3.63335	1.29016
(635.4, 5.53)	0.11925	0.03383	3.52512	1.25992
(319.2, 6.34)	0.17736	0.05035	3.52259	1.25920
(231.1, 2.62)	0.20535	0.05906	3.47671	1.24609
(283.1, 4.96)	0.17190	0.04984	3.44919	1.23814
(766.0, 6.77)	0.13671	0.04032	3.39069	1.22103
(358.0, 6.00)	0.20857	0.06194	3.36714	1.21406
(179.0, 10.16)	0.16841	0.05106	3.29838	1.19343
(209.1, 10.98)	0.13267	0.04090	3.24363	1.17669
(509.3, 5.28)	0.26857	0.08291	3.23925	1.17534
(337.2, 9.32)	0.18169	0.05691	3.19236	1.16076
(423.2, 2.88)	0.16242	0.05097	3.18669	1.15898

[0093] Thus, the reference biomarker profiles of the invention may comprise a combination of features, where the features may be intensities of ions having a *m/z* of about 100 or 150 Da to about 1000 Da as determined by electrospray ionization mass spectrometry in the positive mode, and where the features have a ratio of average normalized intensities in a sepsis-positive reference population versus a SIRS-positive reference population of about 3:1 or higher. Alternatively, the features may have a ratio of average normalized intensities in a sepsis-positive reference population versus a SIRS-positive reference population of about

1:3 or lower. Because these biomarkers appear in biomarker profiles obtained from biological samples taken about 48 hours prior to the onset of sepsis, as determined by conventional techniques, they are expected to be predictors of the onset of sepsis.

1.4.2. Changes in Feature Intensity Over Time

[0094] The examined biomarker profiles displayed features that were expressed both at increasingly higher levels and at lower levels as individuals progressed toward the onset of sepsis. It is expected that the biomarkers corresponding to these features are characteristics of the physiological response to infection and/or inflammation in the individuals. For the reasons set forth above, it is expected that these biomarkers will provide particularly useful predictors for determining the status of sepsis or SIRS in an individual. Namely, comparisons of these features in profiles obtained from different biological samples from an individual are expected to establish whether an individual is progressing toward severe sepsis or whether SIRS is progressing toward normalcy.

[0095] Of the 23 ions listed in TABLE 2, 14 showed a maximum intensity in the time –48 hours population, eight showed a maximum intensity in the time –24 hours population, and one showed a maximum intensity in the time 0 population. A representative change in the intensity of a biomarker over time in biological samples from the sepsis group is shown in FIGURE 4A, while the change in the intensity of the same biomarker in biological samples from the SIRS group is shown in FIGURE 4B. This particular ion, which has a *m/z* of 437.2 Da and a retention time of 1.42 min, peaks in intensity in the sepsis group 48 hours prior to the conversion of these patients to sepsis, as diagnosed by conventional techniques. A spike in relative intensity of this ion in a biological sample thus serves as a predictor of the onset of sepsis in the individual within about 48 hours.

1.4.3. Cross-Validation

[0096] A selection bias can affect the identification of features that inform a decision rule, when the decision rule is based on a large number of features from relatively few biomarker profiles. (*See* Ambroise *et al.*, *Proc. Nat'l Acad. Sci. USA* 99: 6562-66 (2002).) Selection bias may occur when data are used to select features, and performance then is estimated conditioned on the selected features with no consideration made for the variability in the selection process. The result is an overestimation of the classification accuracy. Without compensation for selection bias, classification accuracies may reach 100%, even

when the decision rule is based on random input parameters. (*Id.*) Selection bias may be avoided by including feature selection in the performance estimation process, whether that performance estimation process is 10-fold cross-validation or a type of bootstrap procedure. (*See, e.g.*, Hastie *et al., supra,* at 7.10 – 7.11, herein incorporated by reference.)

[0097] In one embodiment of the present invention, model performance is measured by ten-fold cross-validation. Ten-fold cross-validation proceeds by randomly partitioning the data into ten exclusive groups. Each group in turn is excluded, and a model is fitted to the remaining nine groups. The fitted model is applied to the excluded group, and predicted class probabilities are generated. The predicted class probabilities can be compared to the actual class memberships by simply generating predicted classes. For example, if the probability of sepsis is, say, greater than 0.5, the predicted class is sepsis.

[0098] Deviance is a measure comparing probabilities with actual outcomes. As used herein, "deviance" is defined as:

$$-2\left\{\sum_{\text{sepsis cases}}\ln(P(sepsis))+\sum_{\text{SIRS cases}}\ln(P(SIRS))\right\}$$

where P is the class probability for the specified class. Deviance is minimized when class probabilities are high for the actual classes. Two models can make the same predictions for given data, yet a preferred model would have a smaller predictive deviance. For each of the ten iterations in the ten-fold cross-validation, the predicted deviance is calculated for the cases left out of the model fitting during that iteration. The result is 10 unbiased deviances. Typically, these 10 deviances are summed to create a general summary of model performance (*i.e.*, accuracy) on the total data set. Because in fact 10 different models were fit, cross-validation does not prove the performance of a specific model. Rather, the 10 models were generated by a common modeling process, and cross-validation proved the performance of this process. An eleventh model arising from this process will likely have predictive performance similar to those of the first 10. Use of a ten-fold cross-validation typically results in a model performance of less than 100%, but the performance obtained after ten-fold cross-validation is expected to reflect more closely a biologically meaningful predictive accuracy of the decision rule, when applied to biomarker profiles obtained from samples outside of the training set.

1.4.4. Classification Tree Analysis

[0099] One approach to analyze this data is to use a classification tree algorithm that searches for patterns and relationships in large datasets. A "classification tree" is a recursive partition to classify a particular patient into a specific class (*e.g.*, sepsis or SIRS) using a series of questions that are designed to accurately place the patient into one of the classes. Each question asks whether a patient's condition satisfies a given predictor, with each answer being used to guide the user down the classification tree until a class into which the patient falls can be determined. As used herein, a "predictor" is the range of values of the features—in this Example, ion intensities—of one ion having a characteristic *m/z* and elution profile from a C_{18} column in ACN. The "condition" is the single, specific value of the feature that is measured in the individual's biomarker profile. In this example, the "class names" are sepsis and SIRS. Thus, the classification tree user will first ask if a first ion intensity measured in the individual's biomarker profile falls within a given range of the first ion's predictive range. The answer to the first question may be dispositive in determining if the individual has SIRS or sepsis. On the other hand, the answer to the first question may further direct the user to ask if a second ion intensity measured in the individual's biomarker profile falls within a given range of the second ion's predictive range. Again, the answer to the second question may be dispositive or may direct the user further down the classification tree until a patient classification is ultimately determined.

[00100] A representative set of ion intensities collected from sepsis and SIRS populations at time 0 was analyzed with a classification tree algorithm, the results of which are shown in FIGURE 5. In this case, the set of analyzed ions included those with normalized intensities of less than 0.1. The first decision point in the classification tree is whether the ion having a *m/z* of about 448.5 Daltons and a percent ACN at elution of about 32.4% has a normalized intensity of less than about 0.0414. If the answer to that question is "yes," then one proceeds down the left branch either to another question or to a class name. In this case, if the normalized intensity were less than about 0.0414, then one proceeds to the class name of "SIRS," and the individual is classified as SIRS-positive, but sepsis-negative. If the answer were "no," then one proceeds down the right branch to the next decision point, and so on until a class name is reached. In this example, three decision points were used to predict a class name for an individual. While a single decision point may be used to classify patients as SIRS- or sepsis-positive, additional decision points using other ions generally improved the accuracy of the classification. The skilled artisan will appreciate that many different

classification trees are possible from large datasets. That is, there are many possible combinations of biomarkers that can be used to classify an individual as belonging to a SIRS population or a sepsis population, for example.

1.4.5. Multiple Additive Regression Trees

[00101] An automated, flexible modeling technique that uses multiple additive regression trees (MART) was used to classify sets of features as belonging to one of two populations. A MART model uses an initial offset, which specifies a constant that applies to all predictions, followed by a series of regression trees. Its fitting is specified by the number of decision points in each tree, the number of trees to fit, and a "granularity constant" that specifies how radically a particular tree can influence the MART model. For each iteration, a regression tree is fitted to estimate the direction of steepest descent of the fitting criterion. A step having a length specified by the granularity constant is taken in that direction. The MART model then consists of the initial offset plus the step provided by the regression tree. The differences between the observed and predicted values are recalculated, and the cycle proceeds again, leading to a progressive refinement of the prediction. The process continues either for a predetermined number of cycles or until some stopping rule is triggered.

[00102] The number of splits in each tree is a particularly meaningful fitting parameter. If each tree has only one split, the model looks only at one feature and has no capability for combining two predictors. If each tree has two splits, the model can accommodate two-way interactions among features. With three trees, the model can accommodate three-way interactions, and so forth.

[00103] The value of sets of features in predicting class status was determined for data sets with features and known class status (*e.g.*, sepsis or SIRS). MART provides a measure of the contribution or importance of individual features to the classification decision rule. Specifically, the degree to which a single feature contributes to the decision rule upon its selection at a given tree split can be measured to provide a ranking of features by their importance in determining the final decision rule. Repeating the MART analysis on the same data set may yield a slightly different ranking of features, especially with respect to those features that are less important in establishing the decision rule. Sets of predictive features and their corresponding biomarkers that are useful for the present invention, therefore, may vary slightly from those set forth herein.

[00104] One implementation of the MART technology is found in a module, or "package," for the R statistical programming environment (*see* Venables *et al.*, in *Modern Applied Statistics with S*, 4th ed. (Springer, 2002); www.r-project.org). Results reported in this document were calculated using R versions 1.7.0 and 1.7.1. The module implementing MART, written by Dr. Greg Ridgeway, is called "gbm" and is also freely available for download (*see* www.r-project.org). The MART algorithm is amenable to ten-fold cross-validation. The granularity parameter was set to 0.05, and the gbm package's internal stopping rule was based on leaving out 20% of the data cases at each marked iteration. The degree of interaction was set to one, so no interactions among features were considered. The gbm package estimates the relative importance of each feature on a percentage basis, which cumulatively equals 100% for all the features of the biomarker profile. The features with highest importance, which together account for at least 90% of total importance, are reported as potentially having predictive value. Note that the stopping rule in the fitting of every MART model contributes a stochastic component to model fitting and feature selection. Consequently, multiple MART modeling runs based on the same data may choose slightly, or possibly even completely, different sets of features. Such different sets convey the same predictive information; therefore, all the sets are useful in the present invention. Fitting MART models a sufficient number of times is expected to produce all the possible sets of predictive features within a biomarker profile. Accordingly, the disclosed sets of predictors are merely representative of those sets of features that can be used to classify individuals into populations.

1.4.6. Logistic Regression Analysis

[00105] Logistic regression provides yet another means of analyzing a data stream from the LC/MS analysis described above. "Peak intensity" is measured by the height of a peak that appears in a spectrum at a given *m/z* location. The absence of a peak at a given *m/z* location results in an assigned peak intensity of "0." The standard deviations (SD) of the peak intensities from a given *m/z* location are then obtained from the spectra of the combined SIRS and sepsis populations. If there is no variation in peak intensity between SIRS and sepsis populations (*i.e.*, the SD = 0), the peak intensity is not considered further. Before regression analysis, peak intensities are scaled, using methods well-known in the art. Scaling algorithms are generally described in, Hastie *et al.*, *supra*, at Chapter 11.

[00106] This feature-selection procedure identified 26 input parameters (*i.e.*, biomarkers) from time 0 biomarker profiles, listed in TABLE 6. Although input parameter are ranked in order of statistical importance, lower ranked input parameters still may prove clinically valuable and useful for the present invention. Further, the artisan will understand that the ranked importance of a given input parameter may change if the reference population changes in any way.

TABLE 6

input parameters from time 0 samples

Rank of input parameter importance	*m/z* (Da)	% ACN at elution	Rank of input parameter importance	*m/z* (Da)	% ACN at elution
1	883.6	44.84	14	377.0	25.26
2	718.1	44.94	15	194.1	27.07
3	957.3	44.84	16	413.4	92.04
4	676.1	44.84	17	651.5	59.98
5	766.0	44.77	18	114.2	34.40
6	416.3	40.10	19	607.5	45.21
7	429.4	75.80	20	282.3	37.30
8	820.6	44.84	21	156.2	39.99
9	399.4	90.43	22	127.3	64.68
10	244.2	26.59	23	687.9	41.84
11	593.5	43.51	24	439.5	43.34
12	300.4	59.54	25	462.4	72.70
13	285.3	25.88	26	450.4	64.79

[00107] Using this same logistic regression analysis, biomarkers can be ranked in order of importance in predicting the onset of sepsis using samples taken at time –48 hours. The feature-selection process yielded 37 input parameters for the time –48 hour samples as shown in TABLE 7.

TABLE 7

input parameters from time t-48 hours samples

Rank of input parameter importance	*m/z* (Da)	% ACN at elution	Rank of input parameter importance	*m/z* (Da)	% ACN at elution
1	162.2	28.57	20	379.3	38.63
2	716.2	46.41	21	423.3	39.04
3	980	54.52	22	463.4	87.50
4	136.2	24.65	23	965.3	54.15
5	908.9	57.83	24	265.3	40.10
6	150.2	25.13	25	287.2	40.47
7	948.7	52.54	26	429.4	83.13
8	298.4	25.52	27	886.9	54.42
9	293.3	30.45	28	152.2	28.33
10	188.2	30.65	29	431.4	61.34
11	772.7	47.53	30	335.4	30.72
12	327.4	100.60	31	239.2	43.75
13	524.5	90.30	32	373.4	61.10
14	205.2	33.28	33	771	24.03
15	419.4	87.81	34	555.4	41.43
16	804.8	54.86	35	116.2	24.95
17	496.5	79.18	36	887.2	54.62
18	273.1	29.39	37	511.4	40.95
19	355.4	95.51			

1.4.7. Wilcoxon Signed Rank Test Analysis

[00108] In yet another method, a nonparametric test such as a Wilcoxon Signed Rank Test can be used to identify individual biomarkers of interest. The features in a biomarker profile are assigned a "p-value," which indicates the degree of certainty with which the biomarker can be used to classify individuals as belonging to a particular reference population. Generally, a p-value having predictive value is lower than about 0.05. Biomarkers having a low p-value can be used by themselves to classify individuals. Alternatively, combinations

of two or more biomarkers can be used to classify individuals, where the combinations are chosen on the basis of the relative p-value of a biomarker. In general, those biomarkers with lower p-values are preferred for a given combination of biomarkers. Combinations of at least three, four, five, six, 10, 20 or 30 or more biomarkers also can be used to classify individuals in this manner. The artisan will understand that the relative p-value of any given biomarker may vary, depending on the size of the reference population.

[00109] Using the Wilcoxon Signed Rank Test, p-values were assigned to features from biomarker profiles obtained from biological samples taken at time 0, time –24 hours and time –48 hours. These p-values are listed in TABLES 8, 9 and 10, respectively.

TABLE 8

p-values from time 0 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(179.0, 10.16)	7.701965e-05
2	(512.4, 10.44)	1.112196e-04
3	(371.3, 4.58)	2.957102e-04
4	(592.4, 15.69)	3.790754e-04
5	(363.2, 4.40)	4.630887e-04
6	(679.4, 5.92)	1.261515e-03
7	(835.0, 7.09)	1.358581e-03
8	(377.2, 4.61)	1.641317e-03
9	(490.3, 5.12)	1.959479e-03
10	(265.2, 4.72)	3.138371e-03
11	(627.3, 5.75)	3.438053e-03
12	(266.7, 14.83)	3.470672e-03
13	(774.9, 7.39)	3.470672e-03
14	(142.2, 3.38)	4.410735e-03
15	(142.0, –0.44)	4.443662e-03
16	(231.0, –0.41)	5.080720e-03
17	(451.3, 4.94)	5.096689e-03
18	(753.8, 9.34)	5.097550e-03
19	(399.2, 2.99)	5.217724e-03
20	(534.4, 10.53)	5.877221e-03
21	(978.8, 6.72)	6.448607e-03
22	(539.3, 5.30)	6.651592e-03
23	(492.2, 1.36)	6.697313e-03
24	(730.4, 6.54)	6.724428e-03

25	(842.6, 10.11)	6.724428e-03
26	(622.4, 5.61)	7.249023e-03
27	(331.7, 19.61)	8.137318e-03
28	(564.3, 14.16)	8.419814e-03
29	(415.3, 4.80)	8.475773e-03
30	(229.2, 2.39)	8.604155e-03
31	(118.2, 5.26)	8.664167e-03
32	(410.7, 0.77)	8.664167e-03
33	(733.5, 4.55)	9.271924e-03
34	(503.3, 5.12)	9.413344e-03
35	(453.2, 2.97)	9.802539e-03
36	(534.3, 5.30)	1.089928e-02
37	(459.3, 4.96)	1.100198e-02
38	(337.8, 5.51)	1.136183e-02
39	(525.4, 15.11)	1.136183e-02
40	(495.3, 18.52)	1.282615e-02
41	(763.4, 19.81)	1.282615e-02
42	(256.2, 6.03)	1.286693e-02
43	(319.1, 15.67)	1.286693e-02
44	(548.3, 5.24)	1.286693e-02
45	(858.8, 7.79)	1.287945e-02
46	(671.4, 5.77)	1.310484e-02
47	(353.2, 7.38)	1.323194e-02
48	(844.1, 9.68)	1.333814e-02
49	(421.2, 4.89)	1.365072e-02
50	(506.4, 19.65)	1.438363e-02
51	(393.3, 4.58)	1.459411e-02
52	(473.3, 5.12)	1.518887e-02
53	(189.1, 2.87)	1.602381e-02
54	(528.1, 16.18)	1.603446e-02
55	(137.2, 9.60)	1.706970e-02
56	(163.1, 10.98)	1.706970e-02
57	(176.1, 10.29)	1.706970e-02
58	(179.1, 6.23)	1.706970e-02
59	(271.5, 5.01)	1.706970e-02
60	(272.2, 6.49)	1.706970e-02
61	(399.3, 27.26)	1.706970e-02
62	(467.5, 5.95)	1.706970e-02
63	(478.0, 2.36)	1.706970e-02
64	(481.3, 26.85)	1.706970e-02
65	(931.9, 6.72)	1.706970e-02
66	(970.5, 7.00)	1.706970e-02

67	(763.2, 16.60)	1.730862e-02
68	(544.4, 15.56)	1.732997e-02
69	(666.4, 5.77)	1.750379e-02
70	(337.2, 9.32)	1.812839e-02
71	(407.2, 1.17)	1.852695e-02
72	(597.2, 5.32)	1.895944e-02
73	(333.1, 2.00)	1.930165e-02
74	(490.3, 13.78)	1.989224e-02
75	(139.1, 16.05)	2.026959e-02
76	(991.7, 16.60)	2.046716e-02
77	(814.2, 6.66)	2.121091e-02
78	(665.4, 15.46)	2.127247e-02
79	(875.9, 10.08)	2.127247e-02
80	(144.0, 0.25)	2.137456e-02
81	(622.7, 4.14)	2.178625e-02
82	(377.2, 12.32)	2.240973e-02
83	(509.3, 5.28)	2.243384e-02
84	(349.2, 2.69)	2.252208e-02
85	(302.0, 19.54)	2.266635e-02
86	(411.0, 2.20)	2.303751e-02
87	(296.2, 16.48)	2.373348e-02
88	(299.6, 15.62)	2.440816e-02
89	(162.1, 0.49)	2.441678e-02
90	(372.0, 0.62)	2.472854e-02
91	(377.2, 9.32)	2.514306e-02
92	(979.6, 10.14)	2.530689e-02
93	(417.3, 15.61)	2.550843e-02
94	(281.7, 19.54)	2.563580e-02
95	(276.2, 5.27)	2.598704e-02
96	(229.2, –0.79)	2.626971e-02
97	(346.1, 7.46)	2.654063e-02
98	(356.2, 9.88)	2.654063e-02
99	(616.4, 8.05)	2.683578e-02
100	(850.4, 7.65)	2.697931e-02
101	(495.3, 5.12)	2.712924e-02
102	(446.3, 4.94)	2.739049e-02
103	(476.3, 1.86)	2.770535e-02
104	(520.4, 5.12)	2.774232e-02
105	(428.3, 6.20)	2.808469e-02
106	(536.3, 17.97)	2.863714e-02
107	(860.3, 6.94)	2.894386e-02
108	(762.9, 16.65)	2.958886e-02

109	(788.9, 6.43)	2.967800e-02
110	(970.1, 6.47)	2.967800e-02
111	(853.8, 5.77)	3.039550e-02
112	(913.6, 9.50)	3.039550e-02
113	(407.2, 4.72)	3.041346e-02
114	(335.2, 16.10)	3.047982e-02
115	(331.2, 12.93)	3.075216e-02
116	(512.3, 13.80)	3.075216e-02
117	(895.8, 6.80)	3.084773e-02
118	(120.2, 8.37)	3.110972e-02
119	(238.2, 9.32)	3.110972e-02
120	(506.3, 8.10)	3.110972e-02
121	(949.9, 6.66)	3.115272e-02
122	(176.1, 6.96)	3.161957e-02
123	(664.9, 2.41)	3.275550e-02
124	(551.4, 18.56)	3.290912e-02
125	(459.0, 5.98)	3.389516e-02
126	(811.5, 7.73)	3.389516e-02
127	(919.9, 10.01)	3.414450e-02
128	(547.4, 5.28)	3.444290e-02
129	(895.4, 6.62)	3.460947e-02
130	(132.2, 0.79)	3.549773e-02
131	(944.8, 9.65)	3.567313e-02
132	(730.7, 6.46)	3.581882e-02
133	(529.5, 16.70)	3.666990e-02
134	(449.3, 24.40)	3.687266e-02
135	(465.3, 5.08)	3.725633e-02
136	(481.3, 4.96)	3.956117e-02
137	(250.1, 14.23)	3.982131e-02
138	(565.3, 16.05)	3.982131e-02
139	(559.0, 15.30)	3.994530e-02
140	(555.3, 4.18)	4.078620e-02
141	(568.4, 15.49)	4.118355e-02
142	(120.0, 11.52)	4.145499e-02
143	(120.2, 14.91)	4.145499e-02
144	(167.0, 5.00)	4.145499e-02
145	(173.0, 19.96)	4.145499e-02
146	(324.9, 2.27)	4.145499e-02
147	(328.8, 19.98)	4.145499e-02
148	(345.7, 16.95)	4.145499e-02
149	(407.2, 12.07)	4.145499e-02
150	(478.3, 3.69)	4.145499e-02

151	(484.2, 8.40)	4.145499e-02
152	(502.2, 4.55)	4.145499e-02
153	(597.4, 11.40)	4.145499e-02
154	(612.3, 6.40)	4.145499e-02
155	(700.3, 9.40)	4.145499e-02
156	(730.5, 11.63)	4.145499e-02
157	(771.4, 6.02)	4.145499e-02
158	(811.9, 10.99)	4.145499e-02
159	(859.9, 2.47)	4.145499e-02
160	(450.3, 11.99)	4.145499e-02
161	(619.3, 11.42)	4.165835e-02
162	(102.1, 6.16)	4.238028e-02
163	(717.5, 9.11)	4.238028e-02
164	(606.0, 7.63)	4.317929e-02
165	(627.2, 2.48)	4.317929e-02
166	(252.1, 6.62)	4.318649e-02
167	(657.4, 5.53)	4.332436e-02
168	(635.7, 7.94)	4.399442e-02
169	(167.2, 14.42)	4.452609e-02
170	(812.5, 10.24)	4.528236e-02
171	(575.4, 10.00)	4.533566e-02
172	(379.3, 15.55)	4.644328e-02
173	(468.3, 13.44)	4.644328e-02
174	(295.3, 16.10)	4.721618e-02
175	(715.8, 7.68)	4.736932e-02
176	(810.6, 19.21)	4.759452e-02
177	(159.1, 13.02)	4.795773e-02
178	(435.2, 0.83)	4.795773e-02
179	(443.0, 11.99)	4.795773e-02
180	(468.4, 19.65)	4.795773e-02
181	(909.8, 9.52)	4.795773e-02
182	(647.2, 2.45)	4.838671e-02
183	(564.4, 5.28)	4.958429e-02

TABLE 9

p-values from time –24 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(265.2, 4.72)	0.0003368072
2	(785.5, 9.30)	0.0006770673
3	(685.1, 6.85)	0.0010222902
4	(608.4, 5.39)	0.0014633974
5	(141.1, 5.13)	0.0018265874
6	(652.5, 5.51)	0.0022097623
7	(228.0, 3.12)	0.0029411592
8	(660.1, 3.90)	0.0032802432
9	(235.1, 4.04)	0.0038917632
10	(287.1, 4.72)	0.0045802571
11	(141.2, 1.46)	0.0049063026
12	(553.3, 5.38)	0.0053961549
13	(114.2, 2.49)	0.0060009121
14	(490.3, 5.12)	0.0064288387
15	(142.0, –0.44)	0.0064784467
16	(428.3, 6.20)	0.0064784467
17	(564.4, 5.28)	0.0081876219
18	(678.8, 2.37)	0.0089256763
19	(155.1, 2.87)	0.0091072246
20	(377.2, 4.61)	0.0098626515
21	(221.0, 1.92)	0.0102589726
22	(463.2, 1.88)	0.0102589726
23	(142.2, 3.38)	0.0106568532
24	(231.0, -0.41)	0.0106568532
25	(256.2, 6.03)	0.0106568532
26	(597.2, 2.05)	0.0106568532
27	(638.8, 2.35)	0.0112041041
28	(800.6, 1.53)	0.0112041041
29	(385.3, 24.07)	0.0113535538
30	(578.4, 5.46)	0.0114707005
31	(352.3, 11.76)	0.0115864528
32	(858.2, 10.41)	0.0115864528
33	(889.7, 16.16)	0.0115864528
34	(190.1, 3.99)	0.0120870451
35	(493.3, 26.36)	0.0120870451
36	(608.3, 2.35)	0.0122930750
37	(958.8, 6.36)	0.0127655270
38	(235.0, 0.51)	0.0128665507
39	(739.5, 9.45)	0.0139994021
40	(525.2, 1.92)	0.0141261152
41	(372.4, 11.66)	0.0148592431
42	(415.3, 4.80)	0.0154439839

43	(439.2, 9.40)	0.0154583510
44	(819.0, 2.11)	0.0156979793
45	(459.3, 20.83)	0.0161386158
46	(372.2, 5.10)	0.0169489151
47	(875.4, 19.37)	0.0170124705
48	(989.2, 10.14)	0.0184799654
49	(179.0, 10.16)	0.0190685234
50	(231.0, 6.41)	0.0191486950
51	(460.9, 1.77)	0.0194721634
52	(813.5, 9.83)	0.0194721634
53	(274.2, 4.67)	0.0194863889
54	(158.2, 10.93)	0.0203661514
55	(676.7, 1.07)	0.0208642732
56	(171.2, 25.87)	0.0213201435
57	(520.4, 5.12)	0.0214439678
58	(523.3, 22.32)	0.0216203784
59	(329.0, 1.27)	0.0222231947
60	(585.2, 15.27)	0.0222231947
61	(534.3, 5.30)	0.0224713144
62	(349.2, 2.69)	0.0234305681
63	(263.2, 5.05)	0.0240107773
64	(278.1, 5.24)	0.0240107773
65	(425.9, 6.20)	0.0240107773
66	(575.4, 10.00)	0.0240107773
67	(649.3, 5.75)	0.0240107773
68	(152.1, 1.51)	0.0244163058
69	(785.1, 9.29)	0.0244163058
70	(509.3, 5.28)	0.0257388421
71	(525.4, 15.11)	0.0259747750
72	(261.2, 21.02)	0.0259960666
73	(914.1, 10.04)	0.0260109531
74	(465.3, 5.08)	0.0260926970
75	(433.3, 18.18)	0.0271021410
76	(300.0, 21.90)	0.0275140464
77	(811.6, 19.44)	0.0276109304
78	(710.5, 5.90)	0.0295828987
79	(569.2, 2.00)	0.0302737381
80	(388.3, 4.58)	0.0308414401
81	(173.1, 6.52)	0.0308972074
82	(266.7, 14.83)	0.0308972074
83	(286.2, 12.60)	0.0308972074
84	(619.3, 19.04)	0.0308972074
85	(682.6, 9.44)	0.0308972074
86	(717.3, 17.96)	0.0308972074
87	(920.6, 10.61)	0.0308972074
88	(988.4, 10.46)	0.0308972074
89	(271.1, 15.08)	0.0313675727
90	(740.5, 6.02)	0.0316777607

91	(839.6, 20.85)	0.0316777607
92	(610.9, 2.44)	0.0329765016
93	(179.1, 13.20)	0.0330555292
94	(701.4, 5.63)	0.0330555292
95	(175.1, 8.49)	0.0332024906
96	(279.0, 2.32)	0.0337986949
97	(670.4, 9.09)	0.0337986949
98	(415.3, 15.42)	0.0338750641
99	(183.1, 6.88)	0.0343045905
100	(160.1, 0.50)	0.0344826274
101	(459.3, 4.96)	0.0352364197
102	(305.2, 1.87)	0.0353424937
103	(216.2, 4.54)	0.0363303150
104	(603.3, 6.48)	0.0363303150
105	(914.1, 6.94)	0.0368261384
106	(205.1, 6.75)	0.0368844784
107	(446.3, 4.94)	0.0371476565
108	(513.1, 4.48)	0.0380144912
109	(676.0, 6.65)	0.0382429645
110	(366.1, 0.86)	0.0383351335
111	(227.9, –0.44)	0.0386073936
112	(641.4, 7.27)	0.0387953825
113	(395.2, 24.02)	0.0388820140
114	(929.6, 7.27)	0.0389610390
115	(371.3, 4.58)	0.0392271166
116	(402.2, 1.19)	0.0392271166
117	(127.0, 4.75)	0.0397364228
118	(193.0, 1.36)	0.0404560651
119	(194.0, 1.00)	0.0404560651
120	(379.3, 15.55)	0.0404560651
121	(495.3, 12.82)	0.0404560651
122	(823.4, 9.50)	0.0404560651
123	(235.1, 8.53)	0.0405335640
124	(476.4, 4.96)	0.0421855472
125	(472.5, 11.18)	0.0425955352
126	(693.1, 5.95)	0.0426922311
127	(274.1, 7.80)	0.0428211411
128	(402.2, 12.86)	0.0428660082
129	(746.8, 2.42)	0.0429101967
130	(801.0, 2.11)	0.0429101967
131	(366.7, 5.89)	0.0434178862
132	(458.4, 4.70)	0.0434178862
133	(369.4, 26.36)	0.0440035652
134	(601.0, 0.43)	0.0440035652
135	(249.2, 6.55)	0.0440434139
136	(666.4, 5.77)	0.0444571249
137	(415.4, 12.38)	0.0447164378
138	(652.1, 5.87)	0.0447164378

139	(472.2, 11.12)	0.0453906033
140	(441.4, 24.91)	0.0464361698
141	(575.4, 20.88)	0.0464361698
142	(393.3, 4.58)	0.0464768588
143	(620.7, 0.74)	0.0465716607
144	(842.9, 6.93)	0.0465716607
145	(685.4, 17.53)	0.0468826130
146	(476.3, 1.86)	0.0472378721
147	(399.2, 2.99)	0.0479645296
148	(211.1, 13.48)	0.0488051357
149	(357.3, 9.11)	0.0488051357
150	(313.2, 17.63)	0.0495881957

TABLE 10

p-values from time –48 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(845.2, 6.33)	0.001343793
2	(715.8, 7.68)	0.002669885
3	(745.7, 6.03)	0.002743002
4	(802.4, 8.16)	0.002822379
5	(648.5, –0.24)	0.003721455
6	(745.3, 6.02)	0.005142191
7	(608.4, 5.39)	0.005491954
8	(265.2, 4.72)	0.006272684
9	(505.3, 12.78)	0.006518681
10	(371.3, 4.58)	0.006931949
11	(261.2, 1.26)	0.008001346
12	(971.4, 10.51)	0.008726088
13	(152.1, 1.51)	0.009174244
14	(685.1, 6.85)	0.009704974
15	(456.4, 9.80)	0.010451432
16	(214.2, 15.68)	0.010792220
17	(446.0, 2.54)	0.010792220
18	(346.1, 7.46)	0.011152489
19	(227.0, 23.11)	0.011834116
20	(407.2, 1.17)	0.011946593
21	(435.3, 19.92)	0.011946593
22	(451.3, 4.94)	0.012261329
23	(274.1, 7.80)	0.012266073
24	(869.0, 9.70)	0.012303709
25	(274.2, 4.67)	0.012859736
26	(789.4, 6.11)	0.012890139
27	(576.4, 3.29)	0.013087923
28	(930.0, 9.75)	0.013087923

29	(512.4, 10.44)	0.014315178
30	(878.9, 7.28)	0.014513409
31	(503.3, 5.12)	0.015193810
32	(180.1, 4.54)	0.015226001
33	(209.1, 5.03)	0.015254389
34	(616.2, 11.90)	0.016782325
35	(443.3, 3.41)	0.017490379
36	(572.6, 4.30)	0.017654283
37	(931.9, 6.72)	0.018138469
38	(966.4, 10.49)	0.019031437
39	(541.3, 5.12)	0.019316716
40	(470.3, 10.72)	0.019821985
41	(281.3, 16.88)	0.020436455
42	(407.2, 4.72)	0.021104001
43	(627.2, 2.48)	0.021491454
44	(313.2, 6.31)	0.022912878
45	(173.2, 15.68)	0.023189016
46	(675.6, 5.75)	0.023820433
47	(137.2, 9.60)	0.023895386
48	(357.2, 5.65)	0.023895386
49	(372.0, 0.62)	0.023895386
50	(635.3, 2.38)	0.023895386
51	(743.8, 4.55)	0.023895386
52	(185.2, 6.29)	0.024742907
53	(930.4, 7.60)	0.024770578
54	(564.4, 5.28)	0.024811749
55	(415.2, 9.09)	0.025574438
56	(697.3, 16.10)	0.025714289
57	(657.3, 2.49)	0.025825394
58	(996.1, 9.94)	0.026026402
59	(185.0, 0.10)	0.027530406
60	(333.1, 2.00)	0.027840095
61	(611.3, 6.59)	0.028096875
62	(283.3, 18.53)	0.028392609
63	(506.3, 8.10)	0.028392609
64	(726.4, 5.67)	0.028392609
65	(397.3, 20.91)	0.029361285
66	(311.9, 2.10)	0.029433328
67	(473.3, 8.15)	0.029433328
68	(490.2, 8.85)	0.029433328
69	(493.3, 22.99)	0.029433328
70	(577.2, 3.56)	0.029433328
71	(653.7, 6.16)	0.029433328
72	(757.5, 16.28)	0.029433328
73	(819.0, 2.11)	0.029433328
74	(853.5, 13.13)	0.029433328
75	(889.2, 6.42)	0.029433328
76	(929.6, 10.60)	0.029433328

77	(963.3, 9.70)	0.029433328
78	(982.1, 9.39)	0.029433328
79	(446.3, 4.94)	0.030176399
80	(959.5, 10.86)	0.030176399
81	(169.1, 5.03)	0.030177290
82	(906.7, 9.75)	0.030212739
83	(772.1, 7.79)	0.030482971
84	(857.0, 9.70)	0.030966151
85	(861.8, 9.74)	0.030966151
86	(377.2, 12.32)	0.031285164
87	(229.2, –0.79)	0.031539774
88	(229.2, 2.39)	0.031539774
89	(740.4, 9.58)	0.031759640
90	(958.3, 9.66)	0.031759640
91	(739.5, 18.01)	0.032714845
92	(377.2, 4.61)	0.032818612
93	(144.0, 0.25)	0.032941894
94	(459.3, 4.96)	0.033735985
95	(715.8, 4.37)	0.034116302
96	(649.0, 2.13)	0.034332004
97	(776.3, 6.78)	0.034520017
98	(827.1, 9.58)	0.034662245
99	(439.2, 9.40)	0.035385909
100	(376.0, 2.11)	0.038036916
101	(734.6, 7.21)	0.038036916
102	(402.2, 1.19)	0.038177664
103	(740.5, 6.02)	0.038356830
104	(502.5, 4.01)	0.038481929
105	(694.4, 6.02)	0.039047025
106	(331.0, 0.74)	0.039943461
107	(302.1, 4.44)	0.040965049
108	(836.1, 8.31)	0.041276236
109	(909.4, 9.75)	0.041642229
110	(358.0, 2.13)	0.041676687
111	(502.2, 4.55)	0.042049098
112	(302.2, 0.79)	0.042062826
113	(936.9, 9.51)	0.042143408
114	(492.2, 1.36)	0.042286848
115	(204.2, 5.03)	0.043172669
116	(701.4, 5.63)	0.044132315
117	(373.3, 24.05)	0.045041891
118	(657.4, 5.53)	0.045102516
119	(357.3, 15.86)	0.045170280
120	(670.9, 6.71)	0.045249625
121	(850.0, 7.56)	0.046346695
122	(576.4, 16.02)	0.046573286
123	(670.4, 9.09)	0.046609659
124	(578.4, 5.46)	0.047297957

125	(525.3, 5.12)	0.047503607
126	(926.0, 6.12)	0.047503607
127	(987.3, 9.56)	0.047882538
128	(231.0, –0.41)	0.048437237
129	(608.3, 2.35)	0.048607203
130	(966.7, 10.60)	0.048825822

[00110] A nonparametric test (*e.g.*, a Wilcoxon Signed Rank Test) alternatively can be used to find p-values for features that are based on the progressive appearance or disappearance of the feature in populations that are progressing toward sepsis. In this form of the test, a baseline value for a given feature first is measured, using the data from the time of entry into the study (Day 1 samples) for the sepsis and SIRS groups. The feature intensity in sepsis and SIRS samples is then compared in, for example, time –48 hour samples to determine whether the feature intensity has increased or decreased from its baseline value. Finally, p-values are assigned to the difference from baseline in a feature intensity in the sepsis populations versus the SIRS populations. The following p-values, listed in TABLES 11 – 13, were obtained when measuring these differences from baseline in p-values.

TABLE 11

p-values for features differenced from baseline: time 0 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(991.7, 16.6)	0.000225214
2	(592.4, 15.69)	0.001008201
3	(733.5, 4.55)	0.001363728
4	(173.1, 23.44)	0.001696095
5	(763.2, 16.6)	0.001851633
6	(932.2, 6.72)	0.002380877
7	(842.6, 10.11)	0.002575890
8	(295.9, 15.78)	0.002799236
9	(512.4, 10.44)	0.004198319
10	(551.4, 24.89)	0.005132229
11	(167.1, 10.99)	0.005168091
12	(857.8, 8.21)	0.005209485
13	(763.4, 19.81)	0.005541078
14	(931.9, 6.72)	0.006142506
15	(167.2, 14.42)	0.006349154
16	(510.4, 17.91)	0.006427070
17	(295.3, 16.1)	0.007165849
18	(353.2, 7.38)	0.007255100

19	(653, 6.71)	0.007848203
20	(730.4, 6.54)	0.008402925
21	(142, 0.44)	0.008578959
22	(331.7, 19.61)	0.008807931
23	(386.3, 9.47)	0.009227968
24	(524.4, 19.33)	0.010256841
25	(741.5, 23.22)	0.010329009
26	(272.2, 6.49)	0.010345274
27	(448.3, 9.24)	0.010666648
28	(713.5, 21.99)	0.011150954
29	(353.3, 22.38)	0.011224096
30	(457.2, 0.88)	0.011653586
31	(708.9, 0.37)	0.012197946
32	(256.2, 6.03)	0.013251532
33	(721.4, 23.49)	0.014040014
34	(496.4, 16.6)	0.014612622
35	(634.9, 27.04)	0.015093015
36	(663.3, 2.06)	0.015093015
37	(679.4, 5.92)	0.015176669
38	(521.4, 23.84)	0.015526731
39	(358.3, 4.4)	0.015795031
40	(409.2, 6.95)	0.015875221
41	(537.3, 23)	0.016202704
42	(875.4, 19.37)	0.016372468
43	(875.9, 10.08)	0.016391836
44	(265.2, 9.37)	0.016924737
45	(450.3, 11.99)	0.017293769
46	(329, 1.27)	0.017732659
47	(534.4, 10.53)	0.018580510
48	(616.2, 11.9)	0.018703298
49	(177, 0.93)	0.018855039
50	(772.1, 16.51)	0.018991142
51	(424.2, 6.12)	0.019195215
52	(277.3, 21.72)	0.020633230
53	(333.2, 7.39)	0.020898404
54	(742.8, 4.02)	0.021093249
55	(428.3, 6.2)	0.021697014
56	(946, 10.49)	0.021935440
57	(970.5, 7)	0.021999796
58	(281.7, 19.54)	0.022055564
59	(568.4, 15.49)	0.022208535
60	(700.3, 9.4)	0.022500138
61	(118.2, 5.26)	0.022773904
62	(601.3, 5.46)	0.023578505
63	(818.3, 7.18)	0.023788872
64	(799.4, 9.64)	0.023906673
65	(244.1, 2.22)	0.024125162
66	(145.1, 3.99)	0.024385288

67	(328.8, 19.98)	0.024385288
68	(342.4, 13.41)	0.025034251
69	(356.2, 5.6)	0.025034251
70	(321.3, 19.96)	0.025128604
71	(523.3, 13.8)	0.025164665
72	(504.3, 15.49)	0.025894254
73	(842.3, 10.76)	0.026070176
74	(585.3, 25.35)	0.026196933
75	(176.1, 10.29)	0.027193290
76	(399.3, 27.26)	0.027193290
77	(761.8, 7.89)	0.027193290
78	(909.8, 9.52)	0.027193290
79	(291.2, 12.57)	0.029135281
80	(715.8, 7.68)	0.030440991
81	(546.4, 19.33)	0.030479818
82	(795.5, 20.72)	0.030479818
83	(321, 19.53)	0.030693238
84	(746.8, 10.2)	0.030888031
85	(831.5, 20.87)	0.030888031
86	(872.9, 11.6)	0.030888031
87	(598, 8.58)	0.031026286
88	(407.2, 12.07)	0.031941032
89	(645.3, 13.42)	0.031941032
90	(662.1, 8.16)	0.031941032
91	(179, 10.16)	0.032126841
92	(779.5, 19.79)	0.032301988
93	(171.2, 25.87)	0.032868402
94	(979.6, 10.14)	0.033098647
95	(245.2, 22.24)	0.033117202
96	(370.3, 2.3)	0.033696034
97	(433.3, 5.29)	0.033696034
98	(771.4, 10.01)	0.033696034
99	(876.3, 9.94)	0.033696034
100	(893, 7.09)	0.033919037
101	(669.2, 2.13)	0.034234876
102	(643.3, 5.67)	0.034557232
103	(991.3, 9.72)	0.035680492
104	(577.5, 16.48)	0.036136938
105	(820, 6.38)	0.036179853
106	(856.6, 10.29)	0.036179853
107	(453.2, 6.62)	0.036689053
108	(652.1, 5.87)	0.037082670
109	(944.8, 9.65)	0.037337126
110	(494.4, 14.75)	0.037526457
111	(185, 11.17)	0.037568360
112	(229.2, 0.79)	0.037574432
113	(245.1, 11.44)	0.038031041
114	(279.3, 20.72)	0.038253242

115	(781.5, 20.04)	0.038253242
116	(409.4, 22.56)	0.038673618
117	(315.2, 14.29)	0.039895232
118	(759.5, 9.33)	0.040499878
119	(995.1, 9.94)	0.040516802
120	(848.3, 9.66)	0.040554157
121	(263.3, 22.26)	0.041183545
122	(267.7, 16.55)	0.041183545
123	(544.4, 15.56)	0.041183545
124	(617.5, 17.71)	0.041406719
125	(411.5, 1.06)	0.041454989
126	(597.4, 11.4)	0.041454989
127	(771.4, 6.02)	0.041454989
128	(901.9, 1.03)	0.041454989
129	(415.2, 9.09)	0.041542794
130	(430.3, 9.1)	0.041922297
131	(414.3, 4.29)	0.043298568
132	(414.9, 5.86)	0.043427801
133	(444.2, 6)	0.043665836
134	(505.3, 12.78)	0.043665836
135	(231, 0.41)	0.043722631
136	(370.3, 10.79)	0.044296546
137	(653.5, 19.99)	0.044296546
138	(291.7, 15.37)	0.044815129
139	(531.3, 21.48)	0.044870846
140	(715.4, 5.89)	0.044985107
141	(327.3, 16.98)	0.045218533
142	(499.4, 15.11)	0.046077647
143	(766.2, 15.77)	0.046332971
144	(664.2, 11.84)	0.047191074
145	(567.4, 20.79)	0.047549465
146	(809.6, 21.33)	0.047600425
147	(393.3, 21.08)	0.048014243
148	(754.6, 7.21)	0.048520560
149	(298.3, 24.36)	0.049732041
150	(883.3, 6.69)	0.049768492
151	(468.3, 13.44)	0.049813626
152	(665.4, 15.46)	0.049918030

TABLE 12

p-values for features differenced from baseline: time –24 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(875.4, 19.37)	0.0006856941
2	(256.2, 6.03)	0.0009911606
3	(228, 3.12)	0.0014153532
4	(227.9, 0.44)	0.0015547019
5	(879.8, 4.42)	0.0025072593
6	(858.2, 10.41)	0.0029384997
7	(159, 2.37)	0.0038991631
8	(186.9, 2.44)	0.0045074080
9	(609.1, 1.44)	0.0047227895
10	(996.1, 9.94)	0.0058177265
11	(430.7, 4.21)	0.0063024974
12	(141.1, 5.13)	0.0068343584
13	(839.6, 20.85)	Q.0072422001
14	(956.1, 10.62)	0.0080620376
15	(113.2, 0.44)	0.0081626136
16	(428.3, 6.2)	0.0081962770
17	(802.9, 0.39)	0.0081962770
18	(819, 2.11)	0.0081968739
19	(366.1, 0.86)	0.0084072673
20	(993.5, 9.39)	0.0084773116
21	(919.5, 9.63)	0.0098988701
22	(680.6, 7.39)	0.0105489986
23	(523.3, 22.32)	0.0105995251
24	(668.3, 8.45)	0.0112292667
25	(463.2, 1.88)	0.0113722034
26	(259, 11.71)	0.0115252694
27	(889.7, 16.16)	0.0115864528
28	(810.4, 7.42)	0.0119405153
29	(300, 21.9)	0.0123871653
30	(141.2, 1.46)	0.0124718161
31	(785.5, 9.3)	0.0126735996
32	(660.1, 3.9)	0.0131662199
33	(575.4, 10)	0.0133539242
34	(398.2, 8.89)	0.0133977345
35	(678.8, 2.37)	0.0134811753
36	(779.5, 19.79)	0.0152076628
37	(190.1, 3.99)	0.0153485356
38	(746.8, 2.42)	0.0153591871
39	(407.2, 7.81)	0.0154972293
40	(265.2, 9.37)	0.0163877868
41	(447.8, 6.29)	0.0163877868
42	(472.5, 11.18)	0.0166589145

43	(951.9, 10.21)	0.0169717792
44	(138.2, 10.13)	0.0170020893
45	(739.5, 9.45)	0.0171771560
46	(999, 7.71)	0.0177981470
47	(472.2, 11.12)	0.0178902225
48	(138.1, 1.89)	0.0180631050
49	(842.9, 6.93)	0.0189332371
50	(717.3, 17.96)	0.0193107546
51	(245.2, 5.23)	0.0201247940
52	(666.4, 9.29)	0.0211733529
53	(820, 6.38)	0.0216512533
54	(991.7, 9.21)	0.0219613529
55	(177, 0.93)	0.0223857280
56	(488.3, 9.68)	0.0224061094
57	(119.1, 9.19)	0.0224206599
58	(278.1, 5.24)	0.0240107773
59	(409.2, 6.95)	0.0256235918
60	(369.2, 3.37)	0.0259379108
61	(482.4, 19.26)	0.0261591305
62	(806.6, 21.29)	0.0269790713
63	(637.9, 7.43)	0.0273533420
64	(373.3, 11.45)	0.0277220597
65	(264.2, 8.83)	0.0282234106
66	(909.7, 6.36)	0.0282234106
67	(747.4, 9.38)	0.0287012166
68	(832.9, 6.21)	0.0289271134
69	(155.1, 2.87)	0.0289347031
70	(977.7, 9.56)	0.0298654782
71	(610.9, 2.44)	0.0303741714
72	(235.1, 4.04)	0.0303830303
73	(685.1, 6.85)	0.0303830303
74	(670.4, 9.09)	0.0307328580
75	(346.1, 12.11)	0.0308972074
76	(217.2, 8.66)	0.0309517132
77	(770.9, 16.6)	0.0310937661
78	(163.2, 6.31)	0.0313614024
79	(392.3, 10)	0.0317350792
80	(469.7, 5.98)	0.0317350792
81	(470, 6.32)	0.0317350792
82	(794.9, 9.76)	0.0317350792
83	(357.3, 18.91)	0.0318983292
84	(303.7, 15.73)	0.0325397156
85	(221, 1.92)	0.0328080364
86	(999.5, 7.28)	0.0330940901
87	(637.3, 18.59)	0.0335078063
88	(331, 0.74)	0.0336148466
89	(978.8, 6.72)	0.0338444022
90	(271.1, 15.08)	0.0347235687

91	(801, 2.11)	0.0348606916
92	(599.5, 21.95)	0.0358839090
93	(769.4, 10.46)	0.0371510791
94	(914.1, 6.94)	0.0375945952
95	(363, 26.16)	0.0381998666
96	(235.1, 8.53)	0.0382752828
97	(273.2, 6.31)	0.0390486612
98	(250.1, 14.23)	0.0401201887
99	(585.2, 15.27)	0.0406073368
100	(276.2, 5.27)	0.0414046782
101	(183.1, 6.88)	0.0419461253
102	(430.3, 9.1)	0.0421855472
103	(229.2, 0.79)	0.0424445226
104	(811.6, 19.44)	0.0438285232
105	(126.2, 4.02)	0.0439140255
106	(708.5, 15.79)	0.0439143789
107	(127, 4.75)	0.0442108301
108	(338.2, 7.89)	0.0444291108
109	(391.3, 14.55)	0.0444291108
110	(714.6, 14.02)	0.0444291108
111	(665.3, 9.58)	0.0446481623
112	(875.7, 19.83)	0.0446481623
113	(676, 6.65)	0.0447614386
114	(695.1, 2.71)	0.0448433123
115	(480.2, 8.03)	0.0451624233
116	(754.6, 7.21)	0.0454753333
117	(494.9, 19.41)	0.0454916992
118	(785.1, 9.29)	0.0455064285
119	(265.2, 4.72)	0.0456621220
120	(771.9, 24.52)	0.0460254955
121	(467.2, 8.55)	0.0464130076
122	(869.9, 10.55)	0.0464539626
123	(479.3, 24.87)	0.0473472790
124	(380.3, 24.05)	0.0475242732
125	(194.1, 6.48)	0.0475341652
126	(262.6, 5.7)	0.0475341652
127	(694.2, 11.76)	0.0475341652
128	(695.9, 4.32)	0.0475341652
129	(660.8, 2.32)	0.0475865516
130	(958.8, 6.36)	0.0482703924
131	(504.3, 15.49)	0.0484159645

TABLE 13

p-values for features differenced from baseline: time –48 hours samples

ion number	*m/z* (Da), retention time (min)	p-value
1	(715.8, 7.68)	0.0005303918
2	(919.5, 9.63)	0.0012509535
3	(802.4, 8.16)	0.0016318638
4	(922.5, 7.27)	0.0023943584
5	(741.5, 23.22)	0.0038457139
6	(875.4, 19.37)	0.0044466656
7	(878.9, 7.28)	0.0052374088
8	(996.1, 9.94)	0.0060309508
9	(295.9, 15.78)	0.0070608315
10	(521.4, 23.84)	0.0075730074
11	(676, 6.65)	0.0075742521
12	(703.9, 4.35)	0.0075743621
13	(716.2, 6.62)	0.0078671775
14	(346.1, 7.46)	0.0080100576
15	(551.4, 24.89)	0.0086803932
16	(415.2, 9.09)	0.0088869428
17	(182.1, 2.44)	0.0114906565
18	(310.3, 19.13)	0.0121106698
19	(428.3, 6.2)	0.0124220037
20	(908.6, 10.83)	0.0127529218
21	(715.8, 4.37)	0.0129735339
22	(444.3, 2.8)	0.0135088012
23	(753.3, 9.34)	0.0140485313
24	(779.5, 19.79)	0.0149169860
25	(211.1, 13.48)	0.0149614082
26	(285.2, 19.8)	0.0155513781
27	(441.4, 19.09)	0.0169697745
28	(483.3, 6.17)	0.0171647510
29	(488.3, 6.38)	0.0172240677
30	(616.2, 11.9)	0.0176526391
31	(861.8, 9.74)	0.0185440613
32	(485.3, 23.17)	0.0186867970
33	(435.1, 4.14)	0.0193706655
34	(612.3, 16.87)	0.0193706655
35	(362.3, 5.65)	0.0194196263
36	(227, 23.11)	0.0204130271
37	(883.2, 9.76)	0.0204386696
38	(229.2, 0.79)	0.0205101165
39	(643.3, 5.67)	0.0210117164
40	(980.6, 7.44)	0.0215182605
41	(795.5, 20.72)	0.0218437599
42	(577.2, 3.56)	0.0224776501

43	(152.1, 1.51)	0.0233549892
44	(525.4, 15.11)	0.0234730657
45	(435.3, 19.92)	0.0235646539
46	(299.2, 25.54)	0.0237259148
47	(612.9, 0.36)	0.0245420186
48	(505.3, 12.78)	0.0245629232
49	(986.7, 7.42)	0.0248142595
50	(719.2, 6.07)	0.0252229441
51	(562.3, 19.13)	0.0252471150
52	(552.4, 22.8)	0.0254361708
53	(353.2, 19.3)	0.0266840298
54	(575.4, 16.74)	0.0275127383
55	(845.2, 6.33)	0.0291304640
56	(633.7, 6.14)	0.0301224895
57	(519.3, 13.32)	0.0301986537
58	(205.1, 13.28)	0.0306513410
59	(317.9, 1.41)	0.0306513410
60	(388.3, 9.86)	0.0306513410
61	(471.3, 26.3)	0.0306513410
62	(723.2, 6.69)	0.0320817369
63	(912.5, 10.13)	0.0320817369
64	(965.2, 2.77)	0.0320817369
65	(718.9, 5.76)	0.0322905214
66	(363, 26.16)	0.0330856794
67	(897.1, 9.53)	0.0331382847
68	(227.3, 6.92)	0.0332507087
69	(778.2, 14.75)	0.0335555992
70	(321, 2.35)	0.0337995708
71	(447.8, 6.29)	0.0343295019
72	(536.1, 4.09)	0.0343295019
73	(653.5, 19.99)	0.0343565954
74	(667.4, 21.32)	0.0343565954
75	(982.7, 9.73)	0.0352875093
76	(789.4, 6.11)	0.0364395580
77	(505.3, 18.48)	0.0369258233
78	(277, 0.2)	0.0369277075
79	(285.3, 12.09)	0.0382728484
80	(739.5, 18.01)	0.0382728484
81	(838.9, 0.39)	0.0382728484
82	(400.2, 5.79)	0.0384511838
83	(883.6, 7.04)	0.0384732436
84	(604.3, 19.85)	0.0411740329
85	(287.1, 4.72)	0.0412206143
86	(549.9, 4.23)	0.0415068077
87	(879.8, 4.42)	0.0415426686
88	(721.7, 20.36)	0.0417134604
89	(711.4, 16.81)	0.0417360498
90	(982.1, 9.39)	0.0419790105

91	(971.4, 10.51)	0.0432043627
92	(112.7, 1.05)	0.0452851799
93	(503.3, 14.33)	0.0453240047
94	(173.1, 23.44)	0.0466828436
95	(283.1, 4.96)	0.0466865226
96	(637.4, 6.78)	0.0467959828
97	(597.4, 15.92)	0.0471002889
98	(813.5, 9.83)	0.0480402523
99	(444.2, 6)	0.0486844297
100	(448.3, 9.24)	0.0486916088
101	(502.5, 4.01)	0.0493775335
102	(854.2, 5.79)	0.0493775335

Example 2: Identification of protein biomarkers using quantitative liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS)

2.1. Samples Received and Analyzed

[00111] As above, reference biomarker profiles were obtained from a first population representing 15 patients ("the SIRS group") and a second population representing 15 patients who developed SIRS and progressed to sepsis ("the sepsis group"). Blood was withdrawn from the patients at Day 1, time 0, and time –48 hours. In this case, 50 – 75 μL plasma samples from the patients were pooled into four batches: two batches of five and 10 individuals who were SIRS-positive and two batches of five and 10 individuals who were sepsis-positive. Six samples from each pooled batch were further analyzed.

2.2 Sample Preparation

[00112] Plasma samples first were immunodepleted to remove abundant proteins, specifically albumin, transferrin, haptoglobulin, anti-trypsin, IgG, and IgA, which together constitute approximately 85% (wt%) of protein in the samples. Immunodepletion was performed with a Multiple Affinity Removal System column (Agilent Technologies, Palo Alto, California), which was used according to the manufacturer's instructions. At least 95% of the aforementioned six proteins were removed from the plasma samples using this system. For example, only about 0.1% of albumin remained in the depleted samples. Only an estimated 8% of proteins left in the samples represented remaining high abundance proteins, such as IgM and α-2 macroglobulin. Fractionated plasma samples were then denatured, reduced, alkylated and digested with trypsin using procedures well-known in the art. About 2 mg of digested proteins were obtained from each pooled sample.

2.3. Multidimensional LC/MS

[00113] The peptide mixture following trypsin digestion was then fractionated using LC columns and analyzed by an Agilent MSD/trap ESI-ion trap mass spectrometer configured in an LC/MS/MS arrangement. One mg of digested protein was applied at 10 μL/minute to a micro-flow C_{18} reverse phase (RP1) column. The RP1 column was coupled in tandem to a Strong Cation Exchange (SCX) fractionation column, which in turn was coupled to a C_{18} reverse phase trap column. Samples were applied to the RP1 column in a first gradient of 0 – 10% ACN to fractionate the peptides on the RP1 column. The ACN gradient was followed by a 10 mM salt buffer elution, which further fractionated the peptides into a fraction bound to the SCX column and an eluted fraction that was immobilized in the trap column. The trap column was then removed from its operable connection with the SCX column and placed in operable connection with another C_{18} reverse phase column (RP2). The fraction immobilized in the trap column was eluted from the trap column onto the RP2 column with a gradient of 0 – 10 % ACN at 300 nL/minute. The RP2 column was operably linked to an Agilent MSD/trap ESI-ion trap mass spectrometer operating at a spray voltage of 1000 – 1500 V. This cycle (RP1-SCX-Trap-RP2) was then repeated to fractionate and separate the remaining peptides using a total ACN% range from 0-80% and a salt concentration up to 1M. Other suitable configurations for LC/MS/MS may be used to generate biomarker profiles that are useful for the invention. Mass spectra were generated in an *m/z* range of 200 – 2200 Da. Data dependent scan and dynamic exclusion were applied to achieve higher dynamic range. FIGURE 6 shows representative biomarker profiles generated with LC/MS and LC/MS/MS.

2.4. Data Analysis and Results

[00114] For every sample that was analyzed in the MS/MS mode, about 150,000 spectra were obtained, equivalent to about 1.5 gigabytes of information. In total, some 50 gigabytes of information were collected. Spectra were analyzed using Spectrum Mill v 2.7 software (© Copyright 2003 Agilent Technologies, Inc.). The MS-Tag database searching algorithm (Millennium Pharmaceuticals) was used to match MS/MS spectra against a National Center for Biotechnology Information (NCBI) database of human non-redundant proteins. A cutoff score equivalent to 95% confidence was used to validate the matched peptides, which were then assembled to identify proteins present in the samples. Proteins that were detectable using the present method are present in plasma at a concentration of ~1 ng/mL, covering a dynamic range in plasma concentration of about six orders of magnitude.

[00115] A semi-quantitative estimate of the abundance of detected proteins in plasma was obtained by determining the number of mass spectra that were "positive" for the protein. To be positive, an ion feature has an intensity that is detectably higher than the noise at a given *m/z* value in a spectrum. In general, a protein expressed at higher levels in plasma will be detectable as a positive ion feature or set of ion features in more spectra. With this measure of protein concentration, it is apparent that various proteins are differentially expressed in the SIRS group versus the sepsis group. Various of the detected proteins that were "up-regulated" are shown in FIGURES 7A and 7B, where an up-regulated protein is expressed at a higher level in the sepsis group than in the SIRS group. It is clear from FIGURE 7A that the level at which a protein is expressed over time may change, in the same manner as ion # 21 (437.2 Da, 1.42 min), shown in FIGURE 4. For example, the proteins having GenBank Accession Numbers AAH15642 and NP_000286, which both are structurally similar to a serine (or cysteine) proteinase inhibitor, are expressed at progressively higher levels overtime in sepsis-positive populations, while they are expressed at relatively constant amounts in the SIRS–positive populations. The appearance of high levels of these proteins, and particularly a progressively higher expression of these proteins in an individual over time, is expected to be a predictor of the onset of sepsis. Various proteins that were down-regulated in sepsis-positive populations overtime are shown in FIGURES 8A and 8B. The expression of some of these proteins, like the unnamed protein having the sequence shown in GenBank Accession Number NP_079216, appears to increase progressively or stay at relatively high levels in SIRS patients, even while the expression decreases in sepsis patients. It is expected that these proteins will be biomarkers that are particularly useful for diagnosing SIRS, as well as predicting the onset of sepsis.

Example 3: Identification of Biomarkers Using an Antibody Array

3.1. Samples Received and Analyzed

[00116] Reference biomarker profiles were established for a SIRS group and a sepsis group. Blood samples were taken every 24 hours from each study group. Samples from the sepsis group included those taken on the day of entry into the study (Day 1), 48 hours prior to clinical suspicion of sepsis (time –48 hours), and on the day of clinical suspicion of the onset of sepsis (time 0). In this example, the SIRS group and sepsis group analyzed at time 0 contained 14 and 11 individuals, respectively, while the SIRS group and sepsis group analyzed at time –48 hours contained 10 and 11 individuals, respectively.

3.2. Multiplex Analysis

[00117] A set of biomarkers in each sample was analyzed simultaneously in real time, using a multiplex analysis method as described in U.S. Patent No. 5,981,180 ("the '180 patent"), herein incorporated by reference in its entirety, and in particular for its teachings of the general methodology, bead technology, system hardware and antibody detection. The immunoassay described in the '180 patent is representative of a type of immunoassay that could be used in the methods of the present invention. Furthermore, the biomarkers used herein are not meant to limit the scope of available biomarkers used in the methods of the present invention. For this analysis, a matrix of microparticles was synthesized, where the matrix consisted of different sets of microparticles. Each set of microparticles had thousands of molecules of a distinct antibody capture reagent immobilized on the microparticle surface and was color-coded by incorporation of varying amounts of two fluorescent dyes. The ratio of the two fluorescent dyes provided a distinct emission spectrum for each set of microparticles, allowing the identification of a microparticle within a set following the pooling of the various sets of microparticles. U.S. Patents No. 6,268,222 and No. 6,599,331 also are incorporated herein by reference in their entirety, and in particular for their teachings of various methods of labeling microparticles for multiplex analysis.

[00118] The sets of labeled beads were pooled and were combined with a plasma sample from an individual used in the study. The labeled beads were identified by passing them single file through a flow device that interrogated each microparticle with a laser beam that excited the fluorophore labels. An optical detector then measured the emission spectrum of each bead to classify the beads into the appropriate set. Because the identity of each antibody capture reagent was known for each set of microparticles, each antibody specificity was matched with an individual microparticle that passes through the flow device. U.S. Patent No. 6,592,822 is also incorporated herein by reference in its entirety, and in particular for its teachings of multi-analyte diagnostic system that can be used in this type of multiplex analysis.

[00119] To determine the amount of analyte that bound a given set of microparticles, a reporter molecule was added such that it formed a complex with the antibodies bound to their respective analyte. In the present example, the reporter molecule was a fluorophore-labeled secondary antibody. The fluorophore on the reporter was excited by a second laser having a different excitation wavelength, allowing the fluorophore label on the secondary antibody to

be distinguished from the fluorophores used to label the microparticles. A second optical detector measured the emission from the fluorophore label on the secondary antibody to determine the amount of secondary antibody complexed with the analyte bound by the capture antibody. In this manner, the amount of multiple analytes captured to beads could be measured rapidly and in real time in a single reaction.

3.3. Data Analysis and Results

[00120] For each sample, the concentrations of analytes that bound 162 different antibodies were measured. In this Example, each analyte is a biomarker, and the concentration of each in the sample can be a feature of that biomarker. The biomarkers were analyzed with the various 162 antibody reagents listed in TABLE 14 below, which are commercially available from Rules Based Medicine of Austin, Texas. The antibody reagents are categorized as specifically binding either (1) circulating protein biomarker components of blood, (2) circulating antibodies that normally bind molecules associated with various pathogens (identified by the pathogen that each biomarker is associated with, where indicated), or (3) autoantibody biomarkers that are associated with various disease states.

TABLE 14

(1) Circulating serum components

Alpha-Fetoprotein
Apolipoprotein A1
Apolipoprotein CIII
Apolipoprotein H
β-2 Microglobulin
Brain-Derived Neurotrophic Factor
Complement 3
Cancer Antigen 125
Carcinoembryonic Antigen (CEA)
Creatine Kinase-MB
Corticotropin Releasing Factor
C Reactive Protein
Epithelial Neutrophil Activating Peptide-78 (ENA-78)
Fatty Acid Binding Protein
Factor VII
Ferritin
Fibrinogen
Growth Hormone
Granulocyte Macrophage-Colony Stimulating Factor
Glutathione S-Transferase

Intercellular adhesion molecule 1 (ICAM 1)
Immunoglobulin A
Immunoglobulin E
Immunoglobulin M
Interleukin-10
Interleukin-12 p 40
Interleukin-12 p 70
Interleukin-13
Interleukin-15
Interleukin-16
Interleukin-18
Interleukin-1α
Interleukin-1β
Interleukin-2
Interleukin-3
Interleukin-4
Interleukin-5
Interleukin-6
Interleukin-7
Interleukin-8
Insulin
Leptin
Lipoprotein (a)
Lymphotactin
Macrophage Chemoattractant Protein-1 (MCP-1)
Macrophage-Derived Chemokine (MDC)
Macrophage Inflammatory Protein-1β (MIP-1β)
Matrix Metalloproteinase-3 (MMP-3)
Matrix Metalloproteinase-9 (MMP-9)
Myoglobin
Prostatic Acid Phosphatase
Prostate Specific Antigen, Free
Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES)
Serum Amyloid P
Stem Cell Factor
Serum glutamic oxaloacetic transaminase (SGOT)
Thyroxine Binding Globulin
Tissue inhibitor of metalloproteinase 1 (TIMP 1)
Tumor Necrosis Factor-α (TNF-α)
Tumor Necrosis Factor-β (TNF-β)
Thrombopoietin
Thyroid Stimulating Hormone (TSH)
von Willebrand Factor

(2) Antibodies that bind the indicated pathogen marker

Adenovirus
Bordetella pertussis
Campylobacter jejuni
Chlamydia pneumoniae
Chlamydia trachomatis
Cholera Toxin
Cholera Toxin (subunit B)
Cytomegalovirus
Diphtheria Toxin
Epstein-Barr Virus-Viral Capsid Antigen
Epstein Barr Virus Early Antigen
Epstein Barr Virus Nuclear Antigen
Helicobacter pylori
Hepatitis B Core
Hepatitis B Envelope
Hepatitis B Surface (Ad)
Hepatitis B Surface (Ay)
Hepatitis C Core
Hepatitis C Non-Structural 3
Hepatitis C Non-Structural 4
Hepatitis C Non-Structural 5
Hepatitis D
Hepatitis A
Hepatitis E Virus (orf2 3KD)
Hepatitis E Virus (orf2 6KD)
Hepatitis E Virus (orf3 3KD)
Human Immunodeficiency Virus-1 p24
Human Immunodeficiency Virus-1 gp120
Human Immunodeficiency Virus-1 gp41
Human Papilloma Virus
Herpes Simplex Virus-1/2
Herpes Simplex Virus-1 gD
Herpes Simplex Virus-2 gG
Human T-Cell Lymphotropic Virus 1/2
Influenza A
Influenza A H3N2
Influenza B
Leishmania donovani
Lyme Disease Virus
Mycobacteria pneumoniae
Mycobacteria tuberculosis
Mumps Virus
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3

Polio Virus
Respiratory Syncytial Virus
Rubella Virus
Rubeola Virus
Streptolysin O (SLO)
Trypanosoma cruzi
Treponema pallidum 15KD
Treponema pallidum p47
Tetanus Toxin
Toxoplasma
Varicella zoster

(3) Autoantibodies

Anti-Saccharomyces cerevisiae antibodies (ASCA)
Anti-β-2 Glycoprotein
Anti-Centromere Protein B
Anti-Collagen Type 1
Anti-Collagen Type 2
Anti-Collagen Type 4
Anti-Collagen Type 6
Anti-Complement C1q
Anti-Cytochrome P450
Anti-Double Stranded DNA (ds DNA)
Anti-Histone
Anti-Histone H1
Anti-Histone H2a
Anti-Histone H2b
Anti-Histone H3
Anti-Histone H4
Anti-Heat Shock Cognate Protein 70 (HSC 70)
Anti-Heat Shock Protein 32 (HO)
Anti-Heat Shock Protein 65
Anti-Heat Shock Protein 71
Anti-Heat Shock Protein 90 α
Anti-Heat Shock Protein 90 β
Anti-Insulin
Anti-Histidyl-tRNA Synthetase (JO-1)
Anti-Mitochondrial
Anti-Myeloperoxidase (perinuclear autoantibodies to neutrophil cytoplasmic antigens)
Anti-Pancreatic Islet Cells (Glutamic Acid Decarboxylase Autoantibody)
Anti-Proliferating Cell Nuclear Antigen (PCNA)
Polymyositis-1 (PM-1)
Anti-Proteinase 3 (cytoplasmic autoantibodies to neutrophil cytoplasmic antigens)
Anti-Ribosomal P
Anti-Ribonuclear protein (RNP)
Anti-Ribonuclear protein (a)

Anti-Ribonuclear protein (b)
Anti-Topoisomerase I (Scl 70)
Anti-Ribonucleoprotein Smith Ag (Smith)
Anti-Sjögren's Syndrome A (Ro) (SSA)
Anti-Sjögren's Syndrome B (La) (SSB)
Anti-T3
Anti-T4
Anti-Thyroglobulin
Anti-Thyroid microsomal
Anti-tTG (Tissue Transglutaminase, Celiac Disease)

[00121] Various approaches may used to identify features that can inform a decision rule to classify individuals into the SIRS or sepsis groups. The methods chosen were logistic regression and a Wilcoxon Signed Rank Test.

3.3.1. Analysis of the Data Using Logistic Regression

[00122] Quantitative results from the biomarker immunoassays were analyzed using logistic regression. The top 26 biomarkers for the time 0 populations, which comprise a pattern that distinguishes SIRS from sepsis, are listed in TABLE 15. For the time –48 hours population, the top 14 biomarkers, which comprise a pattern that distinguishes SIRS from sepsis, are listed in TABLE 16. The data in Tables 15 & 16 demonstrate those biomarkers that comprise the patterns that distinguish the SIRS and sepsis groups.

TABLE 15

Biomarkers that comprise a pattern: Time 0 samples

Biomarker	Importance
Myoglobin	0.1958
Matrix Metalloproteinase (MMP)-9	0.1951
Macrophage Inflammatory Protein-1β (MIP-1β)	0.1759
C Reactive Protein	0.1618
Interleukin (IL)-16	0.1362
Herpes Simplex Virus-1/2	0.1302
Anti-Complement C1q antibodies	0.1283
Anti-Proliferating Cell Nuclear Antigen (PCNA) antibodies	0.1271
Anti-Collagen Type 4 antibodies	0.1103
Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)	0.1103

Glutathione S-Transferase (GST)	0.1091
Anti-*Saccharomyces cerevisiae* antibodies (ASCA)	0.1034
Growth Hormone (GH)	0.1009
Polio Virus	0.0999
IL-18	0.0984
Thyroxin Binding Globulin	0.0978
Anti-tTG (Tissue Transglutaminase, Celiac Disease) antibodies	0.0974
Leptin	0.0962
Anti-Histone H2a antibodies	0.0940
β2-Microglobulin	0.0926
Helicobacter pylori	0.0900
Diptheria Toxin	0.0894
Hepatitis C Core	0.0877
Serum Glutamic Oxaloacetic Transaminase	0.0854
Hepatitis C Non-Structural 3	0.0845
Hepatitis C Non-Structural 4	0.0819

TABLE 16

Biomarkers that comprise a pattern: Time –48 hours samples

Biomarker	Importance
Thyroxine Binding Globulin	0.0517
IL-8	0.0414
Intercellular Adhesion Molecule 1 (ICAM 1)	0.0390
Prostatic Acid Phosphatase	0.0387
MMP-3	0.0385
Herpes Simplex Virus – 1/2	0.0382
C Reactive Protein	0.0374
MMP-9	0.0362
Anti – PCNA antibodies	0.0357
IL-18	0.0341
ASCA	0.0341
Lipoprotein (a)	0.0334
Leptin	0.0327

Cholera toxin	0.0326

3.3.2. Analysis of the Data Using a Wilcoxon Signed Rank Test

[00123] A Wilcoxon Signed Rank Test also was used to identify individual protein biomarkers of interest. Biomarkers listed in TABLE 14 were assigned a p-value by comparison of sepsis and SIRS populations at a given time, in the same manner as in Example 1.4.7., TABLES 8 – 10, above. For this analysis, the sepsis and SIRS populations at time 0 (TABLE 17) constituted 23 and 25 patients, respectively; the sepsis and SIRS populations at time –24 hours (TABLE 18) constituted 25 and 22 patients, respectively; and the sepsis and SIRS populations at time –48 hours (TABLE 19) constituted 25 and 19 patients, respectively.

TABLE 17

biomarker p-values from time 0 samples

Biomarker	p-value
IL-6	2.1636e-06
C Reactive Protein	1.9756e-05
TIMP-1	7.5344e-05
IL-10	8.0576e-04
Thyroid Stimulating Hormone	0.0014330
IL-8	0.0017458
MMP-3	0.0032573
MCP-1	0.0050354
Glutathione S-Transferase	0.0056200
MMP-9	0.0080336
β-2 Microglobulin	0.014021
Histone H2a	0.023793
MIP-1β	0.028897
Myoglobin	0.033023
Complement C1q	0.033909
ICAM-1	0.036737
Leptin	0.046272
Apolipoprotein CIII	0.047398

TABLE 18

biomarker p-values from time –24 hours samples

Biomarker	p-value
IL-6	0.00039041
TIMP-1	0.0082532
Complement C1q	0.012980
Thyroid Stimulating Hormone	0.021773
HSC 70	0.031430
SSB	0.033397
MMP-3	0.035187
Calcitonin	0.038964
Thrombopoietin	0.040210
Factor VII	0.040383
Histone H2a	0.042508
Fatty Acid Binding Protein	0.043334

TABLE 19

biomarker p-values from time –48 hours samples

Biomarker	p-value
IL-8	0.0010572
C Reactive Protein	0.0028340
IL-6	0.0036449
ICAM-1	0.0056714
MIP-1β	0.016985
Thyroxine Binding Globulin	0.025183
Prostate Specific Antigen, Free	0.041397
Apolipoprotein A1	0.043747

[00124] In addition, p-values were based on the progressive appearance or disappearance of the feature in populations that are progressing toward sepsis, in the same manner as in Example 1.4.7., TABLES 11 – 13. For this analysis, the population sizes were the same as

shown immediately above, except that the sepsis and SIRS populations at time –48 hours constituted 22 and 18 patients, respectively.

TABLE 20

p-values for features differenced from baseline: time 0 hours samples

Biomarker	p-value
C Reactive Protein	0.0088484
MMP 9	0.022527
T3	0.043963

TABLE 21

p-values for features differenced from baseline: time –24 hours samples

Biomarker	p-value
von Willebrand Factor	0.0047043
HIV1 gp41	0.011768
Pancreatic Islet Cells GAD	0.030731
Creatine Kinase MB	0.043384
Apolipoprotein H	0.046076

TABLE 22

p-values for features differenced from baseline: time –48 hours samples

Biomarker	p-value
Pancreatic Islet Cells GAD	0.00023455
T3	0.0010195
HIV1 p24	0.031107
Hepatitis A	0.045565
Ferritin	0.048698

3.3.3. Analysis of the Data Using Multiple Adaptive Regression Trees (MART)

[00125] Data from protein biomarker profiles obtained from time 0 samples were analyzed using MART, as described above in Example 1.4.5. In this analysis, the time 0 hours sepsis

population consisted of 23 patients and the SIRS population consisted of 25 patients. Features corresponding to all 164 biomarkers listed in TABLE 14 were analyzed. The fitted model included 24 trees, and the model allowed no interactions among the features. Using ten-fold cross-validation, the model correctly classified 17 of 25 SIRS patients and 17 of 23 sepsis patients, giving a model sensitivity of 74% and a specificity of 68%. The biomarkers are ranked in order of importance, as determined by the model, in TABLE 23. All features with zero importance are excluded. Markers indicated with a sign of "1" were expressed at progressively higher levels in sepsis-positive populations as sepsis progressed, while those biomarkers with a sign of "–1" were expressed at progressively lower levels.

TABLE 23

feature importance by MART analysis: time 0 hours samples

Biomarker	Importance	Sign
C Reactive Protein	32.281549	1
Thyroid Stimulating Hormone	11.915463	–1
IL-6	11.284493	1
MCP-1	11.024095	1
β-2 Microglobulin	7.295072	1
Glutathione S-Transferase	5.821976	1
Serum Amyloid P	5.546475	1
IL-10	4.771469	1
TIMP-1	4.161010	1
MIP-1β	3.040239	1
Apolipoprotein CIII	2.858158	–1

Example 4: Identification of Biomarkers Using SELDI-TOF-MS

4.1. Sample Preparation and Experimental Design

[00126] SELDI-TOF-MS (SELDI) provides yet another method of determining the status of sepsis or SIRS in an individual, according to the methods of the invention. SELDI allows a non-biased means of identifying predictive features in biomarker profiles from biological samples. A sample is ionized by a laser beam, and the *m/z* of the ions is measured. The biomarker profile comprising various ions then may be analyzed by any of the algorithms described above.

[00127] A representative SELDI experiment using a WCX2 sample platform, or "chip," is described. Each type of chip adsorbs characteristic biomarkers; therefore, different biomarker profiles may be obtained from the same sample, depending on the particular type of chip that is used. Plasma (500 μL) was prepared from blood collected in a PPTTM Vacutainer™ tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) per conventional protocol. The plasma was divided into 100 μL aliquots and was stored at –80°C. The WCX-2 chip (Ciphergen Biosystems, Inc., Fremont, California) was prepared in a Ciphergen bioprocessor according to the manufacturer protocol, using a Biomek 2000 robot (Beckman Coulter). One WCX-2 chip has eight binding spots. The spots on the chip were successively washed twice with 50 μL of 50% acetonitrile for 5 minutes, then with 50 μL of 10 mM of HCl for 10 minutes, and finally with 50 μL of de-ionized water for 5 minutes. After washing, the chip was conditioned twice with 50 μL of WCX2 buffer for 5 minutes before the introduction of plasma samples. Wash buffers for WCX2 chips, and for other chip types, including H50, IMAC and SAX2/Q10 chips, are given in TABLE 24.

TABLE 24

Chip Type	SELDI Wash Buffer
IMAC3	Phosphate Buffered Saline, pH 7.4, 0.5 M NaCl and 0.1% Triton X-100.
WCX2	20 mM Ammonium acetate of pH 6.0 containing 0.1% Triton X-100.
SAX2/Q10	100mM Ammonium acetate, pH 4.5
H50	0.1 M NaCl, 10% ACN and 0.1% Trifluoroacetic acid

[00128] To each spot on the conditioned WCX-2 chip, 10 μL of the plasma sample and 90 μL of WCX-2 binding buffer (20 mM ammonium acetate and 0.1% Triton X-100, pH 6) were added. After incubation at room temperature for 30 minutes with shaking, the spots were washed twice with 100 μL of the WCX-2 binding buffer, followed by two washes with 100 μL of de-ionized water. The chip was then dried and spotted twice with 0.75 μL of a saturated solution of matrix materials, such as α-cyanohydroxycinnamic acid (99%) (CHCA) or sinapic acid (SPA), in a 50% acetonitrile, 0.5% TFA aqueous solution. The chips with bound plasma proteins were then read by SELDI-TOF-MS using the experimental conditions shown in TABLE 25.

TABLE 25

SELDI reading conditions

Experimental Settings	Matrix:SPA	Matrix:CHCA	
Detector Voltage	2850 V	2850 V	2850 V
Deflector Mass	1000 Da	1000 Da	1000 Da
Digitizer Rate	500 MHz	500 MHz	500 MHz
High Mass	75,000 Da	75,000 Da	75,000 Da
Focus Mass	6000 Da	30,000 Da	30,000 Da
Intensity (low/high)	200/205	160/165	145/150
Sensitivity (low/high)	6/6	6/6	6/6
Fired/kept spots	91/65	91/65	91/65

[00129] TABLES 26 – 49 show p-values for SELDI experiments conducted on plasma samples under the conditions indicated in TABLE 25. In each table, the type of chip is shown, which is WCX-2, H50, Q10 or IMAC. For each chip, experiments were performed with either a CHCA matrix, an SPA matrix at high energy (*see* TABLE 25), or an SPA matrix at low energy. Further, for each matrix, samples from time 0 hours, time –24 hours, and time –48 hours were analyzed. The p-values determined for the listed ions were determined using a nonparametric test, which in this case was a Wilcoxon Signed Rank Test. Only ions with a corresponding p-value of less than 0.05 are listed (blank boxes in the TABLES below indicate those ions in the sample having a p-value not less than 0.05). Finally, in each sample, p-values were assigned to the difference from baseline in a feature intensity in the sepsis populations versus the SIRS populations, which are labeled in the TABLES below as "p-values for features differenced from baseline" (as in Example 1.4.7., *supra*). The *m/z* values listed in the TABLES have an experimental error of about ± 2%.

TABLE 26

SELDI biomarker p-values: WCX-2 chip

Matrix (Energy)	CHCA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	2290.1	0.000438	2579.4	0.001681	2004.6	0.000166
2	3163.9	0.000438	3357.4	0.001681	2004	0.000448
3	6470.6	0.000438	3340.9	0.001826	2005.5	0.000448
4	1773.1	0.000917	1394.6	0.00295	1935.7	0.000916
5	2623.8	0.001253	2195.7	0.003188	1909.1	0.001011
6	4581.4	0.002823	2818.6	0.004009	1892.3	0.001629
7	6474.2	0.00303	17107	0.005392	2003.5	0.001787
8	1645	0.003997	2220.2	0.005392	1939.1	0.002348
9	3065.5	0.004278	18688	0.006229	2035.4	0.002348
10	2775.1	0.004576	2613.3	0.007179	2011.7	0.002567
11	6435.5	0.004893	5827.3	0.007179	2042.4	0.003061
12	3195.9	0.006362	5894.2	0.007701	1916.1	0.003338
13	3781.7	0.006362	5892.8	0.01013	2041.5	0.003637
14	6780.5	0.006362	2813.9	0.011578	1848.6	0.003959
15	1657.1	0.007706	3728.9	0.011578	2041.8	0.004307
16	2579.4	0.007706	1401	0.012367	1722.7	0.005084
17	1628.9	0.008735	1726.1	0.012367	1877.1	0.005084
18	5901.2	0.008735	6673.1	0.013202	1911.2	0.005084
19	6667.5	0.008735	2806	0.014086	6676.7	0.005084
20	2438.8	0.010504	5897.8	0.014086	1878.3	0.005517
21	2793.8	0.010504	37828	0.01502	1879.2	0.005517
22	2811.5	0.010504	6674.5	0.01502	1692	0.005982
23	1627.8	0.01116	2705.9	0.016007	2003.1	0.005982
24	3085.5	0.01116	2793.8	0.016007	2039.2	0.005982
25	3218.6	0.01116	5885.2	0.017049	2042.1	0.005982
26	5885.2	0.01116	6474.2	0.017049	6674.5	0.005982
27	5894.2	0.01185	3331.5	0.018149	2101.2	0.007016
28	2798.3	0.012578	3718.9	0.018149	1879.5	0.00759
29	5897.8	0.012578	5891.2	0.018149	2008.4	0.00759
30	3336.2	0.013343	5901.2	0.020532	1687.5	0.008204
31	3974.5	0.013343	5902.2	0.02182	1689.9	0.008204
32	7483.6	0.013343	5889.9	0.023176	1878.8	0.008861
33	1379.4	0.014149	2039.2	0.026105	4858.8	0.008861
34	3235.8	0.014149	4560.7	0.026105	1855.2	0.009563
35	3238.3	0.014149	5850.4	0.026105	2432	0.009563
36	3761.8	0.014997	3769.5	0.027683	1888.2	0.010314
37	5892.8	0.014997	11639	0.029341	1657.1	0.011115
38	3319.9	0.015888	3346.9	0.029341	1719.7	0.01197
39	1394.6	0.016824	4574.2	0.029341	1879.7	0.01197

40	3333.5	0.017807	6676.7	0.029341	1609.2	0.01288
41	1946.9	0.01884	4567.4	0.031082	2015.1	0.01288
42	2238.6	0.01884	2342.5	0.032909	3333.5	0.01288
43	3299.6	0.01884	2811.5	0.032909	2002.2	0.01385
44	5827.3	0.01884	2340.9	0.034824	2018.1	0.01385
45	3205.2	0.019923	2474.5	0.034824	6673.1	0.01385
46	2274.7	0.021059	2168.3	0.036832	1341.2	0.014882
47	2813.9	0.021059	2683	0.038936	1883.3	0.014882
48	3331.5	0.021059	3038.5	0.038936	3331.5	0.014882
49	3780.6	0.022249	3753.8	0.038936	1380.6	0.01598
50	1724.7	0.023497	2340.1	0.041138	1923.2	0.01598
51	2678.1	0.023497	3412.9	0.041138	3582	0.01598
52	5889.9	0.023497	6470.6	0.041138	1354.4	0.018385
53	2673.4	0.024804	6691.5	0.041138	1605.9	0.018385
54	6635.1	0.026171	1605.1	0.043443	1606.5	0.018385
55	1793.8	0.027603	3450.1	0.043443	1371.1	0.019699
56	2976.7	0.027603	1399.5	0.045854	1940.2	0.019699
57	2359.7	0.029099	1402	0.045854	3085.5	0.019699
58	5891.2	0.029099	7637.9	0.045854	6470.6	0.019699
59	1627	0.030664	4871.3	0.048373	1384.2	0.021093
60	2654.3	0.030664	5810	0.048373	1913.7	0.021093
61	5030.1	0.030664	5867.2	0.048373	2045.1	0.021093
62	5748.8	0.030664	6667.5	0.048373	2051.4	0.021093
63	5962.8	0.030664			1125.7	0.022569
64	3315.7	0.032299			1781.2	0.022569
65	5564.3	0.034006			6780.5	0.022569
66	2538.5	0.035789			1779.1	0.024132
67	6561.5	0.035789			2469.2	0.024132
68	3094.3	0.037649			2775.1	0.025786
69	1827.7	0.039588			1777.8	0.027535
70	5837.7	0.039588			1836.1	0.027535
71	5514.7	0.041611			1420.4	0.031332
72	1472.3	0.043718			2059.5	0.031332
73	2208.4	0.043718			6474.2	0.031332
74	2660.4	0.043718			1694.9	0.03339
75	2951.7	0.043718			1917.4	0.03339
76	1273.2	0.045912			2768.8	0.03339
77	1625.3	0.045912			3126	0.03339
78	1630.7	0.045912			4862.4	0.03339
79	5528.5	0.045912			2029.5	0.035559
80	1626.1	0.048197			1175.8	0.037845
81	2195.7	0.048197			1875.7	0.037845
82	2818.6	0.048197			1880.7	0.037845
83	3758.9	0.048197			1688.3	0.040251
84					2033.4	0.040251
85					5058	0.040251
86					5129.9	0.040251
87					1602.6	0.042783

88					4370.5	0.045445
89					10261	0.048242
90					1991.2	0.048242
91					2062.3	0.048242
92					3485.1	0.048242

TABLE 27

SELDI biomarker p-values: WCX-2 chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	5308.9	0.001309	2802	0.004655	7300.2	0.01197
2	5302.8	0.001416	6777.8	0.005011	7642.6	0.01385
3	5357.6	0.00193	3386.7	0.008254	7651.1	0.01385
4	5335.1	0.002082	5302.8	0.008843	12194	0.014882
5	5324.4	0.002805	37933	0.01013	7653.8	0.014882
6	5316.6	0.003244	7603	0.01013	11591	0.017146
7	5379.4	0.004017	2834.7	0.010833	7624.5	0.018385
8	37933	0.00462	6838.2	0.01502	7658.6	0.019699
9	5312.5	0.006071	7132.1	0.01502	7469.1	0.022569
10	5388.9	0.006071	11676	0.016007	11628	0.027535
11	5222.9	0.008998	74907	0.016007	12385	0.027535
12	5372.2	0.008998	1138	0.018149	7665.2	0.031332
13	5232.4	0.009591	1893.8	0.019309	11635	0.035559
14	11591	0.010217	1005.9	0.023176	3669.3	0.040251
15	11880	0.011577	6819.8	0.023176	4200.7	0.042783
16	11272	0.012314	7126.6	0.024604	4214	0.045445
17	12385	0.014775	7711.6	0.026105	7862.1	0.045445
18	5343	0.014775	2893.6	0.027683	7496.4	0.048242
19	10509	0.015685	5286.1	0.027683	7682.9	0.048242
20	5349.2	0.020991	6604.5	0.027683		
21	5878.5	0.020991	7140.1	0.027683		
22	5295	0.023506	9281	0.027683		
23	5894	0.023506	1009.6	0.029341		
24	11773	0.026274	3588	0.029341		
25	37131	0.026274	29435	0.031082		
26	5260.6	0.027758	30235	0.031082		
27	5902.3	0.027758	3360.7	0.031082		
28	5910.4	0.029312	5277.2	0.031082		
29	5906.8	0.034422	1069.6	0.032909		
30	5254.8	0.036282	50968	0.032909		
31	5277.2	0.036282	6591.3	0.032909		
32	10631	0.044585	7582.4	0.032909		

33	11628	0.04689	1014	0.034824		
34	5240	0.04689	7122.3	0.034824		
35	9487.6	0.04689	5056.1	0.036832		
36	12588	0.049292	7113.7	0.036832		
37	15094	0.049292	73096	0.036832		
38	5271.3	0.049292	3369.2	0.038936		
39	5885.5	0.049292	5324.4	0.038936		
40			6985.9	0.038936		
41			6998.9	0.038936		
42			7682.9	0.038936		
43			1003.5	0.041138		
44			11641	0.041138		
45			3639.3	0.041138		
46			3945.5	0.041138		
47			3952.5	0.041138		
48			7149.2	0.041138		
49			5240	0.043443		
50			6959.8	0.043443		
51			77136	0.043443		
52			11716	0.045854		
53			14244	0.045854		
54			4269.7	0.045854		
55			9194.8	0.048373		

TABLE 28

SELDI biomarker p-values: WCX-2 chip

Matrix (Energy)	**SPA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	3490.7	0.000339	1685.2	0.000848	1882.6	0.002804
2	5356.2	0.001655	6722.9	0.000926	2671.1	0.002804
3	3033.8	0.001788	4584.8	0.001201	2101	0.005084
4	37873	0.001788	12256	0.001423	62628	0.005517
5	5264	0.002606	1182.2	0.001981	2787.9	0.008204
6	7560.1	0.002805	1633.6	0.001981	9900.3	0.008861
7	19083	0.003017	1683.8	0.002148	3077.6	0.01598
8	3681.1	0.004309	1686.4	0.002328	2775.5	0.017146
9	2469.6	0.005302	6938.4	0.002328	5810.7	0.017146
10	2583.7	0.006071	4580	0.002521	2274.5	0.018385
11	2379.3	0.006936	4588.7	0.002521	2635.1	0.021093
12	9126.4	0.007408	6705.1	0.002521	2615.7	0.022569
13	11836	0.007909	9155	0.002521	1679.4	0.024132
14	3980.6	0.007909	1949.5	0.003717	2528.2	0.024132
15	2604.6	0.008998	2553.8	0.003717	1838.9	0.027535

16	2573.3	0.010879	9687.7	0.004009	3410.6	0.027535
17	3084.4	0.010879	1593.2	0.004655	7560.1	0.027535
18	11578	0.013092	1946.2	0.004655	1821.2	0.031332
19	3986	0.013092	9605.1	0.004655	1253.9	0.03339
20	5903.8	0.013092	2799.9	0.005797	1823	0.03339
21	5907.6	0.013092	6750.5	0.006229	3599.6	0.03339
22	5909.7	0.013092	1477.6	0.00669	6697.9	0.03339
23	7554.1	0.013092	2196.2	0.00669	1388.9	0.037845
24	2683.7	0.013912	2735.6	0.00669	1818.3	0.037845
25	5268.7	0.013912	2960.8	0.00669	5268.7	0.037845
26	1627	0.014775	6702.5	0.00669	5903.8	0.040251
27	6969.7	0.014775	1925.8	0.007701	6694.6	0.040251
28	2663.3	0.015685	2811.2	0.007701	11472	0.042783
29	3017.9	0.016642	2193.3	0.008254	11489	0.042783
30	5250.5	0.016642	3042	0.008254	11532	0.042783
31	5906.1	0.016642	2809.6	0.008843	11578	0.042783
32	9129	0.017649	2170.5	0.009468	37873	0.042783
33	2600.8	0.018709	2831.5	0.009468	6699.7	0.042783
34	3977.8	0.018709	3364.2	0.009468	6701	0.042783
35	5321.3	0.018709	4573.6	0.009468	1253.1	0.045445
36	7636.7	0.018709	2809.3	0.01013	7622.6	0.045445
37	9108.6	0.019822	2809.8	0.01013	10098	0.048242
38	2697.6	0.020991	1471.6	0.010833	1863	0.048242
39	7564.6	0.020991	2064.9	0.010833	2055.5	0.048242
40	2815.7	0.022218	2791.7	0.010833	3104.4	0.048242
41	1829.3	0.023506	2801.3	0.010833		
42	11797	0.024858	37873	0.010833		
43	5991.8	0.024858	6508.4	0.010833		
44	2281.6	0.026274	6701	0.010833		
45	2996.8	0.026274	2171.9	0.011578		
46	1898.4	0.029312	4595.5	0.011578		
47	3991.5	0.029312	4865.3	0.011578		
48	1987.2	0.030939	7170.7	0.011578		
49	7244.8	0.030939	1688.5	0.012367		
50	2320.5	0.032642	17749	0.012367		
51	25044	0.032642	2806.4	0.012367		
52	2505.3	0.032642	6699.7	0.012367		
53	4564.4	0.032642	6951.3	0.012367		
54	5900.8	0.032642	1701.2	0.013202		
55	6977.4	0.032642	2795.9	0.013202		
56	1666.5	0.034422	6509.3	0.013202		
57	10098	0.036282	1877.3	0.014086		
58	1995.7	0.038226	19083	0.014086		
59	2582.4	0.038226	2173.6	0.014086		
60	11766	0.040256	3017.9	0.014086		
61	3575.5	0.040256	4600.9	0.014086		
62	5911.6	0.040256	1567.6	0.01502		
63	2546.6	0.042375	2808.7	0.01502		

64	3047.9	0.044585	6697.9	0.01502		
65	8298.4	0.044585	1220.4	0.016007		
66	11472	0.04689	1460.3	0.016007		
67	11732	0.04689	1460.7	0.016007		
68	2151.8	0.04689	2184.9	0.016007		
69	2171.9	0.04689	3025.6	0.016007		
70	2681.6	0.04689	3355.4	0.016007		
71	3021.1	0.04689	3367.9	0.016007		
72	3410.6	0.04689	3871.9	0.016007		
73	3913	0.04689	4900.9	0.016007		
74	4911	0.04689	6506.1	0.016007		
75	9132.4	0.04689	1664	0.017049		
76	4670.1	0.049292	6926.2	0.017049		
77	7566.2	0.049292	3021.1	0.018149		
78			3490.7	0.018149		
79			4592.3	0.018149		
80			9834.1	0.018149		
81			2813.6	0.019309		
82			3362	0.019309		
83			9230.4	0.019309		
84			10661	0.020532		
85			1454.4	0.020532		
86			1595.8	0.020532		
87			2719	0.020532		
88			3030.9	0.020532		
89			5297.9	0.020532		
90			6771.4	0.020532		
91			7106.1	0.020532		
92			97077	0.020532		
93			1234.5	0.02182		
94			1684.7	0.02182		
95			1947.7	0.02182		
96			2803.1	0.02182		
97			6514.8	0.02182		
98			7669.7	0.02182		
99			2180	0.023176		
100			2817.9	0.023176		
101			2841	0.023176		
102			3442.4	0.023176		
103			6502.2	0.023176		
104			2287.5	0.024604		
105			3939.8	0.024604		
106			5215.7	0.024604		
107			1772.5	0.026105		
108			2397.5	0.026105		
109			2692.2	0.026105		
110			3009.7	0.026105		
111			3945.3	0.026105		

112			3973.5	0.026105		
113			9900.3	0.026105		
114			1478.3	0.027683		
115			1690.2	0.027683		
116			2443.3	0.027683		
117			4002.7	0.027683		
118			6192.3	0.027683		
119			6527.3	0.027683		
120			6694.6	0.027683		
121			9639.8	0.027683		
122			1416.4	0.029341		
123			1476.4	0.029341		
124			1699.9	0.029341		
125			3748.9	0.029341		
126			4734.4	0.029341		
127			6566	0.029341		
128			11615	0.031082		
129			1233.7	0.031082		
130			1448.7	0.031082		
131			1863.6	0.031082		
132			2486.9	0.031082		
133			2815.7	0.031082		
134			2826.4	0.031082		
135			11648	0.032909		
136			1181.3	0.032909		
137			1431.3	0.032909		
138			1457.3	0.032909		
139			1479.5	0.032909		
140			2978.7	0.032909		
141			74349	0.032909		
142			8280.7	0.032909		
143			9132.4	0.032909		
144			9994.9	0.032909		
145			2092.8	0.034824		
146			2225	0.034824		
147			1669.8	0.036832		
148			3104.4	0.036832		
149			3499.2	0.036832		
150			6933.9	0.036832		
151			10082	0.038936		
152			1661.8	0.038936		
153			6909.5	0.038936		
154			6929.9	0.038936		
155			11633	0.041138		
156			1938.3	0.041138		
157			2843.4	0.041138		
158			1455.8	0.043443		
159			2440.7	0.043443		

160			2683.7	0.043443		
161			3917.6	0.043443		
162			75273	0.043443		
163			7655	0.043443		
164			1189	0.045854		
165			1432.9	0.045854		
166			1844.6	0.045854		
167			3461.1	0.045854		
168			3465.6	0.045854		
169			3991.5	0.045854		
170			1496.5	0.048373		
171			17459	0.048373		
172			1861.2	0.048373		
173			6543.1	0.048373		
174			6917.4	0.048373		

TABLE 29

SELDI biomarker p-values for features differenced from baseline: WCX-2 chip

Matrix (Energy)	**CHCA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	1273.2	0.000218	2342.5	0.000306	3582.0	7.09E-05
2	1827.7	0.000917	2340.9	0.000648	1855.2	0.000281
3	1332.5	0.00325	1422.1	0.005797	5366.9	0.001064
4	1605.9	0.005962	1737.8	0.012367	1883.3	0.001659
5	1773.1	0.006362	3178.5	0.013202	1888.2	0.002055
6	1158.8	0.007706	3776.7	0.013202	2469.2	0.002533
7	4980.0	0.007706	1627.8	0.018149	1911.2	0.003436
8	4001.1	0.008207	1736.7	0.019309	2041.5	0.003436
9	1147.4	0.009294	4001.1	0.02182	2041.8	0.003436
10	1095.9	0.009883	1860.4	0.023176	2042.1	0.003436
11	6635.1	0.01116	1738.5	0.026105	1083.5	0.003795
12	1198.6	0.01185	1267.0	0.027683	1939.1	0.004187
13	4407.6	0.01185	1793.8	0.027683	2042.4	0.004187
14	4408.0	0.01185	14975.	0.032909	4937.3	0.004187
15	3582.0	0.012578	1523.5	0.032909	5399.9	0.004187
16	1606.5	0.013343	4796.8	0.032909	2011.7	0.004614
17	1173.8	0.014149	2340.1	0.034824	1994.2	0.005078
18	1731.7	0.014149	1628.9	0.038936	2051.4	0.005078
19	1213.0	0.014997	1875.7	0.041138	1371.1	0.006132
20	1605.1	0.014997	5347.5	0.043443	2045.1	0.006132
21	1162.1	0.015888	1627.0	0.045854	1081.3	0.008827
22	1276.6	0.016824	3927.7	0.045854	1625.3	0.008827
23	2109.1	0.016824			1155.3	0.009644

24	2754.9	0.016824			1793.8	0.009644
25	1756.5	0.017807			2029.5	0.009644
26	1461.0	0.01884			1118.9	0.010525
27	1525.2	0.01884			2048.7	0.010525
28	5366.9	0.01884			1940.2	0.011475
29	1146.6	0.019923			1731.7	0.012498
30	1205.3	0.019923			1909.1	0.012498
31	1523.5	0.019923			2015.1	0.012498
32	3238.3	0.019923			2062.3	0.012498
33	1345.4	0.021059			4001.1	0.012498
34	3753.8	0.022249			4862.4	0.012498
35	1315.0	0.023497			5347.5	0.012498
36	3641.1	0.023497			1779.1	0.014781
37	8853.7	0.023497			1781.2	0.014781
38	1172.2	0.024804			2008.4	0.016052
39	2538.5	0.024804			2039.2	0.016052
40	1347.7	0.026171			2116.7	0.016052
41	2202.7	0.026171			1082.7	0.017414
42	1836.1	0.027603			1488.4	0.017414
43	4406.3	0.027603			2885.9	0.017414
44	4466.0	0.027603			3485.1	0.018874
45	1241.4	0.029099			7012.9	0.018874
46	1548.4	0.029099			1991.2	0.020437
47	1724.7	0.029099			1315.0	0.025801
48	6780.5	0.029099			2070.5	0.025801
49	1098.4	0.030664			2880.8	0.025801
50	3703.5	0.030664			1879.5	0.027834
51	4465.4	0.032299			1084.8	0.030000
52	4467.7	0.032299			1879.2	0.030000
53	11700.	0.034006			2059.5	0.030000
54	1462.6	0.034006			1867.4	0.032305
55	3974.5	0.034006			2005.5	0.032305
56	1084.8	0.035789			1138.8	0.034756
57	1089.0	0.035789			1523.5	0.034756
58	1215.0	0.035789			1879.7	0.034756
59	1293.1	0.035789			2018.1	0.034756
60	1799.2	0.035789			1370.2	0.037360
61	3094.3	0.035789			1878.3	0.037360
62	1320.0	0.037649			1293.1	0.040123
63	1860.4	0.037649			1314.6	0.040123
64	1875.7	0.037649			2896.7	0.040123
65	1460.1	0.039588			1232.9	0.043054
66	1747.4	0.039588			1878.8	0.043054
67	2201.8	0.039588			1981.9	0.043054
68	2438.8	0.039588			1997.2	0.043054
69	1172.8	0.041611			4589.5	0.043054
70	1220.5	0.041611			1172.8	0.046158
71	2310.5	0.041611			1329.1	0.046158

72	2579.4	0.043718			1892.3	0.046158
73	4774.0	0.043718			1086.3	0.049444
74	5106.3	0.045912			1111.4	0.049444
75	1155.3	0.048197			14087.	0.049444
76	2055.8	0.048197			1626.1	0.049444
77	6053.8	0.048197			4372.3	0.049444
78	8582.1	0.048197				

TABLE 30

SELDI biomarker p-values for features differenced from baseline: WCX-2 chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	11484.	0.000874	11676.	0.001201	3067.9	0.017414
2	11463.	0.001116	5379.4	0.003717	3588.0	0.017414
3	10509.	0.00242	11716.	0.004655	5006.0	0.020437
4	6864.8	0.002606	8354.6	0.008843	11484.	0.025801
5	11413.	0.002805	8342.3	0.01013	5379.4	0.025801
6	9487.6	0.003244	8347.3	0.01013	11413.	0.027834
7	11880.	0.003743	8384.2	0.01013	3173.1	0.027834
8	3738.5	0.004309	3496.6	0.010833	11591.	0.03736
9	11343.	0.006491	8352.3	0.010833	1229.1	0.040123
10	11591.	0.009591	8360.4	0.010833	11463.	0.043054
11	11525.	0.012314	11525.	0.01502	11716.	0.043054
12	11676.	0.012314	17387.	0.016007	5670.5	0.046158
13	5277.2	0.012314	3639.3	0.016007	11525.	0.049444
14	10452.	0.013912	5858.1	0.016007		
15	11272.	0.014775	5849.2	0.017049		
16	12006.	0.014775	5842.6	0.019309		
17	11641.	0.016642	8421.8	0.019309		
18	11716.	0.016642	11413.	0.020532		
19	11635.	0.017649	1893.8	0.02182		
20	11773.	0.017649	5866.0	0.024604		
21	12588.	0.017649	74907.	0.024604		
22	14629.	0.017649	11484.	0.026105		
23	5873.3	0.019822	11641.	0.027683		
24	11628.	0.020991	8454.3	0.027683		
25	31462.	0.022218	6484.4	0.029341		
26	4122.3	0.023506	66578.	0.029341		
27	5906.8	0.024858	3588.0	0.031082		
28	5910.4	0.024858	73096.	0.031082		
29	28210.	0.026274	1138.0	0.032909		
30	3525.9	0.026274	11463.	0.034824		

31	4964.9	0.026274	1069.6	0.036832		
32	5866.0	0.026274	3610.4	0.036832		
33	5902.3	0.026274	1005.9	0.041138		
34	5858.1	0.027758	11591.	0.041138		
35	5894.0	0.027758	11635.	0.045854		
36	5885.5	0.029312	11880.	0.045854		
37	7059.4	0.029312	3279.6	0.045854		
38	1119.9	0.030939	4356.3	0.045854		
39	4144.2	0.030939	5002.5	0.045854		
40	5286.1	0.030939	11343.	0.048373		
41	5950.5	0.030939	3618.8	0.048373		
42	3777.4	0.032642	8471.9	0.048373		
43	9809.4	0.034422				
44	4138.9	0.036282				
45	7052.8	0.040256				
46	5878.5	0.042375				
47	3369.2	0.044585				
48	7077.7	0.044585				
49	4137.2	0.04689				
50	7318.4	0.04689				
51	5842.6	0.049292				
52	5957.5	0.049292				

TABLE 31

SELDI biomarker p-values for features differenced from baseline: WCX-2 chip

Matrix (Energy)	**SPA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	3681.1	0.001416	17459.	6.46E-05	1607.2	0.001659
2	37873.	0.001532	17749.	0.000371	11489.	0.002283
3	8312.8	0.001532	8315.0	0.000926	1613.6	0.004187
4	11472.	0.001788	8312.8	0.001011	1882.6	0.004614
5	54016.	0.00193	1877.3	0.001102	1665.2	0.006132
6	9126.4	0.00193	8504.1	0.001201	1833.4	0.007373
7	9129.0	0.003244	1182.2	0.001308	1846.3	0.008071
8	11489.	0.004017	17253.	0.001681	2960.8	0.009644
9	1665.2	0.004017	4580.0	0.001681	1565.9	0.010525
10	5855.0	0.004017	8327.3	0.001981	4921.6	0.010525
11	14392.	0.004309	4125.5	0.003444	11661.	0.011475
12	9132.4	0.004309	8545.4	0.003444	1549.1	0.011475
13	6007.8	0.00462	2173.6	0.003717	11648.	0.012498
14	8315.0	0.00462	11489.	0.004321	2073.0	0.013598
15	3511.0	0.004951	1593.2	0.004321	2528.2	0.013598

16	11836.	0.005302	3871.9	0.004321	2307.2	0.014781
17	1879.1	0.005302	8345.6	0.004655	11419.	0.016052
18	4573.6	0.006071	9155.0	0.005392	17459.	0.016052
19	5830.6	0.006936	3036.4	0.005797	3146.8	0.016052
20	1176.9	0.007408	1633.6	0.006229	1585.3	0.017414
21	1180.2	0.007909	3748.9	0.00669	11472.	0.020437
22	11398.	0.008438	1412.8	0.007179	11691.	0.020437
23	5975.9	0.009591	3042.0	0.007179	1582.6	0.020437
24	11691.	0.010879	4573.6	0.007701	1880.7	0.020437
25	5781.7	0.011577	8693.3	0.008843	3241.7	0.020437
26	11732.	0.012314	8398.7	0.009468	5198.9	0.020437
27	19083.	0.012314	8770.5	0.01013	1180.2	0.023895
28	2782.2	0.012314	1154.3	0.010833	1537.9	0.023895
29	1817.3	0.013092	3939.8	0.011578	2274.5	0.023895
30	5770.5	0.013092	1685.2	0.012367	2338.3	0.023895
31	9091.2	0.013092	8789.0	0.012367	2671.1	0.023895
32	9108.6	0.013092	1234.5	0.01502	36974.	0.023895
33	11964.	0.013912	2437.2	0.01502	1563.4	0.025801
34	11444.	0.014775	3442.4	0.01502	1612.1	0.025801
35	2379.3	0.014775	4353.1	0.01502	1852.4	0.025801
36	5864.2	0.014775	8759.4	0.01502	1417.8	0.027834
37	1412.8	0.015685	8781.0	0.01502	1616.6	0.027834
38	2953.5	0.015685	8874.0	0.01502	11532.	0.03
39	5845.6	0.015685	11472.	0.016007	1576.9	0.03
40	8298.4	0.015685	1480.9	0.016007	20146.	0.03
41	11661.	0.016642	1701.2	0.016007	3427.8	0.03
42	1385.0	0.016642	8421.7	0.016007	5837.4	0.032305
43	3530.1	0.016642	2443.3	0.017049	1413.7	0.034756
44	9080.9	0.016642	11633.	0.018149	2335.2	0.034756
45	11648.	0.018709	11691.	0.018149	2758.3	0.034756
46	11895.	0.018709	1460.3	0.018149	2935.4	0.034756
47	1655.0	0.018709	8381.0	0.018149	3744.4	0.034756
48	9087.5	0.018709	11648.	0.019309	1162.6	0.03736
49	1212.5	0.019822	1233.7	0.019309	1534.2	0.03736
50	5356.2	0.019822	2064.9	0.019309	1575.1	0.03736
51	1690.2	0.020991	8815.8	0.019309	1584.3	0.03736
52	3980.6	0.020991	1097.0	0.020532	1602.7	0.03736
53	4117.5	0.020991	11661.	0.02182	17749.	0.03736
54	5886.6	0.020991	9230.4	0.02182	1871.1	0.03736
55	17749.	0.022218	9605.1	0.02182	2090.9	0.03736
56	2369.0	0.022218	11615.	0.023176	4580.0	0.03736
57	4119.1	0.022218	8730.7	0.023176	5845.6	0.03736
58	3516.2	0.023506	1183.1	0.024604	5855.0	0.03736
59	3894.7	0.024858	1416.4	0.024604	1712.0	0.040123
60	9155.0	0.024858	1455.8	0.024604	2066.8	0.040123
61	11532.	0.026274	2440.7	0.024604	1562.6	0.043054
62	2437.2	0.026274	3973.5	0.024604	19909.	0.043054
63	3490.7	0.026274	4697.7	0.024604	9466.5	0.043054

64	3710.4	0.026274	5215.7	0.024604	11895.	0.046158
65	4120.8	0.026274	5464.9	0.024604	1605.5	0.046158
66	17459.	0.027758	5552.3	0.024604	3088.0	0.046158
67	2683.7	0.027758	8298.4	0.024604	3095.6	0.046158
68	5872.8	0.027758	9687.7	0.024604	4710.2	0.046158
69	11633.	0.029312	1477.6	0.026105	5215.7	0.046158
70	4155.9	0.029312	1478.3	0.026105	1510.2	0.049444
71	11797.	0.030939	3439.0	0.026105	1522.8	0.049444
72	33911.	0.030939	11398.	0.027683	5607.0	0.049444
73	5837.4	0.030939	1180.2	0.027683		
74	9064.6	0.030939	1257.5	0.027683		
75	5228.6	0.032642	2170.5	0.027683		
76	3893.0	0.034422	5837.4	0.027683		
77	11578.	0.036282	9004.4	0.027683		
78	1897.2	0.036282	1009.4	0.029341		
79	2151.8	0.036282	11895.	0.029341		
80	3744.4	0.036282	1414.9	0.029341		
81	4580.0	0.036282	1450.6	0.029341		
82	5093.6	0.036282	2171.9	0.029341		
83	6851.5	0.036282	6192.3	0.029341		
84	1160.8	0.038226	8791.2	0.029341		
85	33455.	0.038226	8840.8	0.029341		
86	2686.8	0.040256	1051.4	0.031082		
87	3977.8	0.040256	1206.8	0.031082		
88	5408.3	0.040256	1254.6	0.031082		
89	5998.1	0.040256	13423.	0.031082		
90	7332.1	0.042375	1460.7	0.031082		
91	11766.	0.044585	16690.	0.031082		
92	1666.5	0.044585	1686.4	0.031082		
93	1891.8	0.044585	5781.7	0.031082		
94	3059.3	0.044585	11532.	0.032909		
95	3701.0	0.044585	1434.6	0.032909		
96	11287.	0.049292	1457.3	0.032909		
97	11419.	0.049292	1690.2	0.032909		
98	3109.4	0.049292	2553.8	0.032909		
99			3522.5	0.032909		
100			3605.1	0.032909		
101			5855.0	0.032909		
102			8847.4	0.032909		
103			1181.3	0.034824		
104			1454.4	0.034824		
105			1479.5	0.034824		
106			16980.	0.034824		
107			3062.6	0.034824		
108			3924.2	0.034824		
109			3933.6	0.034824		
110			1253.9	0.036832		
111			1463.1	0.036832		

112			1482.1	0.036832		
113			1595.8	0.036832		
114			3945.3	0.036832		
115			5722.6	0.036832		
116			11444.	0.038936		
117			3331.3	0.038936		
118			3929.1	0.038936		
119			5607.0	0.038936		
120			2180.0	0.041138		
121			4615.2	0.041138		
122			4636.3	0.041138		
123			5845.6	0.041138		
124			1772.5	0.043443		
125			3688.4	0.043443		
126			5408.3	0.043443		
127			1050.8	0.045854		
128			1051.7	0.045854		
129			1081.5	0.045854		
130			11419.	0.045854		
131			1188.4	0.045854		
132			12839.	0.045854		
133			1925.8	0.045854		
134			3362.0	0.045854		
135			5770.5	0.045854		
136			5830.6	0.045854		
137			1938.3	0.048373		
138			2196.2	0.048373		
139			3095.6	0.048373		
140			4336.2	0.048373		
141			9132.4	0.048373		

TABLE 32

SELDI biomarker p-values: H50 chip

Matrix (Energy)	**CHCA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	6694.1	0.000104	3892.3	0.000371	3683.8	0.014882
2	8934.6	0.00037	3458.7	0.000492	4288.3	0.014882
3	9141.2	0.000519	1057	0.00054	4290.5	0.014882
4	8223.8	0.000782	1015.1	0.000648	4471.7	0.014882
5	1298.9	0.001253	5836.1	0.000709	1690.8	0.01598
6	9297.4	0.001353	1315.8	0.000776	12872	0.017146
7	28047	0.002277	28768	0.000776	4289	0.018385

8	4005.1	0.00325	9141.2	0.001102	6694.1	0.018385
9	6442.9	0.00325	5837.6	0.001201	6442.9	0.024132
10	6639.4	0.003483	1033.9	0.001308	3220	0.029382
11	1341.4	0.004278	6639.4	0.001308	6639.4	0.031332
12	1448.5	0.004278	1314.3	0.001423	1748.9	0.03339
13	4719.4	0.004278	5839.4	0.001547	1178.1	0.035559
14	1340.6	0.004893	4418.6	0.001681	9141.2	0.042783
15	28768	0.005229	1034.1	0.001826	8934.6	0.045445
16	1461.8	0.005585	18741	0.001826	4645.9	0.048242
17	9341.7	0.005585	28047	0.001826		
18	3867.5	0.006785	7300.1	0.001826		
19	1456.7	0.007706	2699.3	0.001981		
20	8799.9	0.007706	1000.2	0.002148		
21	4471.7	0.009883	1033.7	0.002148		
22	1706.1	0.010504	1313	0.002328		
23	4109.5	0.010504	14049	0.002328		
24	2959.1	0.012578	5840.9	0.002328		
25	4116.2	0.012578	9479.1	0.002328		
26	3220	0.013343	14500	0.002521		
27	3345.3	0.013343	9376.8	0.002521		
28	1692.9	0.014149	3942.2	0.002728		
29	6898.8	0.014997	5813.3	0.002728		
30	4290.5	0.016824	1032.3	0.003188		
31	12872	0.017807	4467	0.003188		
32	14049	0.01884	6442.9	0.003188		
33	1026.3	0.019923	9297.4	0.003188		
34	4442	0.019923	1014	0.003444		
35	4467	0.021059	3206.4	0.003444		
36	3913.4	0.022249	1016.3	0.003717		
37	4580.6	0.023497	1313.6	0.003717		
38	1339.2	0.024804	1245	0.004009		
39	1422.4	0.024804	1043.5	0.004321		
40	2794.8	0.024804	1001	0.005011		
41	2932.7	0.026171	1142.4	0.005011		
42	4289	0.026171	1318	0.005011		
43	1088.9	0.027603	3896.1	0.005011		
44	18741	0.027603	4471.7	0.005392		
45	2301	0.027603	6694.1	0.005392		
46	3919.9	0.027603	1009.1	0.005797		
47	4675.5	0.027603	1246.5	0.006229		
48	7846.5	0.027603	2712.8	0.006229		
49	9376.8	0.029099	8934.6	0.006229		
50	1342.1	0.030664	1002.6	0.00669		
51	1427.9	0.030664	1127.9	0.007179		
52	14500	0.030664	1249	0.007179		
53	1014	0.032299	1706.1	0.007179		
54	4288.3	0.032299	8799.9	0.007179		
55	4426.9	0.032299	1158.5	0.007701		

56	1341.8	0.034006	1304.5	0.007701		
57	2940.7	0.034006	3329.6	0.007701		
58	1297.4	0.035789	3889.9	0.007701		
59	1433.3	0.035789	1027.7	0.008254		
60	4458	0.035789	14300	0.008254		
61	7009.7	0.035789	9341.7	0.008254		
62	3322.1	0.037649	1129.5	0.008843		
63	7035.6	0.039588	1285.4	0.008843		
64	2992.1	0.041611	12872	0.008843		
65	3942.2	0.041611	1319.2	0.008843		
66	1690.8	0.045912	1328	0.008843		
67	4486.8	0.045912	3888.9	0.008843		
68			5830.2	0.008843		
69			5844.8	0.008843		
70			1312.1	0.009468		
71			3840.3	0.009468		
72			4116.2	0.009468		
73			1012	0.01013		
74			1029.6	0.01013		
75			1054.8	0.01013		
76			1007.9	0.011578		
77			1027.1	0.011578		
78			2907.4	0.011578		
79			6090.8	0.011578		
80			3232.1	0.012367		
81			1010.4	0.013202		
82			1113	0.013202		
83			1301.8	0.013202		
84			5798.6	0.013202		
85			1250.5	0.014086		
86			1286.1	0.014086		
87			1286.7	0.014086		
88			2910.2	0.014086		
89			4426.9	0.014086		
90			4479.1	0.014086		
91			9684.3	0.014086		
92			11626	0.01502		
93			3879.9	0.01502		
94			5759.1	0.01502		
95			1012.9	0.016007		
96			11594	0.016007		
97			4442	0.016007		
98			4694.2	0.016007		
99			1004.9	0.017049		
100			1006.9	0.017049		
101			1011.1	0.017049		
102			1055.1	0.017049		
103			1287.1	0.017049		

104			1298.9	0.017049		
105			2211.2	0.017049		
106			2916.5	0.017049		
107			2922.9	0.017049		
108			3886.3	0.017049		
109			7846.5	0.017049		
110			1028	0.018149		
111			1233.7	0.018149		
112			2729.8	0.018149		
113			3844.1	0.018149		
114			1263.6	0.019309		
115			2902.8	0.019309		
116			3905.9	0.019309		
117			3919.9	0.019309		
118			7035.6	0.019309		
119			1020.5	0.020532		
120			11685	0.020532		
121			1270.2	0.020532		
122			1287.8	0.020532		
123			4580.6	0.020532		
124			4303.4	0.02182		
125			4458	0.02182		
126			12184	0.023176		
127			1287.4	0.023176		
128			4290.5	0.023176		
129			4645.9	0.023176		
130			4675.5	0.023176		
131			1113.6	0.024604		
132			1114.7	0.024604		
133			1289.7	0.024604		
134			3838.6	0.024604		
135			4719.4	0.024604		
136			8223.8	0.024604		
137			1159.4	0.026105		
138			11642	0.026105		
139			3810.5	0.026105		
140			1128.6	0.027683		
141			1275	0.027683		
142			1275.6	0.027683		
143			1361	0.027683		
144			15122	0.027683		
145			3867.5	0.027683		
146			5756.1	0.027683		
147			2119.1	0.029341		
148			3225.5	0.029341		
149			1018.3	0.031082		
150			1160.1	0.031082		
151			2036.2	0.031082		

152			3345.3	0.031082		
153			5753.7	0.031082		
154			1296.6	0.032909		
155			3149.5	0.032909		
156			4464.1	0.032909		
157			7141.1	0.032909		
158			1128.2	0.034824		
159			1296.4	0.034824		
160			1344	0.034824		
161			3770.9	0.034824		
162			3913.4	0.034824		
163			4486.8	0.034824		
164			4682.5	0.034824		
165			5851.1	0.034824		
166			5871.1	0.034824		
167			2003.2	0.036832		
168			2932.7	0.036832		
169			3335.3	0.036832		
170			1131.9	0.038936		
171			3242.6	0.038936		
172			1062.4	0.041138		
173			1319.6	0.041138		
174			2883.5	0.041138		
175			2940.7	0.041138		
176			1112.3	0.043443		
177			1945.9	0.043443		
178			5959.8	0.043443		
179			1019.6	0.045854		
180			2018.3	0.045854		
181			1296.91	0.048373		
182			3899.5	0.048373		
183			4288.3	0.048373		
184			4385.7	0.048373		
185			5764.6	0.048373		

TABLE 33

SELDI biomarker p-values: H50 chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	43045	0.00325	3355.6	1.42E-06	9482	0.00759
2	42800	0.005962	4655.1	0.000277	6896.3	0.008861
3	9482	0.007233	4508.5	0.000306	12870	0.01197
4	6896.3	0.014997	4724.4	0.000592	3048.4	0.031332
5	42693	0.016824	4505.8	0.000648	43634	0.031332
6	10802	0.017807	4759.6	0.000648	10802	0.040251
7	2949.6	0.019923	4680.3	0.000709	3233.2	0.042783
8	34925	0.021059	4516	0.000776	6493.9	0.048242
9	6493.9	0.021059	4873	0.001102		
10	8284	0.021059	4836.6	0.001308		
11	3552.8	0.022249	9034.2	0.001308		
12	10465	0.026171	6127.7	0.001547		
13	73120	0.027603	11773	0.001826		
14	10297	0.035789	9259.8	0.001826		
15	12870	0.035789	4851.1	0.001981		
16	3813.5	0.035789	6096.4	0.001981		
17	14505	0.037649	3813.5	0.002328		
18	6559.8	0.041611	4146	0.002328		
19	7119.7	0.041611	6109.4	0.002328		
20	9158.7	0.043718	6087	0.002521		
21	5942.1	0.048197	6942.8	0.002521		
22			11954	0.002728		
23			7143.1	0.002728		
24			6778	0.003444		
25			7938.5	0.003444		
26			4547	0.003717		
27			9669.7	0.003717		
28			4692.2	0.004321		
29			4825.6	0.004321		
30			6807.4	0.004321		
31			4157.7	0.004655		
32			4532.8	0.004655		
33			13764	0.005392		
34			4522.7	0.005392		
35			5868.8	0.005392		
36			6493.9	0.005392		
37			6514.7	0.005392		
38			9386.5	0.005392		
39			99801	0.005392		

40			3469.4	0.005797		
41			6498.6	0.005797		
42			6499.9	0.006229		
43			6501.7	0.006229		
44			6505.1	0.006229		
45			4611.5	0.00669		
46			6202.5	0.00669		
47			6533.4	0.00669		
48			7083.7	0.00669		
49			7254.9	0.00669		
50			12176	0.007179		
51			4141.6	0.007179		
52			4701.7	0.007179		
53			6150.3	0.007701		
54			6218.5	0.007701		
55			6896.3	0.007701		
56			8296	0.007701		
57			9158.7	0.007701		
58			4633.2	0.008843		
59			8284	0.008843		
60			5889.9	0.01013		
61			6184.5	0.01013		
62			8320.8	0.01013		
63			37619	0.010833		
64			8293	0.010833		
65			5251.9	0.011578		
66			5970.5	0.011578		
67			6685.4	0.011578		
68			63590	0.012367		
69			6559.8	0.012367		
70			7000.7	0.012367		
71			5893.5	0.013202		
72			4481.1	0.01502		
73			6082.1	0.01502		
74			6246.4	0.01502		
75			4892	0.016007		
76			5905.7	0.016007		
77			5906.5	0.016007		
78			6077.2	0.016007		
79			6275.7	0.016007		
80			8297.6	0.016007		
81			12499	0.017049		
82			5907.1	0.017049		
83			7119.7	0.017049		
84			3969.4	0.018149		
85			9482	0.018149		
86			3509.1	0.019309		
87			4792.7	0.019309		

88			5226	0.019309		
89			5903.8	0.019309		
90			5942.1	0.019309		
91			6166.2	0.019309		
92			5898.8	0.020532		
93			5910	0.020532		
94			24366	0.02182		
95			3934.7	0.02182		
96			4142.9	0.02182		
97			4808.4	0.023176		
98			22915	0.026105		
99			3383.3	0.026105		
100			3951.8	0.027683		
101			11652	0.029341		
102			3626.4	0.029341		
103			3826.7	0.029341		
104			5923	0.029341		
105			6001.4	0.029341		
106			12280	0.031082		
107			75442	0.031082		
108			9759.4	0.031082		
109			1230.7	0.032909		
110			5204.1	0.032909		
111			5279	0.032909		
112			6157.8	0.032909		
113			1238.1	0.034824		
114			11131	0.036832		
115			1263.4	0.036832		
116			6068.9	0.036832		
117			23732	0.038936		
118			4420.6	0.038936		
119			4454.7	0.038936		
120			4917.8	0.038936		
121			11399	0.041138		
122			4433.8	0.041138		
123			6033.3	0.041138		
124			8931.7	0.041138		
125			69817	0.043443		
126			11526	0.045854		
127			1290.2	0.045854		
128			40894	0.045854		
129			8377.5	0.045854		

TABLE 34

SELDI biomarker p-values: H50 chip

Matrix (Energy)	SPA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	9170.7	0.000151	1256.6	4.38E-06	2088.9	0.003637
2	9474.9	0.000285	1276.4	1.09E-05	9170.7	0.003637
3	3024.3	0.00037	1227.8	1.24E-05	9474.9	0.005982
4	3030	0.000564	1255.5	1.41E-05	1965.4	0.009563
5	1734.9	0.00116	1225.5	3.67E-05	6563.9	0.009563
6	9636.5	0.001253	1281.4	4.61E-05	12901	0.017146
7	9420.3	0.001574	1275.4	5.17E-05	1956.6	0.017146
8	1716.9	0.001968	3336.5	5.17E-05	7282.6	0.021093
9	9584.5	0.00303	1278	5.78E-05	2838.1	0.024132
10	3041.9	0.003483	2615.5	7.21E-05	1100.7	0.025786
11	35268	0.003997	1229.1	8.04E-05	1132	0.027535
12	3019.4	0.004576	1283.2	8.04E-05	3024.3	0.027535
13	6462.8	0.004576	1259.3	8.96E-05	1154.9	0.029382
14	6563.9	0.004576	1271.3	0.000137	1227.8	0.029382
15	2781.2	0.004893	1281	0.000137	1680.3	0.029382
16	2019.2	0.005229	1281.9	0.000137	2942.9	0.029382
17	4433.9	0.005962	1274.1	0.000152	6462.8	0.029382
18	12901	0.006785	12386	0.000186	1671.3	0.031332
19	2010.8	0.006785	5943.2	0.000186	19918	0.03339
20	2997	0.007706	1272.6	0.000206	1101.1	0.035559
21	5423.5	0.007706	1262.5	0.000228	1688.6	0.035559
22	4115.8	0.009294	1270.3	0.000228	2668.7	0.035559
23	3007.3	0.01185	1299	0.000228	1100.3	0.037845
24	3550.5	0.01185	3335.8	0.000277	6660.6	0.037845
25	3568.8	0.01185	6251.8	0.000277	2862	0.040251
26	3013.4	0.013343	6889	0.000277	1229.1	0.045445
27	3332.4	0.014997	1284.5	0.000306	9300.5	0.045445
28	9334	0.014997	3342	0.000306	2680.7	0.048242
29	3540.2	0.015888	1279.6	0.000337	3567.8	0.048242
30	10130	0.016824	1286.2	0.000337		
31	19918	0.016824	1258.6	0.000371		
32	3813.9	0.016824	1260.6	0.000408		
33	9075.3	0.016824	1236	0.000448		
34	9300.5	0.016824	1254.3	0.000448		
35	7282.6	0.017807	3335	0.000448		
36	1985.3	0.019923	6187.5	0.000448		
37	28070	0.019923	1251.2	0.000492		
38	3037.2	0.021059	1269.2	0.00054		
39	42896	0.021059	4832.1	0.00054		

40	6660.6	0.021059	1253.1	0.000592		
41	8353.7	0.021059	1261.7	0.000592		
42	1729.8	0.022249	1265.3	0.000592		
43	4744.2	0.022249	1280.4	0.000592		
44	4886.7	0.022249	1219.8	0.000648		
45	2657	0.023497	1267.2	0.000648		
46	7109.4	0.023497	3332.4	0.000648		
47	3944.1	0.024804	1263.6	0.000709		
48	1281.4	0.026171	6087.5	0.000709		
49	14780	0.026171	12175	0.000776		
50	9371.9	0.026171	1243.4	0.000776		
51	3880.5	0.027603	1258	0.000776		
52	4536.2	0.027603	11626	0.000848		
53	3688.2	0.029099	1285.4	0.000848		
54	1281.9	0.030664	12088	0.000926		
55	2024.7	0.032299	1301.2	0.000926		
56	28759	0.032299	2442.4	0.000926		
57	28825	0.032299	1290.8	0.001011		
58	3050.7	0.032299	1296.9	0.001011		
59	4446.4	0.032299	4593.6	0.001011		
60	1281	0.034006	1294.7	0.001102		
61	2287.8	0.034006	1295.1	0.001102		
62	2502.7	0.034006	4141.7	0.001102		
63	3962.3	0.034006	11932	0.001201		
64	14194	0.035789	1287.5	0.001201		
65	1731.3	0.035789	6168	0.001201		
66	2757.5	0.035789	6386.4	0.001201		
67	28777	0.035789	12031	0.001308		
68	1117.7	0.039588	1294.3	0.001308		
69	2862	0.039588	1298.5	0.001308		
70	1326.5	0.041611	1245.3	0.001547		
71	14111	0.041611	1289.2	0.001547		
72	2260.5	0.041611	1252.6	0.001681		
73	4320.3	0.041611	4115.8	0.001681		
74	1733.2	0.043718	6209.2	0.001681		
75	2278.6	0.043718	8982.8	0.001681		
76	28307	0.043718	4697.2	0.001826		
77	4164.9	0.043718	1241.2	0.001981		
78	14510	0.045912	1264.4	0.001981		
79	1710	0.048197	3557.3	0.001981		
80			12271	0.002148		
81			1778.8	0.002148		
82			4811	0.002148		
83			5960.9	0.002148		
84			2423.7	0.002328		
85			1209.6	0.002728		
86			1234	0.002728		
87			1293.7	0.002728		

88			1300	0.002728		
89			1323.1	0.002728		
90			3041.9	0.002728		
91			1239.7	0.00295		
92			1241.9	0.00295		
93			4591.4	0.00295		
94			4846.2	0.00295		
95			9474.9	0.00295		
96			9300.5	0.003188		
97			12508	0.003444		
98			1325.3	0.003444		
99			6096	0.003444		
100			1295.7	0.003717		
101			1302.6	0.003717		
102			5825.1	0.004009		
103			6109.3	0.004321		
104			1292.6	0.004655		
105			1298	0.004655		
106			1249.3	0.005011		
107			1309.4	0.005011		
108			1774.7	0.005392		
109			2408.4	0.005392		
110			5072.1	0.005392		
111			1237.5	0.005797		
112			1689.8	0.005797		
113			2413.8	0.005797		
114			4744.2	0.005797		
115			11779	0.006229		
116			4499.6	0.006229		
117			1800.6	0.00669		
118			8865.2	0.00669		
119			10273	0.007179		
120			7109.4	0.007179		
121			9075.3	0.007179		
122			9170.7	0.007179		
123			9334	0.007179		
124			1324.3	0.008254		
125			5843.1	0.008254		
126			1330.1	0.008843		
127			9636.5	0.008843		
128			1311.6	0.009468		
129			9706.4	0.009468		
130			1331	0.01013		
131			1782.7	0.01013		
132			23767	0.01013		
133			2421.1	0.01013		
134			4860.2	0.01013		
135			1312.8	0.010833		

136			2816.8	0.010833		
137			2889.3	0.010833		
138			1109	0.011578		
139			1306.8	0.011578		
140			14111	0.011578		
141			4613.5	0.011578		
142			4876	0.011578		
143			11351	0.012367		
144			2082.2	0.012367		
145			4540.2	0.012367		
146			4796.5	0.012367		
147			9420.3	0.012367		
148			1230.7	0.013202		
149			1307.9	0.013202		
150			1105.7	0.014086		
151			1226.6	0.014086		
152			1303.6	0.014086		
153			1309.8	0.014086		
154			1326.5	0.014086		
155			2403.2	0.014086		
156			1304.8	0.01502		
157			2434.1	0.01502		
158			4994.4	0.01502		
159			1104	0.016007		
160			1310	0.016007		
161			3019.4	0.016007		
162			37418	0.016007		
163			5241.4	0.016007		
164			6660.6	0.016007		
165			9371.9	0.016007		
166			11519	0.017049		
167			1310.5	0.017049		
168			46718	0.017049		
169			4886.7	0.017049		
170			5855.8	0.017049		
171			1315.6	0.018149		
172			1332.2	0.018149		
173			3215.9	0.018149		
174			9930.7	0.018149		
175			11687	0.019309		
176			1223.8	0.019309		
177			1314.3	0.019309		
178			2849.9	0.019309		
179			3348.6	0.019309		
180			1321.8	0.020532		
181			4767.8	0.020532		
182			4968.8	0.020532		
183			6139.2	0.020532		

184			8497	0.020532		
185			2580.5	0.02182		
186			33454	0.02182		
187			3438.9	0.02182		
188			3449.4	0.02182		
189			6462.8	0.02182		
190			9764	0.02182		
191			1117	0.023176		
192			1218.7	0.023176		
193			1222.6	0.023176		
194			1240.9	0.023176		
195			5867.8	0.023176		
196			5906.9	0.023176		
197			1154.9	0.024604		
198			1320.4	0.024604		
199			2024.7	0.024604		
200			1234.8	0.026105		
201			1713.9	0.026105		
202			1780.9	0.026105		
203			1837.8	0.026105		
204			4713.3	0.026105		
205			4873.9	0.026105		
206			5698.7	0.026105		
207			9584.5	0.026105		
208			1058.2	0.027683		
209			1120.4	0.027683		
210			1321	0.027683		
211			2685.4	0.027683		
212			1107.5	0.029341		
213			1121.4	0.029341		
214			1221	0.029341		
215			1224.5	0.029341		
216			1621.1	0.029341		
217			2686.7	0.029341		
218			4555.1	0.029341		
219			6047.3	0.029341		
220			1231.9	0.031082		
221			23126	0.031082		
222			23145	0.031082		
223			3962.3	0.031082		
224			1059.5	0.032909		
225			1308.7	0.032909		
226			1317.2	0.032909		
227			1328.1	0.032909		
228			4628.7	0.032909		
229			1067.1	0.034824		
230			1428.2	0.034824		
231			1060.8	0.036832		

232			11132	0.036832		
233			11550	0.036832		
234			1215	0.036832		
235			1216.3	0.036832		
236			23106	0.036832		
237			2404	0.036832		
238			5075.4	0.036832		
239			5171.3	0.036832		
240			1071	0.038936		
241			1798.8	0.038936		
242			4433.9	0.038936		
243			45039	0.038936		
244			1057.1	0.041138		
245			1086.5	0.041138		
246			1211.6	0.041138		
247			1217.7	0.041138		
248			1238.5	0.041138		
249			28307	0.041138		
250			3217.8	0.041138		
251			3313.1	0.041138		
252			4446.4	0.041138		
253			1110.4	0.043443		
254			1427.6	0.043443		
255			2104.6	0.043443		
256			2679	0.043443		
257			1011.8	0.045854		
258			1085.8	0.045854		
259			11537	0.045854		
260			23420	0.045854		
261			28070	0.045854		
262			2826.3	0.045854		
263			4603.1	0.045854		
264			1100.3	0.048373		
265			1115.1	0.048373		
266			23251	0.048373		
267			40679	0.048373		
268			4371.1	0.048373		
269			4526.6	0.048373		
270			8743.7	0.048373		
271			8937.9	0.048373		

TABLE 35

SELDI biomarker p-values for features differenced from baseline: H50 chip

Matrix (Energy)	CHCA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	3888.9	3.46E-05	1706.1	2.58E-05	12872	2.81E-03
2	3883.4	3.84E-05	3892.3	4.12E-05	3798.2	4.61E-03
3	3889.9	4.71E-05	3942.2	6.46E-05	2910.2	6.13E-03
4	18741	7.03E-05	18741	8.04E-05	3801.5	6.73E-03
5	3886.3	1.25E-04	5836.1	8.96E-05	6898.8	6.73E-03
6	2875.9	1.38E-04	5813.3	9.97E-05	1706.1	8.83E-03
7	28047	1.51E-04	3889.9	1.37E-04	3810.5	8.83E-03
8	2925.5	3.39E-04	5837.6	1.52E-04	1070.8	9.64E-03
9	5709.8	3.39E-04	3888.9	2.06E-04	5696.5	9.64E-03
10	3899.5	4.03E-04	5839.4	2.28E-04	5709.8	1.15E-02
11	14049	5.64E-04	5830.2	3.37E-04	1286.1	1.61E-02
12	1289.7	7.21E-04	5844.8	4.48E-04	2288.7	1.61E-02
13	3867.5	7.21E-04	3840.3	4.92E-04	5557.5	1.61E-02
14	11125	8.47E-04	3458.7	5.40E-04	18741	1.89E-02
15	5666.2	8.47E-04	5840.9	5.92E-04	3805	2.21E-02
16	3849.3	9.17E-04	3883.4	6.48E-04	3847.4	2.39E-02
17	3892.3	9.17E-04	5759.1	6.48E-04	3879.9	2.58E-02
18	4675.5	9.17E-04	11594	7.76E-04	3883.4	2.58E-02
19	2922.9	9.92E-04	11626	7.76E-04	4289	2.58E-02
20	3840.3	9.92E-04	12872	9.26E-04	2269.6	2.78E-02
21	5557.5	9.92E-04	5798.6	1.10E-03	2922.9	2.78E-02
22	5830.2	9.92E-04	11685	1.20E-03	1070.2	3.00E-02
23	1706.1	1.07E-03	11642	1.31E-03	3835.3	3.00E-02
24	3850.1	1.07E-03	14049	1.31E-03	3867.5	3.00E-02
25	3919.9	1.07E-03	5756.1	1.42E-03	3888.9	3.00E-02
26	8223.8	1.07E-03	5851.1	1.68E-03	4288.3	3.00E-02
27	28768	1.16E-03	15122	1.83E-03	4385.7	3.00E-02
28	3805	1.25E-03	3879.9	1.83E-03	3848.4	3.23E-02
29	3810.5	1.25E-03	5753.7	1.83E-03	3899.5	3.23E-02
30	3913.4	1.25E-03	1315.8	1.98E-03	5871.1	3.23E-02
31	6898.8	1.35E-03	3838.6	1.98E-03	8223.8	3.23E-02
32	3848.4	1.46E-03	3886.3	2.15E-03	5813.3	3.48E-02
33	3816.4	1.57E-03	2907.4	2.33E-03	1223.9	3.74E-02
34	3942.2	1.57E-03	3905.9	2.33E-03	15122	3.74E-02
35	3798.2	1.70E-03	2910.2	2.52E-03	2729.8	3.74E-02
36	3830	1.70E-03	28047	2.73E-03	2929.8	3.74E-02
37	3905.9	1.70E-03	3810.5	2.95E-03	3901.4	3.74E-02
38	3879.9	1.83E-03	3835.3	2.95E-03	3849.3	4.31E-02
39	3903.5	1.97E-03	3896.1	2.95E-03	3861.3	4.31E-02

40	3853	2.12E-03	3919.9	2.95E-03	4109.5	4.31E-02
41	25836	2.28E-03	5764.6	3.19E-03	5156.6	4.31E-02
42	3901.4	2.28E-03	5854.7	3.19E-03	5798.6	4.62E-02
43	4486.8	2.28E-03	11453	3.44E-03	14500	4.94E-02
44	3847.4	2.45E-03	14500	3.44E-03	2902.8	4.94E-02
45	3902.6	2.45E-03	11484	3.72E-03	2907.4	4.94E-02
46	3832.1	2.63E-03	1246.5	4.01E-03	3840.3	4.94E-02
47	5836.1	2.63E-03	2916.5	4.01E-03	3850.1	4.94E-02
48	5749.7	2.82E-03	3867.5	4.01E-03	3919.9	4.94E-02
49	6694.1	2.82E-03	9376.8	4.32E-03	4303.4	4.94E-02
50	3820.1	3.03E-03	5749.7	4.66E-03		
51	5753.7	3.03E-03	9479.1	4.66E-03		
52	4479.1	3.25E-03	2932.7	5.01E-03		
53	5756.1	3.48E-03	1289.7	5.39E-03		
54	5837.6	3.48E-03	3225.5	5.39E-03		
55	5744.9	3.73E-03	3232.1	5.39E-03		
56	3838.6	4.00E-03	3899.5	5.39E-03		
57	5724	4.00E-03	14300	5.80E-03		
58	3225.5	4.28E-03	3844.1	5.80E-03		
59	3823.1	4.28E-03	18184	6.23E-03		
60	3835.3	4.28E-03	2875.9	6.23E-03		
61	4005.1	4.28E-03	2883.5	6.69E-03		
62	12872	4.58E-03	3801.5	7.18E-03		
63	14300	4.58E-03	5724	7.18E-03		
64	3826.2	4.58E-03	11508	7.70E-03		
65	5773.1	4.58E-03	5744.9	7.70E-03		
66	5851.1	4.58E-03	8934.6	7.70E-03		
67	3801.5	4.89E-03	3798.2	8.25E-03		
68	11484	5.23E-03	3901.4	8.25E-03		
69	11642	5.23E-03	5770.7	8.25E-03		
70	5813.3	5.23E-03	11402	8.84E-03		
71	2927.5	5.58E-03	5857.1	8.84E-03		
72	5733.6	5.58E-03	7846.5	9.47E-03		
73	8934.6	5.58E-03	12184	1.01E-02		
74	5730.9	5.96E-03	5696.5	1.01E-02		
75	5774.3	5.96E-03	7141.1	1.01E-02		
76	5798.6	5.96E-03	1142.4	1.08E-02		
77	9376.8	5.96E-03	28768	1.08E-02		
78	11453	6.36E-03	3902.6	1.08E-02		
79	5770.7	6.36E-03	3903.5	1.16E-02		
80	11626	6.78E-03	8223.8	1.16E-02		
81	2959.1	6.78E-03	2929.8	1.24E-02		
82	4719.4	6.78E-03	3329.6	1.24E-02		
83	5728	6.78E-03	3805	1.24E-02		
84	5844.8	6.78E-03	5709.8	1.24E-02		
85	11685	7.23E-03	7035.6	1.32E-02		
86	9479.1	7.23E-03	9684.3	1.32E-02		
87	2864.2	7.71E-03	2109.6	1.41E-02		

88	2932.7	7.71E-03	4479.1	1.41E-02		
89	5585.1	7.71E-03	5156.6	1.41E-02		
90	5759.1	7.71E-03	3847.4	1.50E-02		
91	1112.3	8.21E-03	5734.4	1.50E-02		
92	15122	8.21E-03	5773.1	1.50E-02		
93	3844.1	8.21E-03	5871.1	1.50E-02		
94	5696.5	8.21E-03	1304.5	1.60E-02		
95	5734.4	8.21E-03	3913.4	1.60E-02		
96	5839.4	8.21E-03	5791.4	1.70E-02		
97	5840.9	8.21E-03	6442.9	1.70E-02		
98	11594	8.74E-03	7300.1	1.70E-02		
99	2902.8	8.74E-03	9297.4	1.70E-02		
100	5959.8	8.74E-03	2922.9	1.81E-02		
101	3857.6	9.88E-03	3820.1	1.81E-02		
102	5854.7	9.88E-03	5666.2	1.81E-02		
103	4426.9	1.05E-02	1318	1.93E-02		
104	5871.1	1.05E-02	3816.4	1.93E-02		
105	1298.9	1.12E-02	3830	1.93E-02		
106	3821.5	1.12E-02	3848.4	1.93E-02		
107	9141.2	1.12E-02	3909.9	1.93E-02		
108	2679.5	1.19E-02	5730.9	1.93E-02		
109	11402	1.26E-02	1245	2.05E-02		
110	1328	1.26E-02	2196	2.18E-02		
111	2929.8	1.26E-02	3826.2	2.18E-02		
112	5739.1	1.26E-02	4426.9	2.18E-02		
113	1315.8	1.33E-02	5728	2.18E-02		
114	14500	1.33E-02	5733.6	2.18E-02		
115	3724.5	1.33E-02	11125	2.32E-02		
116	5778.6	1.33E-02	3849.3	2.32E-02		
117	3093.8	1.41E-02	4694.2	2.32E-02		
118	3683.8	1.41E-02	5739.1	2.32E-02		
119	3896.1	1.41E-02	5778.6	2.32E-02		
120	6442.9	1.41E-02	2925.5	2.46E-02		
121	18184	1.50E-02	5774.3	2.46E-02		
122	2301	1.50E-02	1015.1	2.61E-02		
123	2828.8	1.59E-02	1328	2.61E-02		
124	5764.6	1.59E-02	2927.5	2.61E-02		
125	1246.5	1.78E-02	3832.1	2.61E-02		
126	1775.7	1.78E-02	5786.5	2.61E-02		
127	11508	1.88E-02	5959.8	2.61E-02		
128	5156.6	1.88E-02	3823.1	2.77E-02		
129	3861.3	1.99E-02	17385	2.93E-02		
130	1319.2	2.11E-02	19852	2.93E-02		
131	1448.5	2.11E-02	2940.7	3.11E-02		
132	2021.1	2.35E-02	6898.8	3.11E-02		
133	8799.9	2.48E-02	1016.3	3.29E-02		
134	3909.9	2.76E-02	17262	3.29E-02		
135	4458	2.91E-02	2902.8	3.29E-02		

136	4467	2.91E-02	3322.1	3.29E-02		
137	1342.1	3.07E-02	4303.4	3.29E-02		
138	7035.6	3.07E-02	3093.8	3.48E-02		
139	9341.7	3.07E-02	6090.8	3.48E-02		
140	1343.1	3.23E-02	9141.2	3.48E-02		
141	9297.4	3.23E-02	1104.4	3.68E-02		
142	12184	3.40E-02	1263.6	3.68E-02		
143	1278.3	3.40E-02	1301.8	3.68E-02		
144	2883.5	3.40E-02	3821.5	3.68E-02		
145	2916.5	3.40E-02	4471.7	3.68E-02		
146	2794.8	3.58E-02	2864.2	3.89E-02		
147	1954.9	3.76E-02	1314.3	4.34E-02		
148	3458.7	3.76E-02	1319.2	4.34E-02		
149	1286.1	3.96E-02	3683.8	4.34E-02		
150	1812.9	3.96E-02	3850.1	4.34E-02		
151	2940.7	3.96E-02	1250.5	4.59E-02		
152	4303.4	3.96E-02	1313	4.59E-02		
153	4471.7	4.16E-02	3853	4.59E-02		
154	6639.4	4.16E-02	1007.9	4.84E-02		
155	1292.2	4.37E-02	8644.4	4.84E-02		
156	5857.1	4.37E-02				
157	1314.3	4.59E-02				
158	1318	4.59E-02				
159	2851.1	4.59E-02				
160	4109.5	4.59E-02				
161	5786.5	4.59E-02				
162	7009.7	4.59E-02				
163	1312.1	4.82E-02				
164	17385	4.82E-02				
165	4580.6	4.82E-02				
166	5791.4	4.82E-02				

TABLE 36

SELDI biomarker p-values for features differenced from baseline: H50 chip

Matrix (Energy)	**SPA matrix (high energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	6493.9	5.64E-04	3355.6	1.23E-04	12870	1.49E-03
2	14505	1.07E-03	6001.4	3.37E-04	6275.7	3.44E-03
3	3436.7	2.12E-03	5898.8	4.08E-04	5596.1	4.19E-03
4	12870	3.73E-03	5970.5	4.08E-04	6246.4	4.19E-03
5	6896.3	4.89E-03	5889.9	5.40E-04	19997	4.61E-03

6	14607	5.23E-03	5893.5	5.40E-04	6184.5	5.58E-03
7	6501.7	5.58E-03	5903.8	7.09E-04	5251.9	6.13E-03
8	14813	5.96E-03	11773	8.48E-04	14065	6.73E-03
9	7318.2	5.96E-03	5905.7	1.10E-03	7119.7	6.73E-03
10	14182	6.36E-03	6033.3	1.20E-03	13173	7.37E-03
11	6499.9	6.36E-03	8296	1.31E-03	14813	7.37E-03
12	6685.4	6.78E-03	6275.7	1.68E-03	39262	7.37E-03
13	11232	7.23E-03	1230.7	1.83E-03	5038.1	8.07E-03
14	37619	7.23E-03	5906.5	1.83E-03	11399	9.64E-03
15	11131	7.71E-03	8293	1.83E-03	14505	1.05E-02
16	28633	8.21E-03	11954	1.98E-03	5106.2	1.05E-02
17	28709	8.21E-03	15211	2.15E-03	11446	1.15E-02
18	6505.1	8.21E-03	5907.1	2.33E-03	20654	1.15E-02
19	8293	8.74E-03	5910	2.52E-03	39776	1.15E-02
20	14411	9.29E-03	6246.4	2.52E-03	1279.1	1.25E-02
21	2949.6	9.29E-03	6778	2.52E-03	1293.7	1.25E-02
22	6498.6	9.29E-03	8297.6	2.73E-03	14607	1.25E-02
23	5942.1	9.88E-03	11526	3.19E-03	5051.9	1.36E-02
24	37067	1.05E-02	6068.9	3.19E-03	7254.9	1.36E-02
25	5834.9	1.05E-02	5942.1	3.44E-03	11131	1.48E-02
26	6068.9	1.05E-02	8284	3.44E-03	5889.9	1.48E-02
27	6514.7	1.05E-02	9259.8	4.66E-03	6001.4	1.48E-02
28	5698.7	1.12E-02	8320.8	5.01E-03	6068.9	1.48E-02
29	9386.5	1.12E-02	11446	5.39E-03	5146.6	1.61E-02
30	1279.1	1.33E-02	11652	5.39E-03	6077.2	1.61E-02
31	5825.3	1.41E-02	11491	6.23E-03	1290.2	1.74E-02
32	6942.8	1.50E-02	13764	6.23E-03	8284	1.74E-02
33	5822.4	1.68E-02	6533.4	6.23E-03	5731.4	1.89E-02
34	5824.3	1.68E-02	40894	6.69E-03	8296	1.89E-02
35	8297.6	1.68E-02	9034.2	6.69E-03	5180.5	2.04E-02
36	5740.9	1.78E-02	14607	7.70E-03	6082.1	2.04E-02
37	5845.4	1.78E-02	5923	8.84E-03	6202.5	2.04E-02
38	6246.4	1.78E-02	1243	1.01E-02	8293	2.04E-02
39	8296	1.88E-02	1263.4	1.01E-02	5740.9	2.39E-02
40	28912	1.99E-02	14411	1.01E-02	7410.9	2.39E-02
41	5743.2	2.11E-02	9482	1.01E-02	14182	2.58E-02
42	6001.4	2.11E-02	23732	1.08E-02	40894	2.58E-02
43	6033.3	2.11E-02	6157.8	1.08E-02	5750.6	2.58E-02
44	29758	2.22E-02	11399	1.16E-02	5743.2	2.78E-02
45	8284	2.22E-02	6166.2	1.16E-02	6157.8	2.78E-02
46	28784	2.35E-02	6514.7	1.16E-02	7318.2	2.78E-02
47	29456	2.35E-02	7143.1	1.16E-02	11232	3.00E-02
48	4106.8	2.35E-02	11131	1.24E-02	8297.6	3.00E-02
49	5736.4	2.35E-02	33462	1.24E-02	12994	3.23E-02
50	5820.4	2.35E-02	3469.4	1.24E-02	24366	3.23E-02
51	6275.7	2.35E-02	6505.1	1.24E-02	5583	3.23E-02
52	1293.7	2.48E-02	1238.1	1.32E-02	6218.5	3.23E-02
53	4873	2.48E-02	14505	1.32E-02	6896.3	3.23E-02

54	5906.5	2.48E-02	24366	1.32E-02	5268	3.48E-02
55	5923	2.48E-02	6493.9	1.32E-02	5161.5	3.74E-02
56	43045	2.62E-02	6501.7	1.32E-02	6338.3	3.74E-02
57	5893.5	2.62E-02	1270.7	1.41E-02	77760	3.74E-02
58	5905.7	2.62E-02	23553	1.41E-02	5970.5	4.01E-02
59	11399	2.76E-02	7254.9	1.41E-02	7358.7	4.01E-02
60	1243	2.76E-02	1287.6	1.50E-02	7453.6	4.01E-02
61	5898.8	2.76E-02	1222.2	1.60E-02	5604	4.31E-02
62	5910	2.76E-02	12499	1.60E-02	5758.1	4.31E-02
63	28460	2.91E-02	1290.2	1.60E-02	5893.5	4.31E-02
64	4680.3	2.91E-02	6150.3	1.60E-02	6499.9	4.31E-02
65	5750.6	2.91E-02	11232	1.70E-02	6505.1	4.31E-02
66	5818.7	3.07E-02	11575	1.70E-02	88472	4.31E-02
67	5907.1	3.07E-02	4516	1.70E-02	23071	4.62E-02
68	5970.5	3.07E-02	1252.7	1.81E-02	2817.9	4.62E-02
69	6394.6	3.07E-02	22915	1.81E-02	5226	4.62E-02
70	7049.2	3.07E-02	6499.9	1.81E-02	6166.2	4.62E-02
71	9158.7	3.07E-02	6942.8	1.81E-02	6493.9	4.62E-02
72	23553	3.23E-02	37619	1.93E-02	6501.7	4.62E-02
73	28063	3.23E-02	3951.8	1.93E-02	6685.4	4.62E-02
74	5903.8	3.23E-02	3509.1	2.05E-02	4299.1	4.94E-02
75	10297	3.40E-02	23071	2.18E-02	5868.8	4.94E-02
76	4825.6	3.40E-02	6498.6	2.18E-02	6096.4	4.94E-02
77	29295	3.58E-02	4508.5	2.32E-02	6109.4	4.94E-02
78	5687.3	3.58E-02	5226	2.32E-02		
79	6077.2	3.58E-02	1293.7	2.46E-02		
80	28264	3.76E-02	1304.5	2.46E-02		
81	4508.5	3.76E-02	6077.2	2.46E-02		
82	11954	3.96E-02	6202.5	2.46E-02		
83	4633.2	3.96E-02	23110	2.61E-02		
84	5765.9	3.96E-02	5868.8	2.61E-02		
85	3552.8	4.16E-02	9669.7	2.61E-02		
86	4112.5	4.16E-02	3934.7	2.77E-02		
87	4001.5	4.37E-02	1211.1	2.93E-02		
88	5849.4	4.37E-02	3826.7	2.93E-02		
89	6807.4	4.37E-02	4655.1	3.11E-02		
90	9259.8	4.37E-02	5797	3.11E-02		
91	9482	4.37E-02	23153	3.29E-02		
92	11773	4.59E-02	6184.5	3.29E-02		
93	4547	4.59E-02	1279.1	3.48E-02		
94	5657	4.59E-02	23235	3.48E-02		
95	5778.8	4.59E-02	3383.3	3.48E-02		
96	5816.4	4.59E-02	5845.4	3.48E-02		
97	6533.4	4.59E-02	7119.7	3.48E-02		
98	4104.6	4.82E-02	3813.5	3.68E-02		
99	4836.6	4.82E-02	5849.4	3.68E-02		
100	5673.2	4.82E-02	28709	3.89E-02		
101	5731.4	4.82E-02	6807.4	3.89E-02		

102	5889.9	4.82E-02	12176	4.11E-02		
103	6184.5	4.82E-02	23182	4.11E-02		
104			14182	4.34E-02		
105			3969.4	4.34E-02		
106			6087	4.34E-02		
107			5818.7	4.59E-02		
108			9759.4	4.59E-02		
109			5811.3	4.84E-02		
110			95452	4.84E-02		

TABLE 37

SELDI biomarker p-values for features differenced from baseline: H50 chip

Matrix (Energy)	**SPA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	9420.3	5.22E-05	11932	5.71E-07	6563.9	5.93E-04
2	6462.8	1.51E-04	12175	2.58E-05	12901	8.46E-04
3	6660.6	1.51E-04	12386	3.27E-05	3580	1.66E-03
4	9170.7	7.82E-04	12508	7.21E-05	1965.4	1.85E-03
5	6563.9	8.47E-04	12031	9.97E-05	2943.8	2.53E-03
6	9764	8.47E-04	6889	1.68E-04	6462.8	2.81E-03
7	6889	9.17E-04	37418	2.77E-04	6889	2.81E-03
8	7366.2	9.17E-04	12088	3.06E-04	19918	3.44E-03
9	5423.5	9.92E-04	6251.8	3.06E-04	8982.8	3.80E-03
10	9636.5	9.92E-04	12271	3.37E-04	4499.6	4.19E-03
11	7109.4	1.07E-03	1283.2	7.76E-04	9474.9	4.19E-03
12	28070	1.16E-03	3336.5	7.76E-04	11932	4.61E-03
13	3705.5	1.16E-03	8982.8	9.26E-04	37418	5.08E-03
14	5317.3	1.83E-03	11779	1.31E-03	7109.4	5.08E-03
15	9474.9	1.97E-03	3335	1.31E-03	2186.4	6.13E-03
16	14314	2.28E-03	4499.6	1.31E-03	4968.8	6.13E-03
17	14194	2.45E-03	5171.3	1.31E-03	1000.5	6.73E-03
18	14780	2.63E-03	3335.8	1.42E-03	3488	6.73E-03
19	1710	2.63E-03	1227.8	1.68E-03	9170.7	6.73E-03
20	28307	2.82E-03	7109.4	1.68E-03	5872.9	8.83E-03
21	4886.7	3.03E-03	4628.7	1.83E-03	9764	8.83E-03
22	5658.7	3.48E-03	1284.5	1.98E-03	1868.3	9.64E-03
23	3580	3.73E-03	3342	1.98E-03	2236	9.64E-03
24	7206.6	3.73E-03	11351	2.33E-03	2558.1	9.64E-03
25	28555	4.28E-03	9474.9	2.52E-03	2944.7	9.64E-03
26	28777	4.28E-03	1270.3	2.73E-03	6660.6	9.64E-03
27	6209.2	4.28E-03	1239.7	2.95E-03	1234	1.05E-02
28	9584.5	4.28E-03	1276.4	2.95E-03	3449.4	1.05E-02

29	9706.4	4.28E-03	4846.2	2.95E-03	5960.9	1.05E-02
30	10130	4.58E-03	4994.4	2.95E-03	6852.6	1.15E-02
31	4446.4	4.58E-03	6187.5	2.95E-03	3387.8	1.36E-02
32	28759	4.89E-03	1265.3	3.19E-03	12386	1.48E-02
33	28825	4.89E-03	5990.8	3.19E-03	3465.1	1.61E-02
34	9371.9	5.23E-03	9764	3.19E-03	1001.8	1.74E-02
35	9930.7	5.23E-03	3449.4	3.44E-03	2862	1.74E-02
36	37418	5.58E-03	11626	3.72E-03	6945.7	1.74E-02
37	5890	5.58E-03	1272.6	3.72E-03	9636.5	1.74E-02
38	1943.8	5.96E-03	1241.2	4.01E-03	11351	1.89E-02
39	2840.2	5.96E-03	1225.5	4.32E-03	20513	1.89E-02
40	4580.7	5.96E-03	5872.9	4.32E-03	2212.3	1.89E-02
41	4968.8	5.96E-03	1269.2	4.66E-03	5867.8	1.89E-02
42	12508	6.36E-03	1289.2	4.66E-03	12271	2.04E-02
43	14045	6.36E-03	1258	5.01E-03	2561.9	2.04E-02
44	12088	6.78E-03	1274.1	5.01E-03	11687	2.21E-02
45	6852.6	6.78E-03	2615.5	5.01E-03	1229.1	2.21E-02
46	19918	7.23E-03	3420.4	5.01E-03	2088.9	2.21E-02
47	3688.2	7.71E-03	9170.7	5.01E-03	2228.3	2.21E-02
48	4320.3	7.71E-03	1275.4	5.39E-03	2668.7	2.21E-02
49	57792	7.71E-03	1285.4	5.80E-03	2942.9	2.21E-02
50	12031	8.74E-03	1286.2	5.80E-03	6251.8	2.21E-02
51	1823	8.74E-03	1290.8	5.80E-03	11053	2.39E-02
52	4499.6	8.74E-03	1301.2	5.80E-03	12088	2.39E-02
53	4873.9	8.74E-03	9930.7	5.80E-03	7442.3	2.39E-02
54	9300.5	8.74E-03	1271.3	6.23E-03	9075.3	2.39E-02
55	8937.9	9.29E-03	3915.8	6.23E-03	11090	2.58E-02
56	12386	9.88E-03	3921.8	6.23E-03	2736.5	2.58E-02
57	28955	1.05E-02	5906.9	6.23E-03	4628.7	2.58E-02
58	8982.8	1.05E-02	8865.2	6.23E-03	11421	2.78E-02
59	12901	1.12E-02	1332.2	6.69E-03	11445	2.78E-02
60	5104.1	1.12E-02	4593.6	6.69E-03	11476	2.78E-02
61	8865.2	1.12E-02	5943.2	6.69E-03	12175	2.78E-02
62	12271	1.19E-02	1287.5	7.18E-03	2605.3	2.78E-02
63	14111	1.19E-02	3919.4	7.18E-03	1003.1	3.00E-02
64	1794.4	1.19E-02	4613.5	7.18E-03	1005.6	3.00E-02
65	29575	1.19E-02	4744.2	7.18E-03	2220.2	3.00E-02
66	9334	1.19E-02	6096	7.18E-03	6209.2	3.00E-02
67	2067.7	1.33E-02	1229.1	7.70E-03	6835.6	3.00E-02
68	1542.1	1.41E-02	1299	7.70E-03	4198	3.23E-02
69	20513	1.41E-02	6209.2	7.70E-03	5658.7	3.23E-02
70	29140	1.41E-02	1261.7	8.25E-03	2174.5	3.48E-02
71	3922.6	1.50E-02	1262.5	8.25E-03	3567.8	3.48E-02
72	4628.7	1.50E-02	1317.2	8.25E-03	3571.3	3.48E-02
73	5872.9	1.50E-02	1333.8	8.25E-03	39141	3.48E-02
74	11932	1.59E-02	3332.4	8.25E-03	1159.5	3.74E-02
75	2186.4	1.59E-02	33454	8.25E-03	12031	3.74E-02
76	1821.3	1.68E-02	9075.3	8.25E-03	1331	3.74E-02

77	42896	1.68E-02	11421	8.84E-03	4744.2	3.74E-02
78	5990.8	1.78E-02	4968.8	8.84E-03	9334	3.74E-02
79	12175	1.88E-02	1241.9	9.47E-03	1217.7	4.01E-02
80	1159.5	1.99E-02	1281.9	9.47E-03	12508	4.01E-02
81	5825.1	1.99E-02	1302.6	9.47E-03	14045	4.01E-02
82	11132	2.11E-02	1245.3	1.01E-02	2227.1	4.01E-02
83	1985.3	2.11E-02	1292.6	1.01E-02	2772.9	4.01E-02
84	4603.1	2.11E-02	1330.1	1.01E-02	5825.1	4.01E-02
85	1530.2	2.22E-02	1259.3	1.08E-02	6187.5	4.01E-02
86	1543.2	2.22E-02	1281	1.08E-02	11132	4.31E-02
87	1796.1	2.22E-02	1314.3	1.08E-02	14780	4.31E-02
88	2287.8	2.22E-02	2082.2	1.08E-02	1671.3	4.31E-02
89	2944.7	2.22E-02	28555	1.08E-02	1945.6	4.31E-02
90	4721.4	2.22E-02	1243.4	1.16E-02	2130.5	4.31E-02
91	3024.3	2.35E-02	1256.6	1.16E-02	2132.5	4.31E-02
92	2634.8	2.48E-02	4141.7	1.16E-02	4185.9	4.31E-02
93	1877	2.62E-02	5731.5	1.16E-02	1000	4.62E-02
94	1176.7	2.76E-02	5825.1	1.16E-02	1152.8	4.62E-02
95	1528.2	2.76E-02	1236	1.24E-02	11626	4.62E-02
96	3799.4	2.76E-02	1281.4	1.24E-02	1233	4.62E-02
97	4198	2.76E-02	1737.1	1.24E-02	1330.1	4.62E-02
98	5906.9	2.76E-02	6168	1.24E-02	1372.8	4.62E-02
99	14510	2.91E-02	8233.8	1.24E-02	15908	4.62E-02
100	4430.3	2.91E-02	1295.1	1.32E-02	1890.3	4.62E-02
101	4433.9	2.91E-02	8497	1.32E-02	2680.7	4.62E-02
102	9075.3	2.91E-02	1258.6	1.41E-02	2945.5	4.62E-02
103	10714	3.07E-02	23075	1.41E-02	5943.2	4.62E-02
104	5761	3.07E-02	1159.5	1.50E-02	7562.2	4.62E-02
105	2491.6	3.23E-02	1315.6	1.50E-02	9420.3	4.62E-02
106	7282.6	3.23E-02	1331	1.50E-02	11570	4.94E-02
107	8497	3.23E-02	23767	1.50E-02	1190.6	4.94E-02
108	11490	3.40E-02	2833.4	1.50E-02	2193.3	4.94E-02
109	11594	3.40E-02	11519	1.60E-02	3099.5	4.94E-02
110	1688.6	3.40E-02	1267.2	1.60E-02	6096	4.94E-02
111	2544.6	3.40E-02	1298.5	1.60E-02	8937.9	4.94E-02
112	3930.3	3.40E-02	14111	1.60E-02		
113	3944.1	3.40E-02	23420	1.60E-02		
114	4335.1	3.40E-02	5658.7	1.60E-02		
115	11742	3.58E-02	6087.5	1.60E-02		
116	13942	3.58E-02	1219.8	1.70E-02		
117	1755.8	3.58E-02	1234	1.70E-02		
118	1965.4	3.58E-02	1294.7	1.70E-02		
119	2833.4	3.58E-02	1296.9	1.70E-02		
120	4185.9	3.58E-02	1733.2	1.70E-02		
121	4924.6	3.58E-02	28070	1.70E-02		
122	1281.9	3.76E-02	11132	1.81E-02		
123	2630.7	3.76E-02	1237.5	1.81E-02		
124	2788.9	3.76E-02	1321.8	1.81E-02		

125	3813.9	3.76E-02	3922.6	1.81E-02		
126	3919.4	3.76E-02	5890	1.81E-02		
127	1540.5	3.96E-02	1226.6	1.93E-02		
128	1545.7	3.96E-02	1260.6	1.93E-02		
129	1668.9	3.96E-02	3313.1	1.93E-02		
130	3420.4	3.96E-02	11445	2.05E-02		
131	4164.9	3.96E-02	11742	2.05E-02		
132	5776.5	3.96E-02	1323.1	2.05E-02		
133	11493	4.16E-02	1713.9	2.05E-02		
134	11626	4.16E-02	1823	2.05E-02		
135	4994.4	4.16E-02	23106	2.05E-02		
136	5804.3	4.16E-02	4115.8	2.05E-02		
137	6251.8	4.16E-02	1778.8	2.18E-02		
138	3921.8	4.37E-02	23126	2.18E-02		
139	4189.7	4.37E-02	1278	2.32E-02		
140	11445	4.59E-02	1319.1	2.32E-02		
141	11476	4.59E-02	14314	2.32E-02		
142	11494	4.59E-02	1806.3	2.32E-02		
143	11779	4.59E-02	3488	2.32E-02		
144	6139.2	4.59E-02	11476	2.46E-02		
145	6835.6	4.59E-02	1293.7	2.61E-02		
146	8402.9	4.59E-02	1294.3	2.61E-02		
147	1531.8	4.82E-02	1734.9	2.61E-02		
148	1753.2	4.82E-02	23251	2.61E-02		
149	2053.4	4.82E-02	4876	2.61E-02		
150	2621.4	4.82E-02	1251.2	2.77E-02		
151	2952.6	4.82E-02	1311.6	2.77E-02		
152	4846.2	4.82E-02	15167	2.77E-02		
153			1689.8	2.77E-02		
154			2104.6	2.77E-02		
155			23145	2.77E-02		
156			5960.9	2.77E-02		
157			11490	2.93E-02		
158			11493	2.93E-02		
159			11504	2.93E-02		
160			1320.4	2.93E-02		
161			1808.7	2.93E-02		
162			3580	2.93E-02		
163			40679	2.93E-02		
164			6109.3	2.93E-02		
165			6386.4	2.93E-02		
166			8743.7	2.93E-02		
167			11494	3.11E-02		
168			1231.9	3.11E-02		
169			1264.4	3.11E-02		
170			1295.7	3.11E-02		
171			1800.6	3.11E-02		
172			4886.7	3.11E-02		

173			11495	3.29E-02		
174			11570	3.29E-02		
175			1255.5	3.29E-02		
176			1304.8	3.29E-02		
177			1335.3	3.29E-02		
178			1337.3	3.29E-02		
179			1762.8	3.29E-02		
180			1782.7	3.29E-02		
181			28307	3.29E-02		
182			11560	3.48E-02		
183			1300	3.48E-02		
184			1309.4	3.48E-02		
185			1309.8	3.48E-02		
186			1310	3.48E-02		
187			5867.8	3.48E-02		
188			6139.2	3.48E-02		
189			11200	3.68E-02		
190			11537	3.68E-02		
191			11568	3.68E-02		
192			1240.9	3.68E-02		
193			4126.9	3.68E-02		
194			6047.3	3.68E-02		
195			11550	3.89E-02		
196			1254.3	3.89E-02		
197			1303.6	3.89E-02		
198			2442.4	3.89E-02		
199			3373.2	3.89E-02		
200			5761	3.89E-02		
201			1298	4.11E-02		
202			1312.8	4.11E-02		
203			1798.8	4.11E-02		
204			2952.6	4.11E-02		
205			3557.3	4.11E-02		
206			45039	4.11E-02		
207			4873.9	4.11E-02		
208			14194	4.34E-02		
209			1760.5	4.34E-02		
210			2963.1	4.59E-02		
211			1252.6	4.84E-02		
212			1310.5	4.84E-02		
213			1321	4.84E-02		
214			1715.6	4.84E-02		
215			1761.1	4.84E-02		
216			2544.6	4.84E-02		
217			2816.8	4.84E-02		
218			3853.1	4.84E-02		
219			4446.4	4.84E-02		
220			5745.1	4.84E-02		

221			9300.5	4.84E-02		

TABLE 38

SELDI biomarker p-values: Q10 chip

Matrix (Energy)	CHCA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	9132	0.001073	1466	0.001011	1209	0.00083
2	7724.8	0.001828	3898.6	0.001011	1310	0.011115
3	11488	0.002118	4675.2	0.001102	1348.4	0.01598
4	6964.3	0.00263	1167.3	0.001547	4962.1	0.018385
5	4962.1	0.004576	8918.2	0.001547	2152.4	0.021093
6	4572	0.004893	1335.4	0.001681	1080.1	0.024132
7	5828.2	0.005962	4512.1	0.001826	1233.1	0.025786
8	13875	0.006785	4632.1	0.001826	2360.3	0.03339
9	10414	0.007706	1002.3	0.001981	1738.1	0.037845
10	5819	0.008207	6964.3	0.002148	1871.7	0.037845
11	8918.2	0.008207	1023.6	0.002328	1104.1	0.040251
12	2087.7	0.009883	1197.9	0.002328	2027.6	0.040251
13	2002.5	0.010504	4361.5	0.002521	1026	0.045445
14	9524.9	0.010504	8674.1	0.003444	1694.3	0.045445
15	1026.9	0.012578	4962.1	0.004321	11488	0.048242
16	1086.9	0.013343	1151.8	0.005011	1197.9	0.048242
17	11687	0.019923	1162.9	0.005392		
18	2178.4	0.019923	1169.9	0.005392		
19	5858.4	0.019923	5199	0.005797		
20	1231.4	0.024804	1008.8	0.006229		
21	1286.6	0.024804	1046.5	0.006229		
22	1336.6	0.024804	2421.1	0.006229		
23	2546.3	0.024804	1261.1	0.00669		
24	5697.8	0.024804	1619.1	0.007179		
25	1018.1	0.026171	4489.9	0.007179		
26	1010	0.027603	5819	0.007701		
27	1330	0.029099	1020.6	0.008254		
28	1027.1	0.030664	1003.6	0.008843		
29	3243.2	0.030664	1336.6	0.008843		
30	1314.2	0.032299	1159.7	0.009468		
31	1027.3	0.034006	9524.9	0.009468		
32	1113.2	0.034006	1137.2	0.01013		
33	1843	0.035789	5828.2	0.010833		
34	1056.1	0.037649	1145.9	0.012367		
35	1115.3	0.039588	1179.2	0.012367		
36	1036.2	0.041611	1343.5	0.012367		

37	1271.3	0.041611	1014.5	0.014086		
38	1652.3	0.041611	1029.5	0.014086		
39	1784.6	0.043718	1324.7	0.014086		
40	8202.5	0.043718	4203.8	0.014086		
41	1791.8	0.045912	4424.1	0.014086		
42	1297.7	0.048197	1101.3	0.01502		
43	4720.4	0.048197	1337.3	0.01502		
44			1001.1	0.018149		
45			1834.9	0.018149		
46			1465.5	0.019309		
47			6894.9	0.019309		
48			2014.2	0.020532		
49			1059	0.02182		
50			1302.2	0.02182		
51			1447.4	0.023176		
52			1016.1	0.024604		
53			1026.9	0.024604		
54			1038.1	0.024604		
55			1157	0.024604		
56			1262.8	0.024604		
57			1466.8	0.024604		
58			1018.8	0.026105		
59			2918.8	0.026105		
60			1005.3	0.027683		
61			1031.8	0.027683		
62			2300.1	0.027683		
63			1042.6	0.029341		
64			1126.4	0.029341		
65			1142.5	0.029341		
66			1164.9	0.031082		
67			1049	0.032909		
68			1318.1	0.034824		
69			2016.4	0.034824		
70			1010	0.036832		
71			2315.8	0.036832		
72			9132	0.036832		
73			1036.2	0.038936		
74			1092.5	0.038936		
75			1134.3	0.038936		
76			1159	0.038936		
77			1261.7	0.038936		
78			2456.3	0.038936		
79			2107.7	0.041138		
80			1017.1	0.043443		
81			2247.9	0.043443		
82			1007.2	0.045854		
83			1803.2	0.045854		
84			4455.8	0.045854		

85			4474.1	0.045854		
86			1010.8	0.048373		

TABLE 39

SELDI biomarker p-values: Q10 chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	9487.7	2.52E-05	5309.4	0.00054	41779	0.001227
2	9242.4	3.84E-05	3340	0.002521	3357.6	0.006481
3	8981.3	7.03E-05	12354	0.004655	3803.3	0.01598
4	3424.7	9.42E-05	4997.2	0.006229	3289.9	0.018385
5	9527.9	0.000114	22360	0.007179	5518.9	0.019699
6	9386	0.000138	5650.4	0.008254	6768.8	0.035559
7	14058	0.000311	5299.5	0.008843	1454.1	0.045445
8	9078.4	0.000519	5325.1	0.009468	4775.5	0.048242
9	14777	0.000665	66640	0.013202	89344	0.048242
10	8869.3	0.000847	85778	0.013202		
11	7041.3	0.000917	11759	0.014086		
12	8258.7	0.000917	5006.7	0.014086		
13	9019.6	0.000917	5230.5	0.014086		
14	8276	0.00116	3245.2	0.01502		
15	7014.2	0.00146	13423	0.016007		
16	8281.8	0.00146	5246.4	0.017049		
17	7076.4	0.001968	1454.1	0.018149		
18	7060.3	0.002277	5066.1	0.018149		
19	6505.7	0.002448	73372	0.018149		
20	6986.9	0.002448	23190	0.019309		
21	8885.9	0.002448	3743.5	0.019309		
22	59238	0.00263	5278.1	0.019309		
23	8293.1	0.00263	6049.8	0.02182		
24	10017	0.002823	23390	0.023176		
25	27849	0.002823	5020.5	0.023176		
26	6489.6	0.00303	6929.1	0.024604		
27	13015	0.00325	3900.8	0.029341		
28	6975.9	0.003732	6972.8	0.029341		
29	8302.9	0.003732	6973.4	0.029341		
30	5472.3	0.003997	6974.1	0.029341		
31	8288.1	0.003997	80860	0.029341		
32	7089.7	0.004576	9242.4	0.029341		
33	14246	0.005229	6965.9	0.031082		
34	23190	0.005229	6975.9	0.031082		
35	8327.5	0.005229	11634	0.032909		

36	13423	0.005585	1379.7	0.032909		
37	6974.1	0.005585	3182.2	0.032909		
38	6950.1	0.005962	4976.1	0.032909		
39	6970.7	0.005962	5088.2	0.032909		
40	6973.4	0.005962	6959.8	0.032909		
41	7137.3	0.005962	8281.8	0.032909		
42	10354	0.006362	6970.7	0.034824		
43	21192	0.006362	5003.2	0.036832		
44	6972.8	0.006362	7060.3	0.036832		
45	8794.2	0.006362	7041.3	0.038936		
46	11220	0.006785	71073	0.038936		
47	13906	0.006785	44823	0.041138		
48	6496	0.006785	5102.4	0.041138		
49	23390	0.007233	5659.8	0.041138		
50	80860	0.007233	5885.5	0.041138		
51	7105	0.008207	6950.1	0.041138		
52	6954.2	0.008735	6968	0.041138		
53	7147.5	0.008735	5921.1	0.043443		
54	9769	0.009294	5984.7	0.043443		
55	3493.7	0.009883	7266.2	0.043443		
56	6687.9	0.009883	13906	0.045854		
57	6968	0.010504	6986.9	0.045854		
58	8381.4	0.010504	7014.2	0.045854		
59	6501.9	0.01116	8276	0.045854		
60	8238.3	0.01185	3357.6	0.048373		
61	1395.5	0.013343	4479.7	0.048373		
62	6477.9	0.013343	7105	0.048373		
63	6527.2	0.013343	8981.3	0.048373		
64	6768.8	0.013343				
65	6959.8	0.013343				
66	7124.9	0.013343				
67	6965.9	0.014149				
68	6698.4	0.014997				
69	6916.5	0.014997				
70	6929.1	0.014997				
71	6940.5	0.014997				
72	12354	0.015888				
73	28220	0.017807				
74	6705	0.01884				
75	6728.4	0.021059				
76	6557.6	0.022249				
77	1016.8	0.024804				
78	28401	0.024804				
79	41779	0.026171				
80	1638.7	0.027603				
81	3760.8	0.027603				
82	73372	0.027603				
83	5255.8	0.029099				

84	24106	0.030664				
85	5261.4	0.030664				
86	66640	0.030664				
87	7169.9	0.030664				
88	1403	0.032299				
89	3563.1	0.032299				
90	5033.3	0.032299				
91	5054.2	0.032299				
92	54069	0.034006				
93	7222.4	0.034006				
94	1017.3	0.035789				
95	6484.5	0.035789				
96	8425.2	0.035789				
97	89344	0.035789				
98	29193	0.037649				
99	5265.3	0.039588				
100	6890.8	0.039588				
101	1008.3	0.041611				
102	1617.1	0.043718				
103	5042.3	0.043718				
104	7240.2	0.043718				

TABLE 40

SELDI biomarker p-values: Q10 chip

Matrix (Energy)	**SPA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	13932	8.33E-06	4651.2	0.000448	2622.4	7.07E-06
2	6983.2	1.47E-05	4652.9	0.000448	1854.3	0.000498
3	9540.9	3.12E-05	4653.8	0.000448	3220.1	0.000916
4	10319	3.84E-05	1646.7	0.00054	2180	0.001114
5	9184.1	3.84E-05	4652	0.00054	3338.8	0.001483
6	9468.2	0.000125	4650.5	0.000592	1209.5	0.002146
7	9652.8	0.000138	4649	0.000848	9103.4	0.003959
8	14136	0.000166	2968	0.001011	1908.8	0.004307
9	7084.9	0.000182	4976	0.001102	3224.6	0.004307
10	9365	0.000238	11669	0.001423	1637	0.004681
11	1820.9	0.000311	2960.6	0.001681	3834.7	0.007016
12	13810	0.00037	2773	0.002328	1671.2	0.00759
13	1714	0.000403	1651.1	0.002521	1891.2	0.008204
14	13917	0.000438	11691	0.003188	2232	0.008204
15	9919.6	0.000477	4658.3	0.003188	2968	0.008861
16	7060.1	0.000519	23273	0.003717	4100.8	0.009563

17	8853.5	0.000564	3389.5	0.003717	2743.2	0.010314
18	14018	0.000612	23751	0.004009	1596.6	0.01197
19	1712.5	0.000612	23066	0.004321	1702.9	0.01197
20	7203.3	0.000612	2558.9	0.004321	1909.7	0.01197
21	13894	0.000665	11565	0.004655	2236.9	0.01197
22	8807.4	0.000665	11516	0.005392	1620.3	0.01288
23	2191.1	0.000782	4647.3	0.006229	8853.5	0.01288
24	13947	0.000847	2904.6	0.00669	1621.9	0.01385
25	9103.4	0.000847	11433	0.007701	2409.2	0.01385
26	6919.9	0.000992	3117.3	0.007701	3793.5	0.01385
27	13959	0.00116	1184.5	0.008843	1597.8	0.014882
28	14281	0.00116	11862	0.008843	2752.2	0.014882
29	1706.2	0.00116	23471	0.009468	2861.3	0.014882
30	2176.1	0.00116	4140.8	0.009468	28959	0.014882
31	13985	0.00146	2766.3	0.01013	3110.8	0.014882
32	14081	0.00146	1633	0.010833	1866.1	0.01598
33	7319.5	0.001697	3313.7	0.011578	2718.2	0.01598
34	13900	0.001828	2266.2	0.012367	1592.8	0.017146
35	1705.8	0.001828	2765.4	0.012367	2554.3	0.017146
36	1686.8	0.002118	4973.7	0.012367	1905.1	0.018385
37	13902	0.002277	3347.9	0.013202	1879.8	0.019699
38	13963	0.002448	46073	0.013202	2960.6	0.019699
39	1928.7	0.00263	9184.1	0.013202	1624.5	0.021093
40	1192.3	0.002823	3402.1	0.014086	2208.7	0.021093
41	1705.6	0.00303	4332.7	0.014086	3313.7	0.021093
42	13905	0.00325	4778.6	0.014086	2139.3	0.022569
43	4755.9	0.00325	66483	0.014086	1626.2	0.024132
44	1707.4	0.003483	9103.4	0.014086	2540.8	0.024132
45	3113.7	0.003483	11727	0.017049	3076.7	0.024132
46	1737.9	0.003732	1365.9	0.018149	4129.4	0.024132
47	4741.6	0.003732	3256.3	0.018149	9652.8	0.024132
48	2206.6	0.003997	11484	0.019309	1828	0.025786
49	13828	0.004278	1770.4	0.019309	1595.5	0.027535
50	13843	0.004576	2547.9	0.019309	1599.6	0.027535
51	8904.5	0.004893	4987.9	0.019309	1618	0.027535
52	11862	0.005229	1668.7	0.02182	2443.5	0.027535
53	13876	0.005229	1762.9	0.02182	8733.3	0.027535
54	3544.1	0.005229	1835.7	0.02182	1191	0.029382
55	10132	0.005585	4111.7	0.02182	1568.8	0.029382
56	11691	0.005585	1970.1	0.023176	17425	0.029382
57	1886.2	0.005585	2876.6	0.023176	10682	0.031332
58	21103	0.005585	1656.9	0.024604	12908	0.031332
59	1203.3	0.005962	18608	0.024604	1593.6	0.031332
60	8733.3	0.005962	3391	0.024604	1598.7	0.031332
61	8965.1	0.005962	1652.3	0.026105	1646.7	0.031332
62	1884.9	0.006362	3000	0.026105	2730.2	0.031332
63	4040.1	0.006362	4379.4	0.026105	3186.7	0.031332
64	41641	0.006362	11603	0.027683	4728.1	0.031332

65	53658	0.006362	1208.5	0.027683	1591.5	0.03339
66	1194.9	0.006785	2870	0.027683	1600.9	0.03339
67	13037	0.007233	3170.1	0.027683	2276.1	0.03339
68	1883.9	0.007233	13917	0.029341	2687.2	0.03339
69	23066	0.007706	3558.7	0.029341	9365	0.03339
70	39932	0.007706	4376.2	0.029341	1567.6	0.035559
71	4270.6	0.007706	4380.1	0.029341	1633	0.035559
72	1136.4	0.008207	5232.3	0.029341	4621.6	0.035559
73	7016.5	0.008207	11399	0.031082	8904.5	0.035559
74	1147.4	0.008735	1648.4	0.031082	11862	0.037845
75	1715.7	0.008735	2640.5	0.031082	1573.8	0.037845
76	11603	0.009294	4972.6	0.031082	1589.9	0.037845
77	1701.6	0.009883	1655.2	0.032909	3449.9	0.037845
78	1709.1	0.009883	3236.9	0.032909	1603.7	0.040251
79	1847.5	0.009883	7203.3	0.032909	1641.9	0.040251
80	1888	0.009883	2553	0.034824	1911.1	0.040251
81	23273	0.010504	4122.7	0.034824	2253.9	0.040251
82	1190	0.01116	1447.4	0.036832	2898.1	0.040251
83	1005.1	0.01185	2963.4	0.036832	3647.8	0.040251
84	1153	0.01185	1964.9	0.038936	4140.8	0.040251
85	28959	0.01185	2458	0.038936	1188.8	0.042783
86	1202	0.012578	13796	0.041138	1570.4	0.042783
87	1832	0.012578	1629	0.041138	1594.6	0.042783
88	2189.6	0.012578	4378.9	0.041138	3381.2	0.042783
89	4274	0.012578	10880	0.043443	1608.7	0.045445
90	13781	0.013343	1765.3	0.043443	2773	0.045445
91	9752.3	0.013343	1800.6	0.043443	2550.9	0.048242
92	1134.5	0.014149	2119.8	0.045854	3213.2	0.048242
93	15011	0.014149	2957.7	0.045854	8807.4	0.048242
94	1710.8	0.014149	1017.4	0.048373		
95	1720.5	0.014149	1089.4	0.048373		
96	1911.1	0.014149	13792	0.048373		
97	5018.8	0.014149	1809.1	0.048373		
98	1692	0.014997	2040.5	0.048373		
99	4806.2	0.014997	5803.4	0.048373		
100	5138.3	0.014997	8400.5	0.048373		
101	6880.3	0.014997				
102	8274.6	0.014997				
103	1149.7	0.015888				
104	13792	0.015888				
105	3224.6	0.015888				
106	13148	0.016824				
107	1717.8	0.016824				
108	1137.8	0.017807				
109	1151.9	0.017807				
110	1256.4	0.017807				
111	13786	0.017807				
112	13789	0.017807				

113	13796	0.017807				
114	1901.4	0.017807				
115	11466	0.01884				
116	1696.9	0.01884				
117	1700.2	0.01884				
118	7121.4	0.01884				
119	1146.3	0.019923				
120	1685	0.019923				
121	1724.3	0.019923				
122	1983.3	0.019923				
123	3343	0.019923				
124	3766.6	0.019923				
125	1679.4	0.021059				
126	1690.3	0.021059				
127	1718.6	0.021059				
128	13790	0.022249				
129	3014.2	0.022249				
130	3201.4	0.022249				
131	3456.1	0.022249				
132	4728.1	0.022249				
133	1154.1	0.023497				
134	1167.6	0.023497				
135	1727.1	0.023497				
136	7429.4	0.023497				
137	10682	0.024804				
138	1765.3	0.024804				
139	2519	0.024804				
140	3110.8	0.024804				
141	4129.4	0.024804				
142	2749.6	0.026171				
143	28290	0.026171				
144	3209	0.026171				
145	11433	0.027603				
146	1627.9	0.027603				
147	1705.2	0.027603				
148	1762.9	0.027603				
149	2631	0.027603				
150	2766.3	0.027603				
151	1356.5	0.029099				
152	1629	0.029099				
153	1717.3	0.029099				
154	4140.8	0.029099				
155	1016.6	0.030664				
156	1133.1	0.030664				
157	1148.4	0.030664				
158	1420.8	0.030664				
159	1702.9	0.030664				
160	1014.3	0.032299				

161	1135.5	0.032299				
162	1150.7	0.032299				
163	1199.3	0.032299				
164	1392.9	0.032299				
165	2588.8	0.032299				
166	28087	0.032299				
167	3574.9	0.032299				
168	4155.8	0.032299				
169	6471.6	0.032299				
170	1017.4	0.034006				
171	1021.6	0.034006				
172	11669	0.034006				
173	1358.8	0.034006				
174	1850.1	0.034006				
175	12908	0.035789				
176	1688.5	0.035789				
177	2935	0.035789				
178	2992.8	0.035789				
179	1125.7	0.037649				
180	1144.6	0.037649				
181	1387.5	0.037649				
182	1618	0.037649				
183	4272.4	0.037649				
184	1020.1	0.039588				
185	1132.2	0.039588				
186	1339.7	0.039588				
187	2171.7	0.039588				
188	2898.1	0.039588				
189	3438.2	0.039588				
190	4866.1	0.039588				
191	77930	0.039588				
192	1018.6	0.041611				
193	1139.2	0.041611				
194	1140	0.041611				
195	1193.8	0.041611				
196	1257.1	0.041611				
197	1670.4	0.041611				
198	1785.8	0.041611				
199	1795.8	0.041611				
200	1933.8	0.041611				
201	3578.8	0.041611				
202	1142.5	0.043718				
203	1599.6	0.043718				
204	1725.6	0.043718				
205	2304.4	0.043718				
206	23471	0.043718				
207	2803.1	0.043718				
208	1011.1	0.045912				

209	1118	0.045912				
210	15376	0.045912				
211	2326.1	0.045912				
212	4280.3	0.045912				
213	1161.5	0.048197				
214	1304.8	0.048197				
215	1340.8	0.048197				
216	1595.5	0.048197				
217	2147.1	0.048197				

TABLE 41

SELDI biomarker p-values for features differenced from baseline: Q10 chip

Matrix (Energy)	**CHCA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	2546.3	0.000612	8918.2	0.001681	2477.9	0.001487
2	9132	0.000665	1445.3	0.001826	1209	0.004187
3	1778.9	0.00146	1466	0.003188	1197.9	0.008071
4	5858.4	0.002448	4424.1	0.004655	9132	0.008071
5	8918.2	0.00325	1465.5	0.00669	6784.5	0.011475
6	6784.5	0.003732	2280.9	0.007701	4720.4	0.014781
7	1457.2	0.003997	8674.1	0.008254	8918.2	0.018874
8	1086.9	0.005585	1167.3	0.011578	1348.4	0.020437
9	1269.5	0.005585	4512.1	0.011578	1444.6	0.020437
10	1445.3	0.005585	6784.5	0.011578	1847	0.023895
11	1443.4	0.006785	1145.9	0.014086	1871.7	0.023895
12	1746.2	0.007233	1385.2	0.014086	1137.2	0.032305
13	5772	0.007233	2918.8	0.01502	1393.3	0.032305
14	7724.8	0.008735	1723	0.016007	9524.9	0.032305
15	1741.6	0.012578	1164.9	0.017049	1179.2	0.034756
16	1486.7	0.013343	1466.8	0.018149	1307.8	0.03736
17	5697.8	0.014997	1197.9	0.020532	1694.3	0.03736
18	5819	0.014997	1834.9	0.020532	1629.7	0.043054
19	11488	0.015888	1003.6	0.02182	2288.9	0.046158
20	1784.6	0.015888	1218.6	0.023176	15116	0.049444
21	9365.8	0.015888	3834.6	0.024604		
22	1115.3	0.017807	7090.4	0.024604		
23	1458.5	0.017807	9132	0.024604		
24	1660.1	0.01884	1169.9	0.029341		
25	1471.2	0.021059	1463.9	0.029341		
26	2002.5	0.023497	1238.7	0.031082		
27	4648.9	0.023497	1652.3	0.031082		
28	1210.4	0.024804	9524.9	0.031082		

29	1286.6	0.027603	2663.7	0.032909		
30	1500.9	0.027603	5858.4	0.032909		
31	6964.3	0.027603	6964.3	0.034824		
32	4572	0.030664	1135.4	0.038936		
33	1996.5	0.032299	1067.8	0.045854		
34	1274.2	0.037649	1453.4	0.045854		
35	1488.9	0.037649	1343.5	0.048373		
36	6636.1	0.037649				
37	1446.1	0.039588				
38	1806.3	0.039588				
39	1440.1	0.041611				
40	1500.5	0.041611				
41	23326	0.041611				
42	5828.2	0.043718				
43	1018.8	0.045912				
44	1231.4	0.045912				
45	4675.2	0.045912				
46	9524.9	0.045912				
47	16747	0.048197				
48	1838.6	0.048197				

TABLE 42

SELDI biomarker p-values for features differenced from baseline: Q10 chip

Matrix (Energy)	**SPA matrix (high energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	12354	0.000114	5874.3	0.003444	5518.9	9.47E-05
2	1395.5	0.000917	3182.2	0.004009	1221.1	0.002533
3	11634	0.000992	12354	0.004321	41779	0.005583
4	8981.3	0.001968	5864	0.005011	3803.3	0.007373
5	23190	0.002823	11759	0.00669	12354	0.009644
6	10017	0.003483	5896.3	0.00669	1200.1	0.010525
7	5827.2	0.003483	5902.5	0.007179	5847.2	0.012498
8	23390	0.004576	11634	0.007701	1183.8	0.016052
9	46588	0.004893	5885.5	0.007701	11634	0.020437
10	5847.2	0.005585	5847.2	0.008843	1355.5	0.023895
11	5864	0.005962	5957.6	0.01013	3357.6	0.025801
12	6505.7	0.005962	5975.3	0.010833	4885.4	0.027834
13	23585	0.007233	3900.8	0.01502	51391	0.027834
14	11759	0.007706	3340	0.016007	29193	0.03
15	5902.5	0.007706	5891.5	0.016007	7997.9	0.03
16	9019.6	0.007706	1454.1	0.017049	8008	0.03
17	6640.1	0.008207	5937.8	0.017049	4890.3	0.03736

18	6477.9	0.008735	6003.7	0.017049	1120.4	0.040123
19	9769	0.009294	5993.7	0.019309	11759	0.040123
20	5921.1	0.009883	5947.8	0.020532	1226.4	0.043054
21	5957.6	0.009883	5827.2	0.023176	5332.9	0.043054
22	3424.7	0.01116	5921.1	0.031082	1100.7	0.046158
23	6557.6	0.01116	5838.3	0.032909	7650.7	0.046158
24	41779	0.01185	5984.7	0.032909	1125.9	0.049444
25	24106	0.012578	1459.6	0.038936	5762.4	0.049444
26	6484.5	0.012578	3668.3	0.038936	5792.4	0.049444
27	6489.6	0.012578	5325.1	0.038936		
28	6496	0.012578	5309.4	0.043443		
29	6874.5	0.012578	6049.8	0.043443		
30	9078.4	0.012578	5792.4	0.048373		
31	1638.7	0.013343				
32	1165.5	0.014149				
33	6501.9	0.014149				
34	6853.1	0.016824				
35	1176.8	0.017807				
36	6698.4	0.01884				
37	1170.3	0.019923				
38	14777	0.019923				
39	5838.3	0.019923				
40	5874.3	0.021059				
41	8258.7	0.022249				
42	5776.9	0.023497				
43	13015	0.024804				
44	6527.2	0.024804				
45	6687.9	0.024804				
46	1193.9	0.026171				
47	29193	0.026171				
48	6705	0.026171				
49	8276	0.026171				
50	1146.1	0.027603				
51	1582.9	0.027603				
52	1588.3	0.027603				
53	1617.1	0.027603				
54	8281.8	0.027603				
55	11220	0.029099				
56	1568	0.029099				
57	6728.4	0.029099				
58	1600.7	0.030664				
59	7347.4	0.030664				
60	8302.9	0.030664				
61	1179.5	0.032299				
62	1399.5	0.032299				
63	5792.4	0.032299				
64	5947.8	0.032299				
65	8327.5	0.032299				

66	8885.9	0.032299				
67	3743.5	0.035789				
68	6890.8	0.035789				
69	1575.8	0.037649				
70	5885.5	0.037649				
71	5891.5	0.037649				
72	6003.7	0.037649				
73	9386	0.037649				
74	6916.5	0.041611				
75	1348.6	0.043718				
76	8293.1	0.043718				
77	1167.6	0.045912				
78	8288.1	0.045912				
79	3650	0.048197				

TABLE 43

SELDI biomarker p-values for features differenced from baseline: Q10 chip

Matrix (Energy)	**SPA matrix / (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	1714	6.37E-05	2968	0.000592	1877.7	0.000281
2	9919.6	8.56E-05	4332.7	0.000776	17425	0.000362
3	2665.9	0.000261	1749.1	0.001547	1671.2	0.000753
4	8965.1	0.000564	1117	0.002328	1733.1	0.000753
5	13932	0.000612	1208.5	0.00295	2180	0.001659
6	5138.3	0.00146	3081.9	0.004321	2968	0.001659
7	9540.9	0.001574	1766.2	0.006229	1714	0.001847
8	1190	0.00263	2291.4	0.006229	4759.9	0.003108
9	1727.1	0.00303	4111.7	0.006229	6551.3	0.005583
10	1706.2	0.003483	1102.3	0.00669	12908	0.006132
11	1766.2	0.003483	1103	0.00669	17293	0.007373
12	2588.8	0.003732	4649	0.007179	4956.9	0.008071
13	9184.1	0.003732	4650.5	0.007179	4242	0.008827
14	1147.4	0.003997	1118	0.007701	1908.8	0.009644
15	4293.1	0.003997	1123.3	0.007701	1919.3	0.009644
16	8733.3	0.003997	1344.7	0.007701	7429.4	0.009644
17	9468.2	0.004278	1102.7	0.008843	1701.6	0.012498
18	1148.4	0.004893	1101.3	0.009468	3449.9	0.013598
19	6551.3	0.004893	1314.9	0.009468	1380.4	0.016052
20	2176.1	0.005229	1475	0.009468	1756.9	0.016052
21	1913.3	0.005585	1660.4	0.009468	2601.6	0.016052
22	3343	0.005962	1964.9	0.01013	8904.5	0.016052
23	1159.4	0.006362	1470.9	0.010833	8965.1	0.016052

24	1883.9	0.006362	17293	0.010833	2181.9	0.017414
25	1117	0.006785	3402.1	0.010833	2420.6	0.017414
26	1142.5	0.006785	11275	0.012367	3076.7	0.017414
27	1155.4	0.006785	1656.9	0.012367	1241.1	0.018874
28	1795.8	0.006785	2119.8	0.012367	1949	0.020437
29	13947	0.007233	1099.2	0.013202	4100.8	0.020437
30	4759.9	0.007233	1479.7	0.013202	1792.5	0.023895
31	2147.1	0.007706	1761.4	0.013202	1986.8	0.023895
32	8274.6	0.007706	1482.7	0.014086	2547.9	0.023895
33	11862	0.008207	3779.3	0.014086	3343	0.023895
34	1707.4	0.008207	1100.2	0.016007	4806.2	0.023895
35	1149.7	0.008735	1327.7	0.016007	11466	0.025801
36	1720.5	0.008735	2432.6	0.016007	1905.1	0.025801
37	1737.9	0.008735	4651.2	0.016007	1847.5	0.027834
38	1709.1	0.009294	4652	0.016007	4621.6	0.027834
39	2539.2	0.009294	1103.6	0.017049	1225.5	0.032305
40	1132.2	0.009883	1344.2	0.017049	1247.8	0.032305
41	1785.8	0.009883	1346	0.017049	2086.6	0.032305
42	5018.8	0.009883	1527.4	0.017049	2208.7	0.032305
43	1118	0.010504	2656.8	0.017049	2261	0.032305
44	11466	0.010504	1097.8	0.018149	1199.3	0.03736
45	1153	0.010504	1104.7	0.018149	1720.5	0.03736
46	11565	0.010504	1316.1	0.018149	1973.9	0.03736
47	1712.5	0.010504	1326.7	0.018149	2253.9	0.03736
48	2012	0.010504	1334.6	0.018149	2889.4	0.03736
49	8853.5	0.010504	1529.3	0.018149	1208.5	0.040123
50	3081.9	0.01116	1751.3	0.018149	1222.9	0.040123
51	3197.3	0.01116	2355.6	0.018149	1254.5	0.040123
52	12908	0.01185	2765.4	0.018149	1255.6	0.040123
53	1156.1	0.012578	1116.6	0.019309	3233.6	0.040123
54	1166.2	0.012578	1349.2	0.019309	1352.2	0.043054
55	1167.6	0.012578	2558.9	0.019309	1660.4	0.043054
56	1391.1	0.012578	1083.6	0.020532	1820.9	0.043054
57	1742.4	0.012578	1307.1	0.020532	1981.8	0.043054
58	1814.9	0.012578	1526	0.020532	2056.9	0.043054
59	1820.9	0.012578	1119.6	0.02182	1209.5	0.046158
60	4806.2	0.012578	1499.4	0.02182	1727.1	0.046158
61	10319	0.013343	1533.4	0.02182	1780	0.046158
62	1725.6	0.013343	1087.7	0.023176	1891.2	0.046158
63	3220.1	0.013343	1116.2	0.023176	1931	0.046158
64	9752.3	0.013343	1313.7	0.023176	2658.9	0.046158
65	1116.6	0.014149	17425	0.023176	2861.3	0.046158
66	1160.1	0.014149	2181.9	0.023176	8733.3	0.046158
67	13810	0.014149	2553	0.023176	1239.8	0.049444
68	1701.6	0.014149	2766.3	0.023176	1270.8	0.049444
69	4886.6	0.014149	1330.4	0.024604	2319	0.049444
70	1151.9	0.014997	1343.7	0.024604	2409.2	0.049444
71	1160.9	0.014997	1399.1	0.024604	4122.7	0.049444

72	23066	0.014997	1324.5	0.026105	4364.9	0.049444
73	1144.6	0.015888	1342.1	0.026105		
74	1161.5	0.015888	1510.4	0.026105		
75	1724.3	0.016824	4652.9	0.026105		
76	2206.6	0.017807	1084.2	0.027683		
77	1116.2	0.01884	1086.1	0.027683		
78	1164.8	0.01884	1532.3	0.027683		
79	2326.1	0.01884	1535.2	0.027683		
80	3438.2	0.01884	2326.1	0.027683		
81	4766.1	0.01884	2346	0.027683		
82	1121	0.019923	2547.9	0.027683		
83	3766.6	0.019923	3044.6	0.027683		
84	11275	0.021059	1298.6	0.029341		
85	2438.8	0.021059	1491.9	0.029341		
86	2749.6	0.021059	1733.1	0.029341		
87	7429.4	0.021059	1743.8	0.029341		
88	1146.3	0.022249	1767.2	0.029341		
89	1710.8	0.022249	2353.6	0.029341		
90	3014.2	0.022249	1297.3	0.031082		
91	3313.7	0.022249	1299.7	0.031082		
92	4270.6	0.022249	1325.9	0.031082		
93	1756.9	0.023497	1487.9	0.031082		
94	4866.1	0.023497	1526.6	0.031082		
95	1387.5	0.024804	1122.3	0.032909		
96	1735.7	0.024804	11565	0.032909		
97	28290	0.024804	11669	0.032909		
98	1157.7	0.026171	1256.4	0.032909		
99	1163.7	0.026171	1341.8	0.032909		
100	1980.4	0.026171	1481.5	0.032909		
101	5803.4	0.026171	1492.8	0.032909		
102	6471.6	0.026171	1501	0.032909		
103	1705.6	0.027603	1086.8	0.034824		
104	17425	0.027603	1115	0.034824		
105	1749.1	0.027603	1312.7	0.034824		
106	1765.3	0.027603	1496.2	0.034824		
107	2968	0.027603	1531	0.034824		
108	4973.7	0.027603	1553.8	0.034824		
109	1327.7	0.029099	1755.5	0.034824		
110	1679.4	0.029099	1780	0.034824		
111	1705.8	0.029099	2916.1	0.034824		
112	1759.5	0.029099	1461.9	0.036832		
113	1780	0.029099	1467.9	0.036832		
114	2443.5	0.029099	1502.7	0.036832		
115	2803.1	0.029099	1085	0.038936		
116	46073	0.029099	1262.6	0.038936		
117	4668.4	0.029099	1290.7	0.038936		
118	4688.6	0.029099	1294.7	0.038936		
119	1139.2	0.030664	1300.8	0.038936		

120	1143.2	0.030664	1462.8	0.038936		
121	13828	0.030664	1469.1	0.038936		
122	1436.4	0.030664	1474.1	0.038936		
123	1700.2	0.030664	1509.5	0.038936		
124	2832	0.030664	1548.9	0.038936		
125	1122.3	0.032299	1765.3	0.038936		
126	1162.5	0.032299	3347.9	0.038936		
127	1119.6	0.034006	5803.4	0.038936		
128	1131.8	0.034006	1261.2	0.041138		
129	13148	0.034006	1329.3	0.041138		
130	2195.7	0.034006	1518.3	0.041138		
131	4111.7	0.034006	1795.8	0.041138		
132	1123.3	0.035789	2754	0.041138		
133	1145.4	0.035789	4653.8	0.041138		
134	1767.2	0.035789	1254.5	0.043443		
135	23273	0.035789	1255.6	0.043443		
136	28959	0.035789	1308.4	0.043443		
137	4364.9	0.035789	1524.7	0.043443		
138	1715.7	0.037649	1547.6	0.043443		
139	2437	0.037649	1106.1	0.045854		
140	3201.4	0.037649	1107.6	0.045854		
141	3205.2	0.037649	1521.2	0.045854		
142	1115.7	0.039588	1744.6	0.045854		
143	11691	0.039588	2773	0.045854		
144	1888	0.039588	3000	0.045854		
145	4280.3	0.039588	1071.7	0.048373		
146	1124.5	0.041611	1072.7	0.048373		
147	1877.7	0.041611	1082.9	0.048373		
148	2232	0.041611	1114.3	0.048373		
149	2365.9	0.041611	1115.7	0.048373		
150	3704.3	0.041611	1192.3	0.048373		
151	1101.3	0.043718	1270.8	0.048373		
152	1134.5	0.043718	1279.5	0.048373		
153	1154.1	0.043718	1282.6	0.048373		
154	13037	0.043718	1461	0.048373		
155	1717.8	0.043718	1466	0.048373		
156	2181.9	0.043718	2429.5	0.048373		
157	3209	0.043718	4647.3	0.048373		
158	1136.4	0.045912				
159	1686.8	0.045912				
160	1928.7	0.045912				
161	1963	0.045912				
162	1981.8	0.045912				
163	2188.4	0.045912				
164	4040.1	0.045912				
165	4598	0.045912				
166	5867.4	0.045912				
167	8807.4	0.045912				

168	2004.9	0.048197				
169	53658	0.048197				

TABLE 44

SELDI biomarker p-values: IMAC chip

Matrix (Energy)	CHCA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	1978.3	0.000339	3240	0.00054	2141.5	0.001629
2	1176.8	0.001253	3301.3	0.001308	1109.8	0.004681
3	1870.5	0.00325	2330.7	0.001423	2977.4	0.005517
4	2707	0.00325	3233	0.003444	1526.1	0.006481
5	2483.7	0.004576	3835.3	0.003717	1514.8	0.007016
6	1997.7	0.006785	3341.9	0.004321	5073.2	0.007016
7	3082	0.008735	3239	0.004655	5806	0.007016
8	1218.9	0.01185	2111.8	0.005011	5673.6	0.008204
9	1319.2	0.012578	3338.3	0.005797	5883.4	0.008204
10	2977.4	0.013343	2356.3	0.00669	5760	0.009563
11	1530.1	0.015888	2797.6	0.007701	1110.3	0.01197
12	2691.7	0.015888	3332.7	0.008254	1112.3	0.01385
13	2572	0.016824	3339.8	0.008254	1124.7	0.01385
14	1768.9	0.017807	3349.5	0.008254	1137.2	0.01598
15	6959	0.017807	2125.9	0.009468	25550	0.01598
16	1581.5	0.01884	1659.2	0.01013	1111.4	0.017146
17	1767.5	0.01884	3844.2	0.01013	1965.7	0.017146
18	2111.8	0.01884	5858.7	0.011578	3028.3	0.017146
19	2675.9	0.01884	6460.1	0.011578	2386.8	0.018385
20	1483.4	0.019923	2682.3	0.012367	1193.9	0.024132
21	1702.9	0.021059	6676.8	0.012367	1526.8	0.024132
22	1995	0.023497	6699.1	0.014086	1839.7	0.027535
23	1494.1	0.024804	1628.4	0.01502	3144.5	0.027535
24	1528.1	0.024804	2572	0.01502	3286.3	0.027535
25	3338.3	0.024804	3361.1	0.016007	3658.8	0.027535
26	9534.5	0.026171	2818.4	0.017049	1095.6	0.029382
27	2038.6	0.027603	4145.4	0.019309	1485.5	0.029382
28	2890.3	0.027603	6440.7	0.019309	1541.6	0.029382
29	2676.3	0.029099	3222.9	0.020532	1110.8	0.031332
30	1173.6	0.030664	3241.1	0.020532	1816.4	0.031332
31	2350.6	0.030664	2086.5	0.02182	1072.1	0.03339
32	2785.1	0.030664	6636.9	0.02182	5899	0.03339
33	4650.5	0.030664	1487.5	0.023176	1108.2	0.035559
34	1159.7	0.032299	5673.6	0.023176	2147.1	0.035559
35	1485.5	0.032299	1470.9	0.024604	3460.8	0.035559
36	25550	0.032299	2036.4	0.024604	5312.5	0.035559

37	3144.5	0.032299	3324.9	0.024604	1138.6	0.037845
38	1145.5	0.034006	6959	0.024604	1483.4	0.037845
39	1932.9	0.034006	6648.5	0.026105	1503.6	0.037845
40	1967.8	0.035789	1483.4	0.027683	1070.2	0.040251
41	4646.1	0.037649	2811.1	0.027683	1094.6	0.040251
42	1867.9	0.039588	1482.7	0.029341	1128.9	0.042783
43	3151	0.039588	1963.5	0.029341	1528.1	0.042783
44	3154.1	0.039588	2227.9	0.029341	1084.7	0.045445
45	5893.4	0.039588	6674.2	0.029341	1105.4	0.045445
46	1293.8	0.041611	1532.1	0.031082	1126	0.045445
47	1408.7	0.041611	2673.5	0.031082	1341	0.045445
48	1758.2	0.041611	3035.8	0.031082	2824.7	0.045445
49	1920.8	0.041611	3310.3	0.031082		
50	2399.1	0.043718	4191.5	0.031082		
51	2804	0.043718	1055	0.034824		
52	2858.4	0.045912	3137.7	0.034824		
53	2973.8	0.045912	1191	0.036832		
54	2361.8	0.048197	1403.7	0.036832		
55	5673.6	0.048197	5826.7	0.036832		
56	5858.7	0.048197	2970.1	0.038936		
57			3279.7	0.038936		
58			1055.5	0.041138		
59			2584.2	0.041138		
60			3778.4	0.041138		
61			4646.1	0.041138		
62			5914.3	0.041138		
63			2223.8	0.043443		
64			3216.8	0.043443		
65			4069.6	0.043443		
66			4343.4	0.043443		
67			2643.8	0.045854		
68			3313.6	0.045854		
69			1054.2	0.048373		
70			2327.6	0.048373		
71			2509.2	0.048373		
72			2734.4	0.048373		
73			3383.6	0.048373		

TABLE 45

SELDI biomarker p-values: IMAC chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	9585.6	0.000665	1020.8	0.001547	9248.4	0.001629
2	11505	0.001253	1018	0.007179	6727.5	0.004681
3	9248.4	0.001253	4032	0.020532	6726.6	0.005084
4	11634	0.002118	6707.7	0.023176	6722.9	0.005982
5	11530	0.003997	4028.8	0.024604	11287	0.010314
6	9387.3	0.003997	17506	0.027683	6732.5	0.010314
7	11758	0.005585	4132.2	0.031082	9268.9	0.010314
8	12083	0.005962	4022.3	0.036832	6741.1	0.01197
9	11611	0.007233	4142.1	0.036832	3184.4	0.01598
10	11652	0.007706	6903.1	0.036832	9601.6	0.01598
11	11779	0.009883	6688	0.038936	9284.5	0.017146
12	11568	0.010504	6501.1	0.041138	6737.8	0.019699
13	9284.5	0.010504	4019.9	0.043443	6715	0.024132
14	9384.2	0.01185	6699.1	0.043443	6748.3	0.025786
15	11437	0.012578	6737.8	0.043443	11342	0.027535
16	9626.4	0.014149	6715	0.045854	9078.3	0.027535
17	9470.5	0.014997	6741.1	0.045854	6558.5	0.03339
18	11197	0.015888	8950.8	0.045854	10465	0.035559
19	6189.1	0.015888	1022.7	0.048373	6538.5	0.035559
20	9268.9	0.016824	3740.9	0.048373	9626.4	0.035559
21	6193.1	0.01884			6756.7	0.040251
22	11040	0.019923			9048.9	0.042783
23	14017	0.021059			6545.8	0.048242
24	39807	0.024804				
25	9302	0.026171				
26	11255	0.029099				
27	2605.4	0.029099				
28	6040.4	0.029099				
29	6274.8	0.029099				
30	11845	0.030664				
31	5944.5	0.030664				
32	11287	0.032299				
33	6067.8	0.032299				
34	9516	0.032299				
35	9735.7	0.032299				
36	11702	0.034006				
37	5860.6	0.034006				
38	5920	0.034006				
39	1225.6	0.037649				

40	5910.1	0.037649				
41	74001	0.037649				
42	5933.5	0.039588				
43	12381	0.041611				
44	7253.8	0.043718				
45	9391.4	0.043718				
46	7144.3	0.045912				
47	6252	0.048197				
48	7161.6	0.048197				
49	7165.1	0.048197				

TABLE 46

SELDI biomarker p-values: IMAC chip

Matrix (Energy)	SPA matrix (low energy)					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	1850	0.001353	2570.6	2.91E-05	1229.6	0.009563
2	1191	0.00325	6608.7	0.000306	1001	0.027535
3	2255	0.003997	3353.8	0.000926	2399.2	0.040251
4	1675.2	0.006362	2115.1	0.003188	33884	0.040251
5	2203.7	0.007233	6485.2	0.003717	2411.1	0.042783
6	1190.6	0.014149	2079.5	0.00669	2470.1	0.045445
7	2395.8	0.014149	2622.8	0.007701	3171.9	0.045445
8	2115.1	0.016824	2978.1	0.01013		
9	2036.1	0.01884	6816.7	0.013202		
10	3366.4	0.023497	2841	0.014086		
11	13947	0.024804	2819.7	0.01502		
12	2472.4	0.032299	1805.5	0.016007		
13	39764	0.034006	1586.1	0.017049		
14	3067.3	0.037649	6686.5	0.018149		
15	1191.5	0.041611	2559.4	0.02182		
16	1982.7	0.043718	2499.2	0.023176		
17	2407.1	0.045912	2808.3	0.023176		
18	2815.1	0.045912	1220	0.024604		
19			1404.8	0.024604		
20			1817.6	0.024604		
21			6787.8	0.024604		
22			6745.1	0.026105		
23			5005.5	0.029341		
24			2807.4	0.031082		
25			2160.8	0.032909		
26			3004.7	0.032909		
27			6462.1	0.032909		

28			6910.5	0.032909		
29			1600.9	0.034824		
30			2685.8	0.034824		
31			3429.6	0.034824		
32			1900	0.036832		
33			2770.8	0.036832		
34			1611.3	0.038936		
35			1911.5	0.038936		
36			4563	0.038936		
37			1242.4	0.041138		
38			2157.4	0.041138		
39			1217.6	0.043443		
40			6575.1	0.043443		
41			6850.8	0.043443		
42			1406.7	0.045854		
43			2826.7	0.045854		
44			3740	0.045854		
45			1568	0.048373		

TABLE 47

SELDI biomarker p-values for features differenced from baseline: IMAC chip

Matrix (Energy)	**CHCA matrix (low energy)**					
Samples:	**Time 0 hours**		**Time –24 hours**		**Time –48 hours**	
Ion No.	***m/z***	**p**	***m/z***	**p**	***m/z***	**p**
1	1978.3	8.56E-05	3301.3	0.000648	1137.2	0.000144
2	2111.8	0.000665	2111.8	0.001102	1116.5	0.002283
3	2086.5	0.00116	6648.5	0.001423	1575	0.002533
4	2858.4	0.001353	2673.5	0.002148	1978.3	0.002533
5	1352.9	0.008735	3233	0.002521	1118.3	0.004187
6	1319.2	0.01185	4145.4	0.002728	2600.9	0.004614
7	1222.8	0.013343	3240	0.00295	1557.5	0.005583
8	1792.9	0.013343	3008.3	0.004009	4377.2	0.006132
9	2483.7	0.014149	3239	0.004009	1514.8	0.007373
10	1242.9	0.014997	4726.3	0.004009	1115.3	0.008071
11	1284.5	0.014997	3259.4	0.004321	1126	0.008071
12	1310.1	0.014997	3213.6	0.008254	1342.1	0.008827
13	4478.1	0.017807	3835.3	0.008254	1629.8	0.009644
14	1670.7	0.01884	11198	0.008843	1880.2	0.009644
15	1494.1	0.019923	2223.8	0.01013	4094.2	0.009644
16	1711.1	0.019923	3339.8	0.01013	1642.5	0.010525
17	2633.5	0.019923	2670.4	0.010833	1102.9	0.011475
18	3082	0.019923	1479.3	0.013202	1117.3	0.012498
19	2179.4	0.021059	2970.1	0.013202	1128.9	0.012498

20	1288.5	0.023497	2330.7	0.014086	2029.6	0.012498
21	1917.4	0.023497	3242.5	0.014086	1141.2	0.013598
22	2804	0.023497	3310.3	0.016007	1758.2	0.013598
23	1642.5	0.024804	6440.7	0.016007	4646.1	0.013598
24	1758.2	0.026171	3137.7	0.017049	1101.3	0.014781
25	4650.5	0.026171	3241.1	0.018149	2515	0.014781
26	1287.4	0.027603	6460.1	0.018149	1102.5	0.016052
27	3008.3	0.027603	2589.8	0.019309	1124.7	0.016052
28	1763.1	0.030664	1557.5	0.020532	5673.6	0.016052
29	1932.9	0.030664	3313.6	0.020532	1851.9	0.017414
30	1842.7	0.032299	1230.1	0.02182	1895.5	0.017414
31	3349.5	0.032299	13467	0.02182	3717	0.017414
32	1270.7	0.034006	1457	0.02182	1101.8	0.018874
33	1602.4	0.034006	3460.8	0.02182	1513.8	0.018874
34	1882.1	0.034006	3921.3	0.02182	4639.7	0.018874
35	1674.7	0.035789	6628.3	0.02182	4657.2	0.018874
36	1723.1	0.035789	1670.7	0.023176	1399.2	0.022109
37	2964.2	0.035789	1470.9	0.024604	1835.4	0.022109
38	3154.1	0.035789	1610.6	0.024604	1593.9	0.023895
39	3603.8	0.035789	3242	0.024604	5276.2	0.023895
40	1283.5	0.039588	3246.5	0.024604	2386.8	0.025801
41	1449.6	0.039588	3315.4	0.024604	1099.2	0.027834
42	2299.2	0.039588	3332.7	0.026105	1121.9	0.027834
43	1218.9	0.041611	3778.4	0.026105	1685.4	0.027834
44	1500	0.041611	2590.4	0.027683	4643.2	0.027834
45	1685.4	0.041611	3222.9	0.027683	5073.2	0.027834
46	2174.5	0.041611	3349.5	0.027683	1112.3	0.03
47	2563.4	0.041611	3844.2	0.027683	1127.4	0.03
48	3714	0.041611	6699.1	0.027683	1094.6	0.032305
49	4657.2	0.045912	3496.8	0.029341	1222.8	0.032305
50	1995	0.048197	3954.8	0.029341	1576.7	0.032305
51			5858.7	0.029341	1628.9	0.032305
52			2036.4	0.031082	1878.1	0.032305
53			4191.5	0.031082	1109.8	0.034756
54			5338.2	0.031082	1169.8	0.034756
55			5673.6	0.031082	1862.2	0.034756
56			6959	0.031082	1108.2	0.03736
57			1674.7	0.032909	1121.1	0.03736
58			2074.3	0.032909	1139.8	0.03736
59			4377.2	0.034824	1630.6	0.03736
60			1691.3	0.036832	1111.4	0.040123
61			2734.4	0.036832	1892.2	0.040123
62			3717	0.036832	2141.5	0.040123
63			4596.2	0.036832	2250.2	0.040123
64			6674.2	0.036832	4441	0.040123
65			1820.2	0.038936	1105.4	0.043054
66			2078	0.038936	1110.3	0.043054
67			3216.8	0.038936	1168.4	0.043054

68			3338.3	0.038936	1541.6	0.043054
69			22302	0.041138	1573.5	0.043054
70			3724.9	0.041138	1503.6	0.046158
71			14006	0.045854	1518.2	0.046158
72			1844.8	0.045854	1572.3	0.046158
73			2572	0.045854	1826.2	0.046158
74			4646.1	0.045854	2107.2	0.046158
75			6636.9	0.045854	1457	0.049444
76			6663.7	0.045854	1459.2	0.049444
77			1503.6	0.048373	1573	0.049444
78			2682.3	0.048373	1932.9	0.049444
79			3595.6	0.048373	4072.9	0.049444
80			7008.2	0.048373	6631	0.049444

TABLE 48

SELDI biomarker p-values for features differenced from baseline: IMAC chip

Matrix (Energy)	SPA matrix (high energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	11505	0.000151	1020.8	0.006229	1002.4	0.018874
2	11530	0.001253	12247	0.007701	11040	0.022109
3	11634	0.001828	1250.2	0.016007	3184.4	0.023895
4	11568	0.001968	3925	0.019309	9339.7	0.025801
5	11779	0.002448	3920.5	0.031082	4118.5	0.043054
6	12083	0.002448	11530	0.038936	1000.7	0.046158
7	12247	0.002448	11758	0.038936	13170	0.046158
8	2605.4	0.00263	11779	0.038936	11568	0.049444
9	3103.1	0.003997	11505	0.041138	7765.9	0.049444
10	11652	0.004278	28285	0.041138	7772.9	0.049444
11	11702	0.004278	11702	0.043443		
12	11758	0.004278				
13	11611	0.004576				
14	12381	0.005229				
15	11845	0.005585				
16	9104.1	0.01116				
17	2800.5	0.022249				
18	6826.1	0.022249				
19	6827.9	0.022249				
20	1182	0.029099				
21	10246	0.039588				
22	6377.8	0.043718				
23	11437	0.045912				

TABLE 49

SELDI biomarker p-values for features differenced from baseline: IMAC chip

Matrix (Energy)	SPA matrix (low energy)					
Samples:	Time 0 hours		Time –24 hours		Time –48 hours	
Ion No.	*m/z*	p	*m/z*	p	*m/z*	p
1	2646.6	0.001073	2622.8	0.001981	2880.4	0.000362
2	1675.2	0.00146	1198.6	0.003444	2523.9	0.003436
3	11571	0.001574	11571	0.004655	1920.1	0.011475
4	1850	0.002823	1217.9	0.005011	2244.9	0.012498
5	2871.7	0.004576	1242.4	0.006229	2808.3	0.017414
6	2036.1	0.006362	11751	0.007179	1881.6	0.020437
7	2448.2	0.007706	1361	0.011578	1024.6	0.022109
8	11751	0.009883	1217.6	0.012367	3171.9	0.025801
9	2034.2	0.014997	3165.4	0.013202	4108.7	0.025801
10	2472.4	0.016824	1543.9	0.014086	31457	0.034756
11	1235.7	0.017807	2363.5	0.016007	1141.4	0.043054
12	2160.8	0.017807	1287.6	0.017049	1642.2	0.046158
13	2221.3	0.019923	2978.1	0.018149	3004.7	0.046158
14	5993.7	0.021059	2559.4	0.019309	11571	0.049444
15	2407.1	0.023497	1920.1	0.020532	2214.6	0.049444
16	1817.6	0.024804	1560.6	0.02182	2434.1	0.049444
17	2484.8	0.024804	1003.8	0.023176		
18	2203.7	0.026171	1220	0.024604		
19	2255	0.026171	1292.4	0.024604		
20	5866.1	0.030664	1360	0.024604		
21	2053.3	0.032299	1318.4	0.027683		
22	3345.6	0.032299	2841	0.029341		
23	2214.6	0.034006	1288.9	0.031082		
24	2028.6	0.037649	1379.4	0.032909		
25	2062.1	0.037649	1261.6	0.034824		
26	2719.1	0.037649	1270.4	0.034824		
27	1230.7	0.045912	1301.7	0.034824		
28	9645.7	0.045912	1586.1	0.034824		
29			1805.5	0.034824		
30			1005.7	0.038936		
31			1244	0.038936		
32			2118	0.038936		
33			1832.1	0.041138		
34			2059.5	0.041138		
35			3212.4	0.041138		
36			1260.7	0.043443		
37			3572.4	0.043443		
38			1257.3	0.045854		
39			1259.5	0.045854		

40			2214.6	0.045854		
41			2570.6	0.045854		
42			2880.4	0.045854		
43			1284.4	0.048373		

[00130] MART analysis was performed on the data from SELDI analysis set forth in TABLES 26 – 49, as described at Example 1.4.5., *supra*. TABLE 50 shows the results of two SELDI experiments from time 0 samples in which the accuracy of the classification meets or exceeds about 60%.

TABLE 50

MART analysis of SELDI data

Time (hours)	Chip Type	Matrix	Laser Energy	Sensitivity	Specificity	Accuracy	Markers (m/z)
0	H50	CHCA	Low	67%	64%	65%	9297.4
0	Q10	SPA	Low	88%	76%	82%	9540.9, 6983.2, 9184.1, 9468.2, 1928.7, 3000

[00131] Having now fully described the invention with reference to certain representative embodiments and details, it will be apparent to one of ordinary skill in the art that changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

What is claimed is:

1. A method of determining the status of sepsis in an individual, comprising:

(a) obtaining a first biomarker profile from a first biological sample taken from the individual; and

(b) comparing the individual's first biomarker profile to a reference biomarker profile obtained from a reference population;

wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison determines the status of sepsis in the individual.

2. A method of determining the status of sepsis in an individual, comprising:

(a) obtaining a first biomarker profile at a single point in time from the individual; and

(b) comparing the individual's first biomarker profile to a reference biomarker profile;

wherein the comparison of the biomarker profiles determines the status of sepsis in the individual with an accuracy of at least about 60%.

3. A method of determining the status of sepsis in an individual, comprising comparing (i) a first biomarker profile generated from a first biological sample taken from the individual at a single point in time with (ii) a reference biomarker profile generated from a reference population, wherein the comparison comprises applying a decision rule that determines the status of sepsis in the individual.

4. A method of determining the status of sepsis in an individual, comprising:

(a) obtaining a first biomarker profile from a first biological sample taken from the individual; and

(b) comparing the individual's first biomarker profile to a reference biomarker profile obtained from biological samples from a reference population,

wherein the reference population is selected from the group consisting of a normal reference population, a SIRS-positive reference population, an infected/SIRS-negative reference population, a sepsis-positive reference population, a reference population at a stage in the progression of sepsis, a SIRS-positive reference population confirmed as having sepsis by

conventional techniques after about 0 – 36 hours, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 36 – 60 hours, and a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 60 – 84 hours, and wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison determines the status of sepsis in the individual.

5. A method of determining the status of sepsis in an individual, comprising comparing a measurable characteristic of at least one biomarker between (i) a first biomarker profile obtained from a first biological sample from the individual and (ii) a biomarker profile obtained from biological samples from a reference population, wherein the comparison classifies the individual as belonging or not belonging to the reference population, and wherein the comparison determines the status of sepsis in the individual.

6. A method of determining the status of sepsis in an individual, comprising:

(a) selecting at least two features from a set of biomarkers in a first biomarker profile generated from a first biological sample of an individual; and

(b) comparing the features to a set of the same biomarkers in a reference biomarker profile generated from biological samples from a reference population,

wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population with an accuracy of at least about 60%, and wherein the comparison determines the status of sepsis in the individual.

7. A method of determining the status of sepsis in an individual, comprising:

(a) determining an abundance or changes in an abundance of at least two biomarkers in a first biomarker profile obtained from a first biological sample from the individual, and

(b) comparing the abundance or the changes in the abundance of the at least two biomarkers in the individual's first biomarker profile to an abundance or changes in an abundance of these biomarkers in a reference biomarker profile obtained from biological samples from a reference population,

wherein the comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison determines the status of sepsis in the individual.

8. A method of determining the status of sepsis in an individual, comprising determining an abundance or a change in an abundance of at least one biomarker of a first biomarker profile obtained from a first biological sample from the individual as compared to an abundance or change in an abundance of the at least one biomarker of a reference biomarker profile obtained from biological samples from a (i) SIRS-positive reference population that contracted sepsis and (ii) a SIRS-positive reference population that did not, wherein the biomarkers are selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

9. The method of claim 2, wherein the individual's first biomarker profile is from a first biological sample from the individual, and the reference biomarker profile is from biological samples taken from the reference population.

10. The method of any one of claims 1 and 3 – 9, wherein the biological sample is selected from the group consisting of blood, saliva, serum, plasma, urine, stool, cerebral spinal fluid, cells, a cellular extract, a tissue sample, and a tissue biopsy.

11. The method of claim 1, further comprising:

(a) obtaining a second biomarker profile from a second biological sample taken from the individual; and

(b) comparing the individual's second biomarker profile to the reference biomarker profile;

wherein the second comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the second comparison determines the status of sepsis in the individual.

12. The method of any one of claims 1 and 3 – 9, further comprising repeating the method at least once, wherein a separate biomarker profile is obtained from the individual from a separate biological sample taken each time the method is repeated.

13. The method of claim 12, wherein the biological samples from the individual are taken about 24 hours apart.

14. The method of any one of claims 1 – 8, wherein the determining the status of sepsis in the individual comprises predicting the onset of sepsis in the individual.

15. The method of claim 14, wherein the onset of sepsis is predicted at least about 24 hours prior to the determination of sepsis in the individual using conventional techniques.

16. The method of claim 14, wherein the onset of sepsis is predicted at least about 48 hours prior to the determination of sepsis in the individual using conventional techniques.

17. The method of claim 14, wherein the onset of sepsis is predicted at least about 96 hours prior to the determination of sepsis in the individual using conventional techniques.

18. The method of any one of claims 1 – 8, wherein the determining the status of sepsis in the individual comprises determining the progression of sepsis in the individual.

19. The method of any one of claims 1 – 8, wherein the determining the status of sepsis in the individual comprises diagnosing sepsis in the individual.

20. The method of any one of claims 1 – 2 and 4 – 8, wherein the comparison comprises applying a decision rule.

21. The method of claim 3 or claim 20, wherein applying the decision rule comprises using a data analysis algorithm.

22. The method of claim 21, wherein the data analysis algorithm comprises the use of a classification tree.

23. The method of claim 21, wherein the data analysis algorithm is nonparametric.

24. The method of claim 23, wherein the data analysis algorithm detects differences in a distribution of feature values.

25. The method of claim 24, wherein the nonparametric algorithm comprises using a Wilcoxon Signed Rank Test.

26. The method of claim 21, wherein the data analysis algorithm comprises using a multiple additive regression tree.

27. The method of claim 21, wherein the data analysis algorithm is a logistic regression.

28. The method of claim 21, wherein the data analysis algorithm comprises at least two input parameters.

29. The method of claim 28, wherein the data analysis algorithm comprises at least five input parameters.

30. The method of claim 29, wherein the data analysis algorithm comprises at least ten input parameters.

31. The method of claim 30, wherein the data analysis algorithm comprises at least twenty input parameters.

32. The method of claim 21, wherein the data analysis algorithm uses at least two of the features set forth in any one of TABLES 15 – 23 and 26 – 50 as input parameters.

33. The method of claim 20, wherein the decision rule determines the status of sepsis in the individual with an accuracy of at least about 60%.

34. The method of claim 33, wherein the decision rule determines the status of sepsis in the individual with an accuracy of at least about 70%.

35. The method of claim 34, wherein the decision rule determines the status of sepsis in the individual with an accuracy of at least about 80%.

36. The method of claim 35, wherein the decision rule determines the status of sepsis in the individual with an accuracy of at least about 90%.

37. The method of claim 33, wherein the determination of the status of sepsis in the individual is made at least about 48 hours prior to clinical suspicion that the individual had sepsis, as determined using conventional techniques.

38. The method of claim 33, wherein the decision rule has been subjected to ten-fold cross-validation.

39. The method of any one of claims 1 – 8, wherein the reference biomarker profile is obtained from a population comprising a single individual.

40. The method of any one of claims 1 – 8, wherein the reference biomarker profile is obtained from a population comprising at least two individuals.

41. The method of claim 40, wherein the reference biomarker profile is obtained from a population comprising at least 20 individuals.

42. The method of any one of claims 1 – 3 and 5 – 8, wherein the reference biomarker profile is obtained from a population selected from the group consisting of a normal reference population, a SIRS-positive reference population, an infected/SIRS-negative reference population, a sepsis-positive reference population, a reference population at a stage in the progression of sepsis, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 0 – 36 hours, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 36 – 60 hours, and a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 60 – 84 hours.

43. The method of any one of claims 1 and 3 – 9, further comprising comparing a second biomarker profile from the individual with a reference biomarker profile, wherein the second biomarker profile is obtained from a second biological sample taken from the individual.

44. The method of claim 43, wherein the second biological sample from the individual is taken about 24 hours after the first biological sample is taken from the individual.

45. The method of claim 43, wherein the second biomarker profile is compared to a different reference biomarker profile than the first biomarker profile.

46. The method of any one of claims 1 and 3 – 9, wherein the individual's first biomarker profile and the reference biomarker profile comprise a measurable aspect of at least one nucleic acid.

47. The method of claim 46, wherein the nucleic acid is an mRNA.

48. The method of any one of claims 1 – 8, wherein the individual's first biomarker profile and the reference biomarker profile comprise a measurable aspect of at least one polypeptide.

49. The method of claim 48, wherein measurement of said measurable aspect comprises contacting the at least one polypeptide with an antibody or a functional fragment thereof that specifically binds the at least one polypeptide.

50. The method of claim 49, wherein said antibody or a functional fragment thereof is detectably labeled.

51. The method of claim 50, wherein the label is an amplifiable nucleic acid.

52. The method of claim 49, wherein the at least one polypeptide is present in blood.

53. The method of claim 49, wherein the at least one polypeptide is a cell surface protein.

54. The method of claim 49, wherein the at least one polypeptide is a component of a pathogen.

55. The method of claim 49, wherein the at least one polypeptide is an antibody that binds a component of a pathogen.

56. The method of claim 49, wherein the at least one polypeptide is an autoantibody.

57. The method of any one of claims 1 and 3 – 9, comprising contacting proteins from the biological sample obtained from the individual with an array of antibodies, wherein the antibodies of the array are immobilized.

58. The method of any one of claims 1 and 3 – 9, wherein said biological sample is fractionated prior to said obtaining of said individual's first biomarker profile.

59. The method of any one of claims 1 – 8, wherein at least one separation method is used to obtain said individual's first biomarker profile.

60. The method of claim 59, wherein at least two separation methods are used to obtain said individual's first biomarker profile.

61. The method of claim 59, wherein said at least one separation method comprises mass spectrometry.

62. The method of claim 61, wherein said mass spectrometry is selecting from the group consisting of electrospray ionization mass spectrometry (ESI-MS), ESI-MS/MS, ESI-MS/$(MS)^n$, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), desorption/ionization on silicon (DIOS), secondary ion mass spectrometry (SIMS), quadrupole time-of-flight (Q-TOF), atmospheric pressure chemical ionization mass spectrometry (APCI-MS), APCI-MS/MS, APCI-$(MS)^n$, atmospheric pressure photoionization mass spectrometry (APPI-MS), APPI-MS/MS, and

APPI-$(MS)^n$, quadrupole mass spectrometry, fourier transform mass spectrometry (FTMS), and ion trap mass spectrometry, where n is an integer greater than zero.

63. The method of claim 62, wherein the at least one separation method comprises SELDI-TOF-MS.

64. The method of claim 59, wherein the at least one separation method is selected from the group consisting of chemical extraction partitioning, ion exchange chromatography, reverse phase liquid chromatography, isoelectric focusing, one-dimensional polyacrylamide gel electrophoresis (PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), thin-layer chromatography, gas chromatography, liquid chromatography, and any combination thereof.

65. The method of claim 59, wherein at least two different separation methods are used to obtain said individual's biomarker profile.

66. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and reference biomarker profile comprise a measurable aspect of an infectious agent or a component thereof.

67. The method of claim 66, wherein said component is selected from the group consisting of a viral coat protein, a lipopolysaccharide and lipoteichoic acid.

68. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker that is informative of the state of the immune system in response to infection.

69. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker selected from the group consisting of hormones, autoantibodies, growth factors, transcription factors, cell surface markers, and soluble proteins derived from cells.

70. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker associated with bacteremia.

71. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker associated with macrophage lysis.

72. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker associated with a sepsis pathway.

73. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of an autoantibody.

74. The method of any one of claims 1 – 8, wherein said individual's first biomarker profile and said reference biomarker profile comprise a measurable aspect of a biomarker associated with a physiological condition selected from the group consisting of tissue hypoxia, multiple organ dysfunction, and metabolic acidosis.

75. A method of predicting the onset of sepsis in an individual, comprising:

(a) measuring an aspect of at least two features in a biomarker profile, wherein the biomarker profile comprises at least two biomarkers selected from the set of biomarkers set forth in any one of TABLES 15 – 23 and 26 – 50; and

(b) comparing the measured aspect of said at least two features with the value of a corresponding aspect of the same at least two features in a reference population,

wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison predicts the onset of sepsis in the individual.

76. The method of claim 75, wherein said prediction of the onset of sepsis is made about 12 – 36 hours prior to the onset of sepsis, where the onset of sepsis is determined by conventional techniques.

77. The method of claim 75, wherein said prediction of the onset of sepsis is made about 36 – 60 hours prior to the onset of sepsis, where the onset of sepsis is determined by conventional techniques.

78. The method of claim 75, wherein said prediction of the onset of sepsis is made about 60 – 84 hours prior to the onset of sepsis, where the onset of sepsis is determined by conventional techniques.

79. A method of diagnosing SIRS in an individual, comprising:

(a) obtaining a first biomarker profile from a first biological sample taken from the individual; and

(b) comparing the individual's first biomarker profile to a reference biomarker profile obtained from a reference population,

wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison diagnoses SIRS in the individual.

80. A method of diagnosing SIRS in an individual, comprising:

(a) obtaining a biomarker profile at a single point in time from the individual; and

(b) comparing the individual's biomarker profile to a reference biomarker profile,

wherein the comparison of the biomarker profiles can diagnose SIRS in the individual with an accuracy of at least about 60%.

81. A method of diagnosing SIRS in an individual, comprising comparing (i) a first biomarker profile generated from a first biological sample taken from the individual at a single point in time with (ii) a reference biomarker profile generated from a reference population, wherein the comparison comprises applying a decision rule that determines the status of SIRS in the individual.

82. A method of diagnosing SIRS in an individual, comprising:

(a) obtaining a first biomarker profile from a first biological sample taken from the individual; and

(b) comparing the individual's first biomarker profile to a reference biomarker profile obtained from biological samples from a reference population,

wherein the reference population is selected from the group consisting of a normal reference population, a SIRS-positive reference population, and an infected/SIRS-negative reference population, a sepsis-positive reference population, a reference population at a stage in the progression of sepsis, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 0 – 36 hours, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 36 – 60 hours, and a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 60 – 84 hours, and wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison diagnoses SIRS in the individual.

83. A method of diagnosing SIRS in an individual, comprising comparing a measurable characteristic of at least one biomarker between (i) a first biomarker profile obtained from a first biological sample from the individual and (ii) a biomarker profile obtained from biological samples from a reference population, wherein the comparison classifies the individual as belonging or not belonging to the reference population, and wherein the comparison diagnoses SIRS in the individual.

84. A method of diagnosing SIRS in an individual, comprising:

(a) selecting at least two features from a set of biomarkers in a biomarker profile generated from a first biological sample of an individual; and

(b) comparing the features to a set of the same biomarkers in a biomarker profile generated from biological samples from a reference population,

wherein a single such comparison is capable of classifying the individual as belonging to or not belonging to the reference population with an accuracy of at least about 60%, and wherein the comparison diagnoses SIRS in the individual.

85. A method of diagnosing SIRS in an individual, comprising:

(a) determining an abundance or change in an abundance of at least two biomarkers contained in a first biological sample of an individual, and

(b) comparing the abundance or change in an abundance of the biomarkers in the individual's biological sample to an abundance of these biomarkers in biological samples from a reference population,

wherein the comparison is capable of classifying the individual as belonging to or not belonging to the reference population, and wherein the comparison diagnoses SIRS in the individual.

86. A method of diagnosing SIRS in an individual, comprising determining the abundance or a change in abundance of at least one biomarker obtained from a biological sample from the individual as compared to an abundance or change in an abundance of the at least one biomarker obtained from biological samples from a normal reference population, wherein the biomarkers are selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

87. A method of isolating a biomarker, wherein said biomarker can be used to generate a biomarker profile to diagnose or predict sepsis, said method comprising:

(a) obtaining a reference biomarker profile, said reference biomarker profile obtained from a population of individuals;

(b) identifying a feature of said reference biomarker profile, wherein said feature is predictive or diagnostic of sepsis or one of the stages of sepsis;

(c) identifying a biomarker that corresponds with said feature; and

(d) isolating said biomarker.

88. A biomarker profile comprising at least two features that contribute to the classification of an individual as belonging to a reference population with an accuracy of at least about 60%, based on a comparison with the reference population, wherein the reference population is selected from the group consisting of a normal reference population, a SIRS-positive reference population, an infected/SIRS-negative reference population, a sepsis-positive reference population, a reference population at a stage in the progression of sepsis, a SIRS-positive reference population confirmed as having sepsis by conventional techniques

after about 0 – 36 hours, a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 36 – 60 hours, and a SIRS-positive reference population confirmed as having sepsis by conventional techniques after about 60 – 84 hours.

89. A kit, comprising at least two biomarkers selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

90. A kit, comprising a set of antibodies or functional fragments thereof that specifically bind at least two biomarkers selected from the group consisting of the biomarkers listed in any one of TABLES 15 – 23 and 26 – 50.

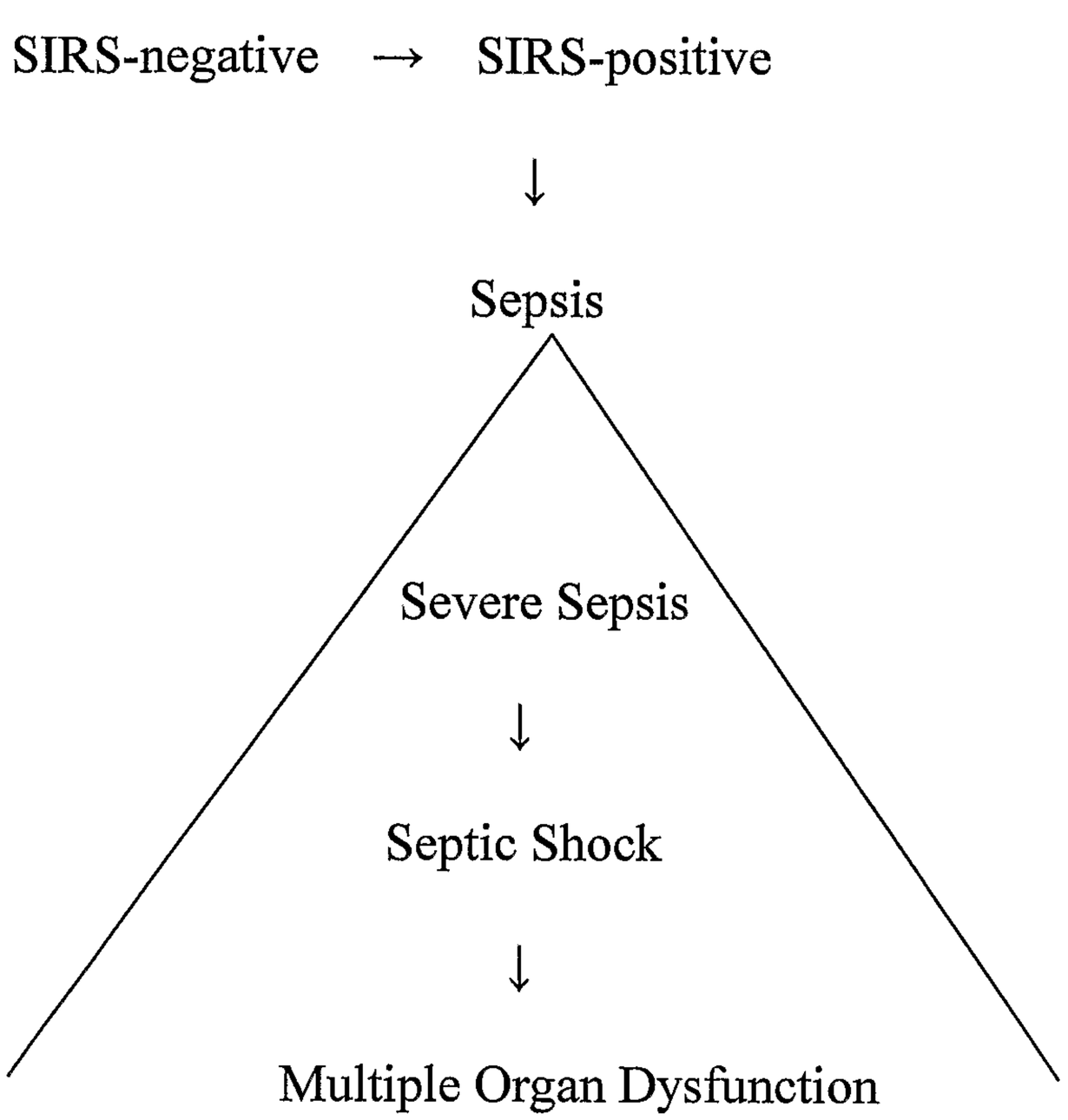

SIRS-negative
SIRS-positive
Sepsis
Severe Sepsis
Septic Shock
Multiple Organ Dysfunction

FIGURE 1

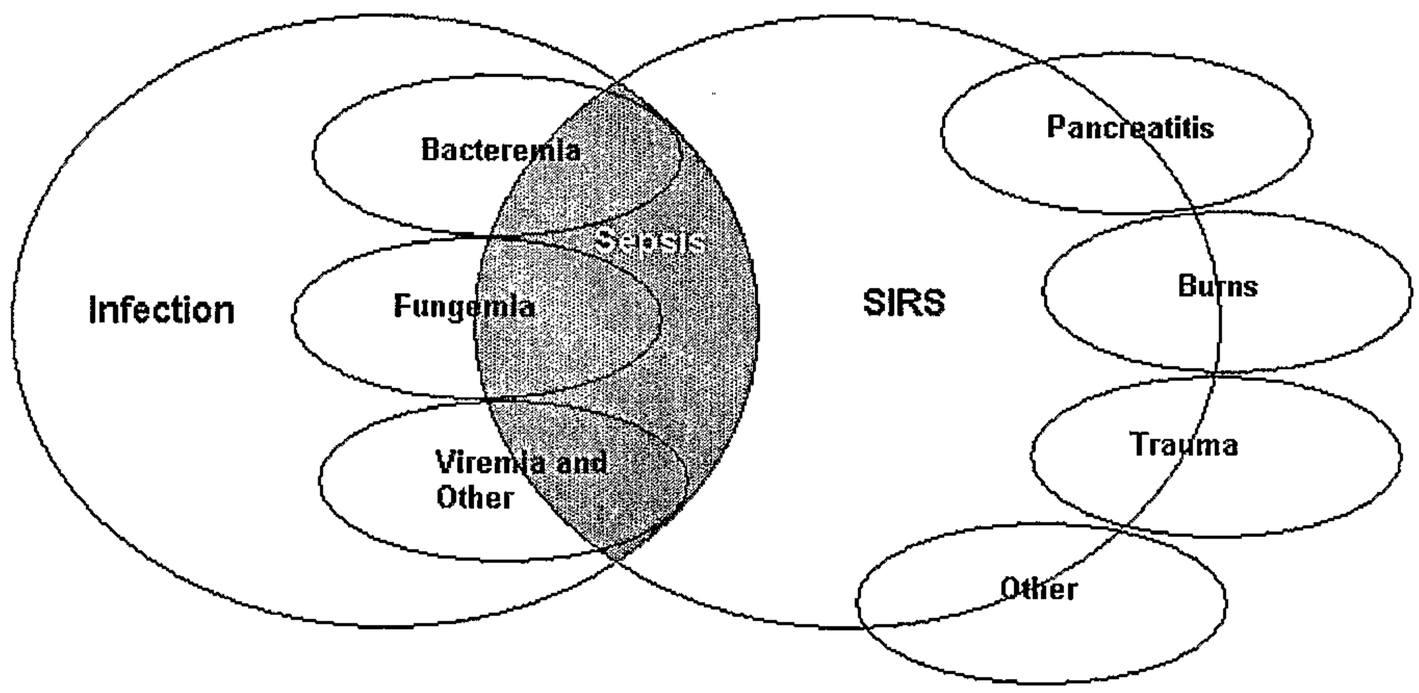

Infection
Bacteremia
Sepsis
Fungemia
Viremia and
Other
SIRS
Pancreatitis
Burns
Trauma
Other

FIGURE 2

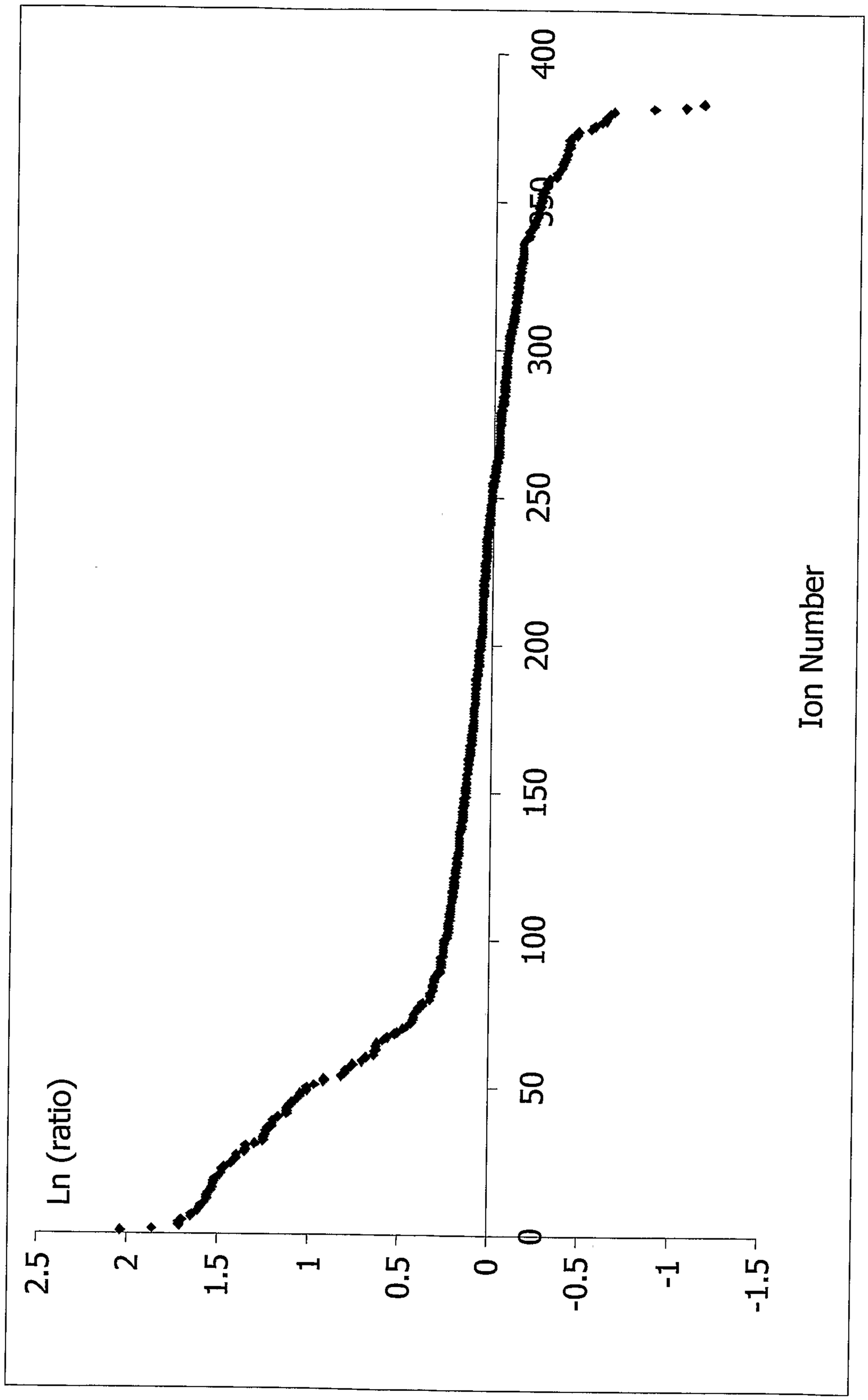
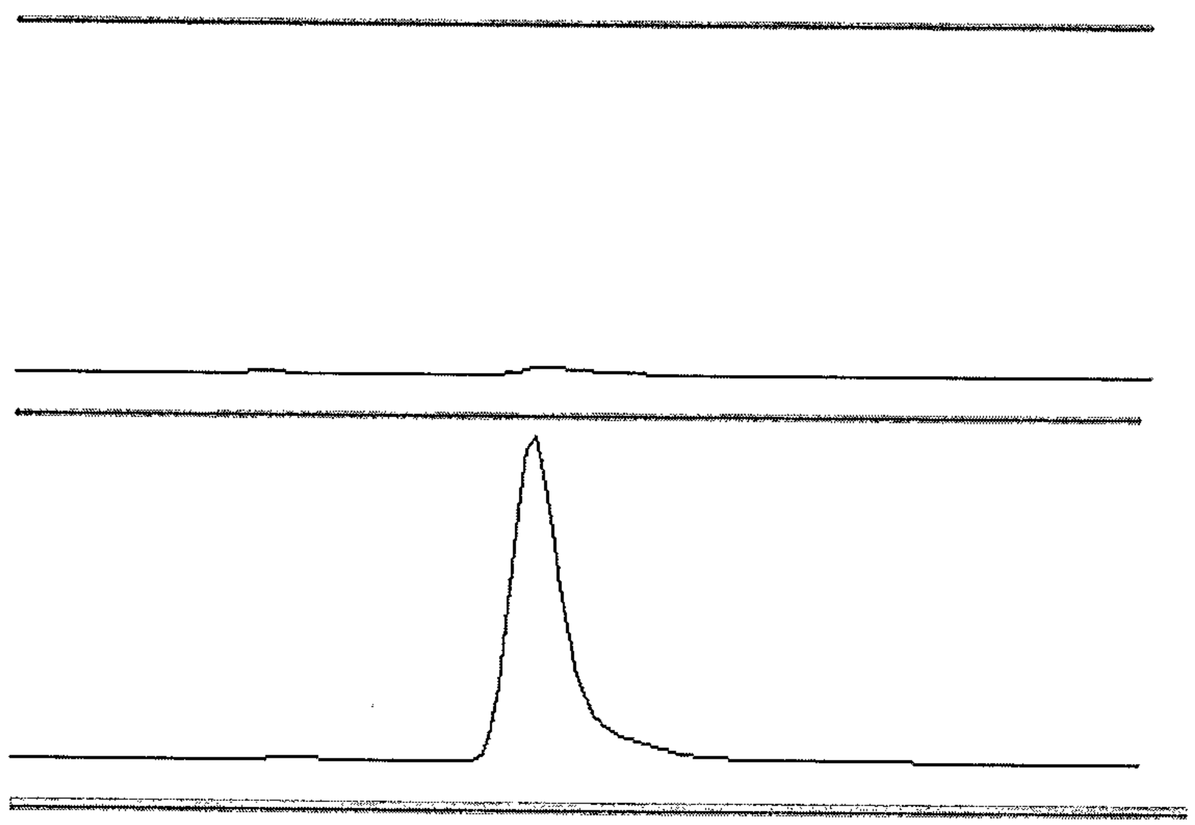

Ln (ratio)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
50
100
150
200
250
300
350
400
Ion Number

FIGURE 3

Presence of ion # 21 (437.2 Da, 1.42 min) in Biological Samples from the Sepsis Group



Day 1 samples

Time –48 hour samples

Time –24 hour samples

Time 0 hour samples

FIGURE 4A

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WO 2004/044554 PCT/US2003/036019 CA 02505902 2005-05-12

Presence of ion # 21 (437.2 Da, 1.42 min) in Biological Samples from the SIRS Group

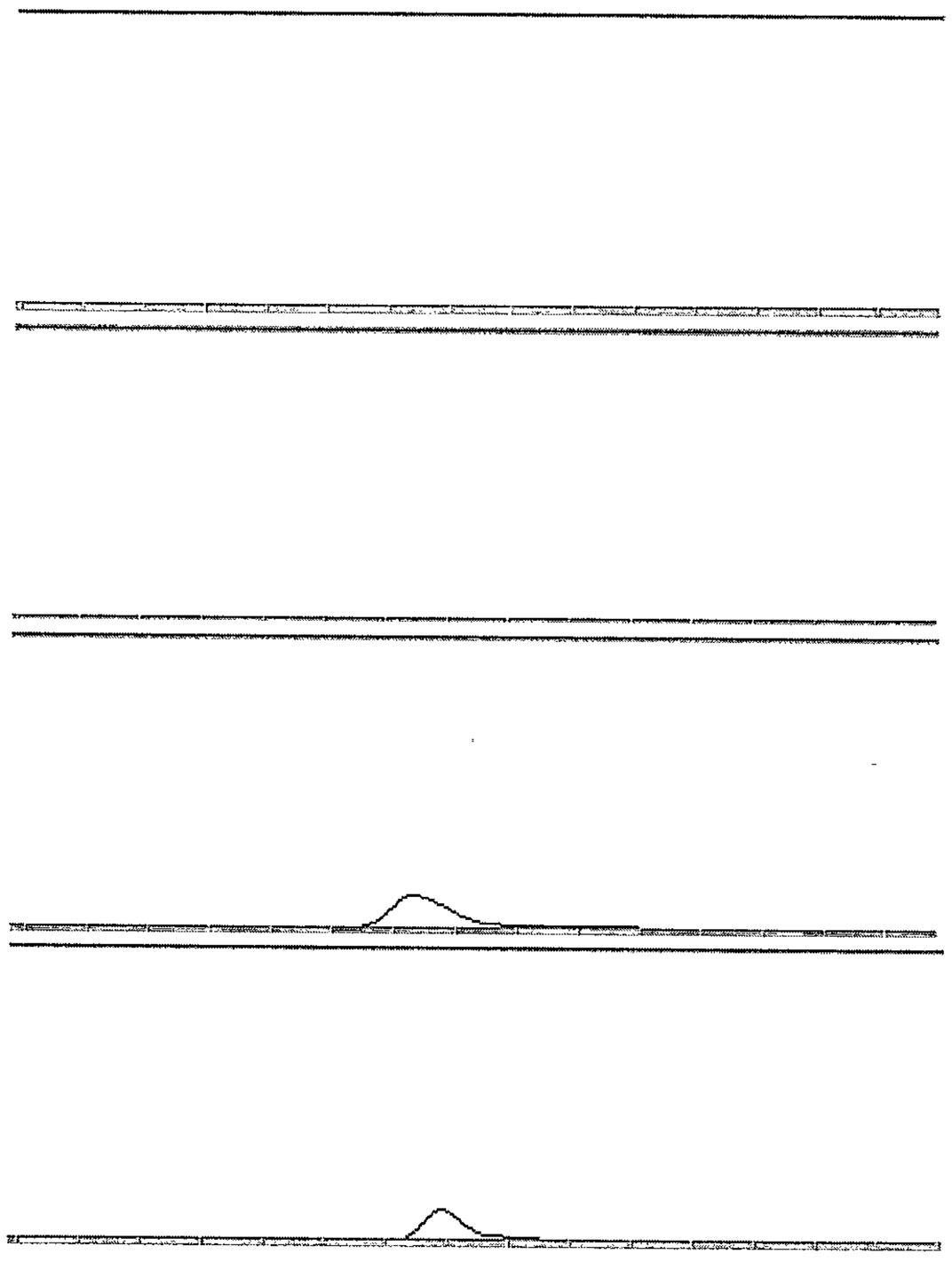


FIGURE 4B

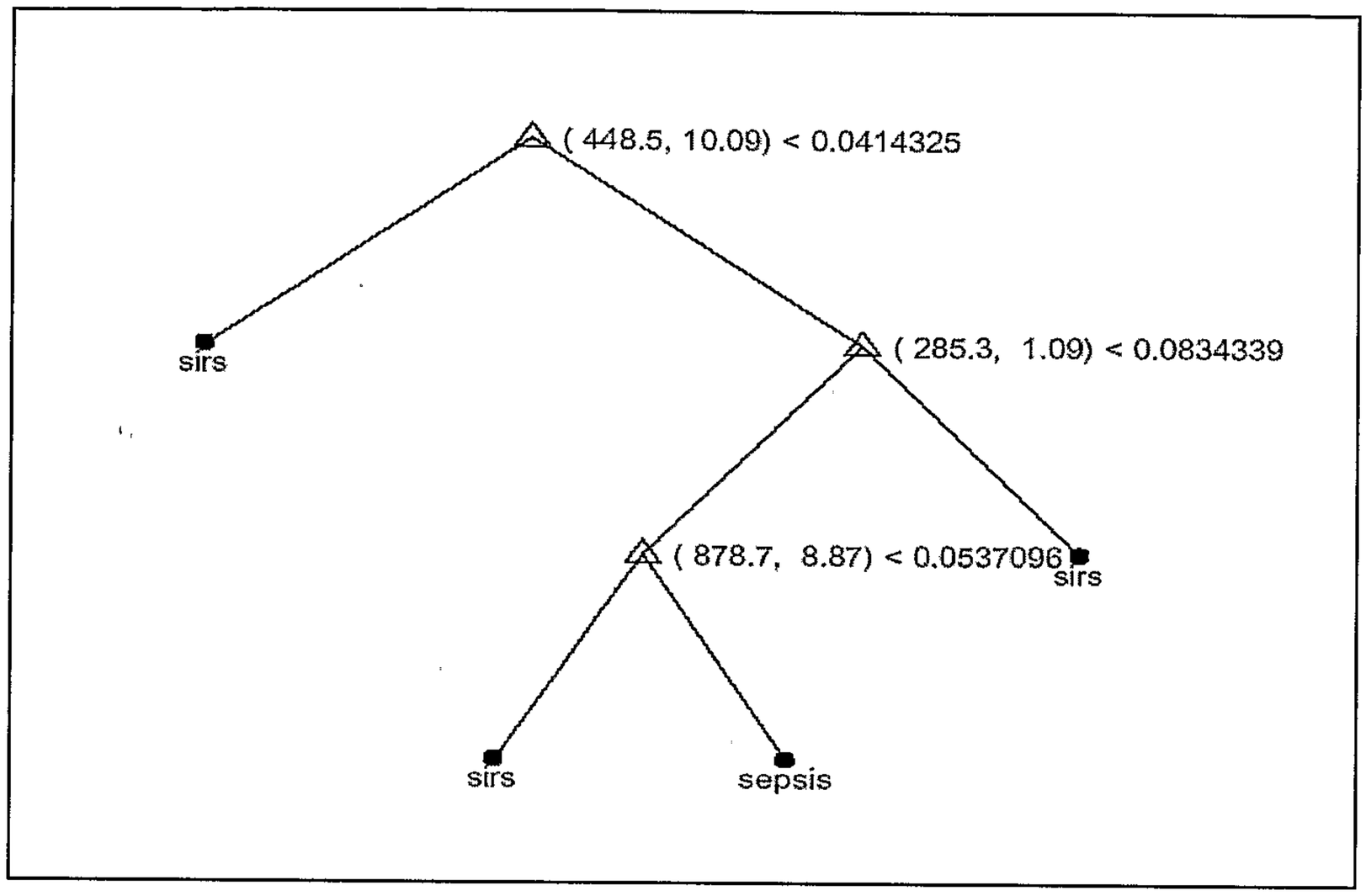
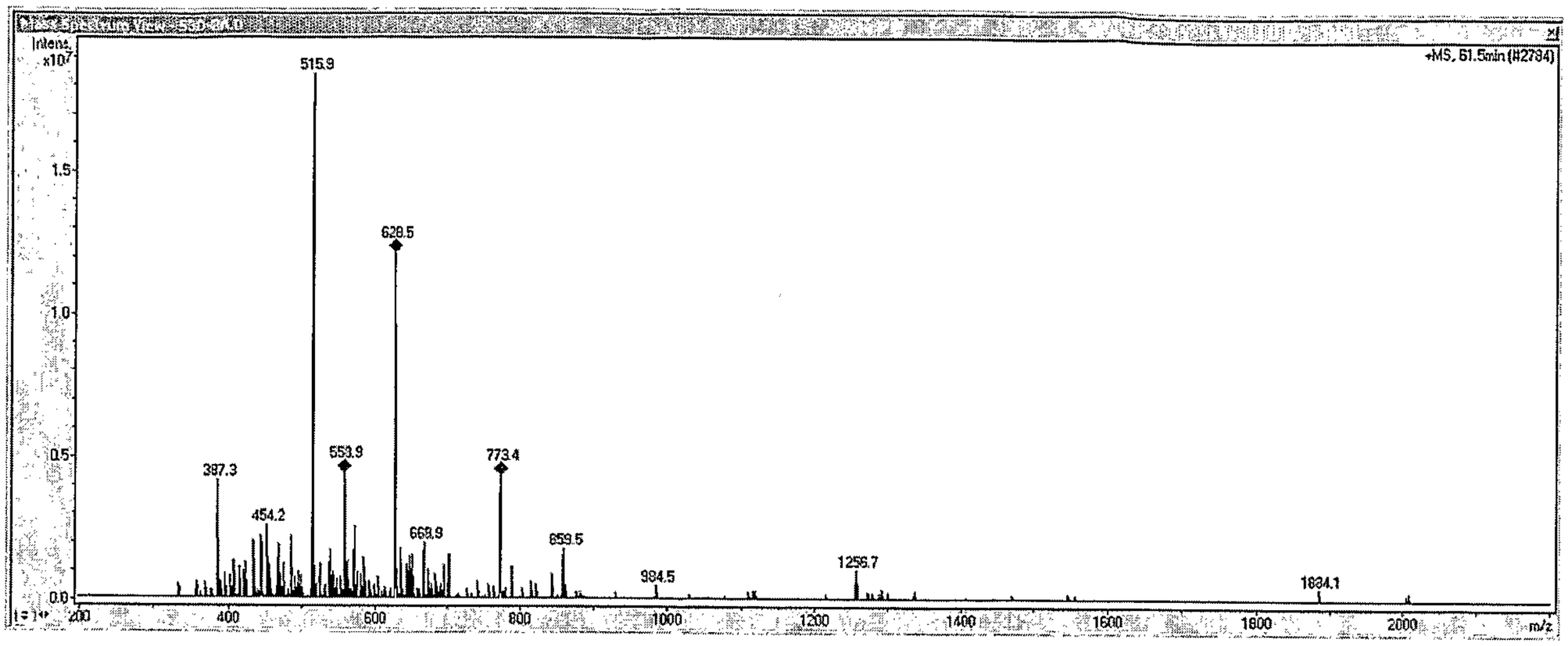
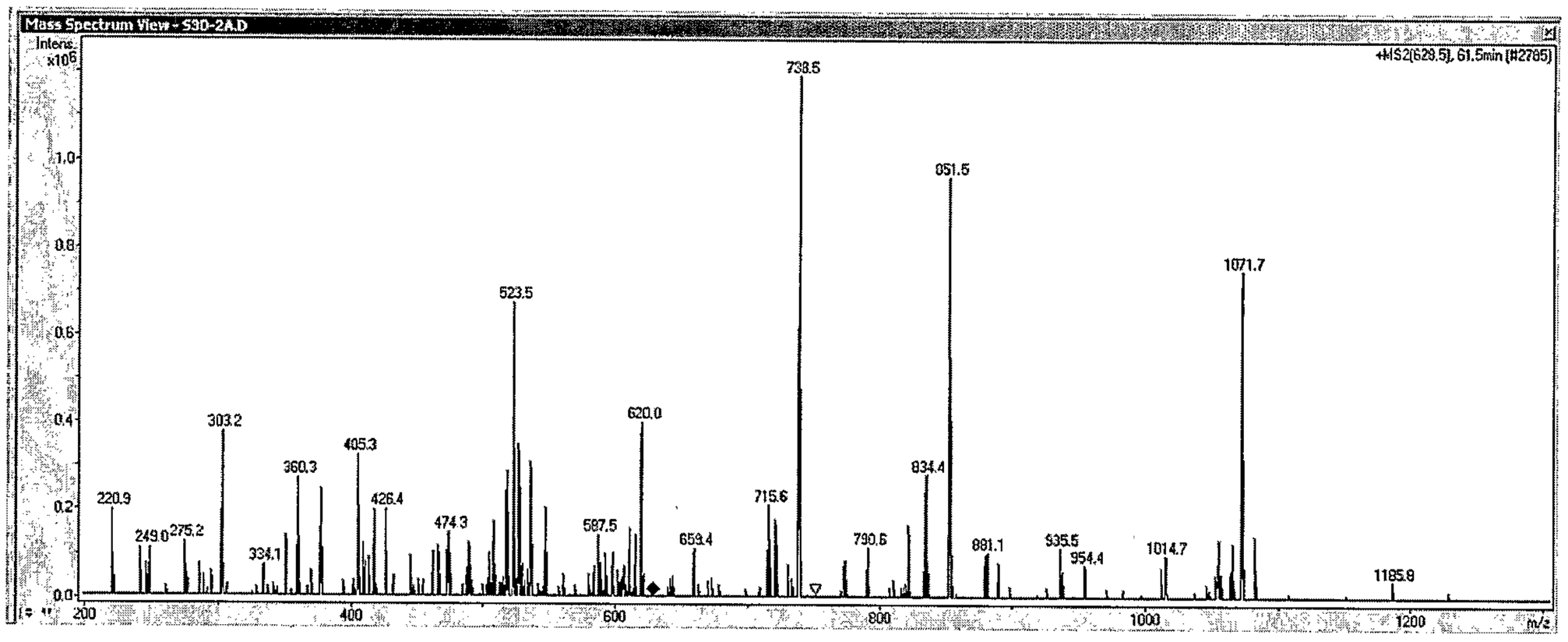

(448.5, 10.09) < 0.0414325
sirs
(285.3, 1.09) < 0.0834339
(878.7, 8.87) < 0.0537096
sirs
sirs
sepsis

FIGURE 5



LC/MS



LC/MS/MS

FIGURE 6

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Up-Regulated Proteins

SIRS			Sepsis			Protein Identifier	GenBank Accession Number
Day 1	T –48 hours	T 0 hours	Day 1	T –48 hours	T 0 hours		
127	81	80	125	371	508	15990507	AAH15642
308	190	229	98	615	659	21361198	NP_000286
10	16	13	43	20	26	4505881	NP_000292
35	15	16	23	25	39	2851501	P19827
48	39	35	101	81	58	72059	NBHUA2
62	44	29	20	47	68	4557327	NP_000033
125	64	44	147	151	142	36308	CAA39974
306	276	170	228	361	408	1197209	CAA29229
22	201	317	169	702	519	1197209	CAA29229
12	4	1	15	9	28	627517	BAA34292
30	10	12	10	58	37	4502149	AAH15642
7	2	1	9	8	3	4504165	NP_000168
33	37	25	50	43	67	2521983	BAA22652
7	11	14	34	85	26	4504345	NP_000508
0	0	14	0	31	33	5031777	NP_005521
0	1	0	6	15	12	1351236	P05543
2	1	0	4	8	4	2144886	C1HUQB

FIGURE 7A

GenBank Accession Number	Protein Identifier	Protein Description
AAH15642	15990507	Similar to serine (or cysteine) proteinase inhibitor, clade A (α –1 antiproteinase, antitrypsin), member 1
NP_000286	21361198	Similar to serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1
NP_000292	4505881	Plasminogen precursor [Contains Angiostatin]
P19827	2851501	Inter- α –trypsin inhibitor heavy chain H1 precursor (ITI heavy chain H1) (Serum-derived hyaluronan-associated protein) (SHAP)
NBHUA2	72059	Leucine-rich α –2-glycoprotein
NP_000033	4557327	Apolipoprotein H precursor
CAA39974	36308	SAAB1 β
CAA29229	1197209	α –1-acid glycoprotein 1 precursor
CAA29229	1197209	α –1-acid glycoprotein
BAA34292	627517	Lipopolysaccharide-binding protein
AAH15642	4502149	Apolipoprotein A-II precursor (Apo-AII) (ApoA-II)
NP_000168	4504165	Gelsolin precursor, plasma (Actin-depolymerizing factor) (ADF) (Brevin) (AGEL)
BAA22652	2521983	α 2-HS glycoprotein
NP_000508	4504345	Hemoglobin α chain
NP_005521	5031777	Isocitrate dehydrogenase [NAD] subunit α, mitochondrial precursor (Isocitric dehydrogenase)
P05543	1351236	Thyroxine-binding globulin precursor (T4-binding globulin)
C1HUQB	2144886	Complement subcomponent C1q chain B precursor

FIGURE 7B

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Down-Regulated Proteins

SIRS			Sepsis			Protein Identifier	GenBank Accession Number
Day 1	T –48 hours	T 0 hours	Day 1	T –48 hours	T 0 hours		
72	49	39	40	9	18	4504489	NP_000403
17	55	26	15	16	9	4502261	NP_000479
0	18	23	19	2	1	13376417	NP_079216
16	6	3	5	0	0	19344010	AAH25681
8	5	5	7	1	2	18490598	AAH22256
21	10	6	4	3	5	15705411	AAL05629
16	10	0	3	0	2	4505047	NP_002336
12	17	6	0	5	4	4557323	NP_000031
13	15	9	6	0	0	4502157	NP_001636
0	9	16	4	0	0	3868933	BAA34292
5	10	10	2	2	0	13169436	AAK13574
4	5	2	0	0	0	14009346	AAK50336
3	8	0	0	0	1	21040475	AAH30580
3	1	9	1	0	0	6912502	NP_036346
24	35	6	6	2	4	27697129	AAH41761

FIGURE 8A

CA 02505902 2005-05-12

GenBank Accession Number	Protein Identifier	Protein Description
NP_000403	4504489	Histidine-rich glycoprotein precursor (Histidine-proline rich glycoprotein) (HPRG)
NP_000479	4502261	Serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1; Antithrombin-III precursor (ATIII) (PRO0309)
NP_079216	13376417	Unnamed protein product
AAH25681	19344010	Insulin-like growth factor binding protein, acid labile subunit
AAH22256	18490598	Lipopolysaccharide binding protein
AAL05629	15705411	Peptidoglycan recognition protein L precursor
NP_002336	4505047	Lumican precursor (Keratan sulfate proteoglycan lumican) (KSPG lumican)
NP_000031	4557323	Apolipoprotein C-III precursor (Apo-CIII)
NP_001636	4502157	Apolipoprotein C-I precursor (Apo-CI)
BAA34292	3868933	α1-acid glycoprotein
AAK13574	13169436	Forkhead homolog
AAK50336	14009346	nGAP-like protein
AAH30580	21040475	Unknown (protein for MGC:26123)
NP_036346	6912502	UDP-GlcNAc:α-1,3-D-mannoside β-1,4-N-acetylglucosaminyltransferase IV
AAH41761	27697129	Similar to dedicator of cyto-kinesis 1

FIGURE 8B

SIRS-negative → SIRS-positive

↓

Sepsis

Severe Sepsis

↓

Septic Shock

↓

Multiple Organ Dysfunction