

TITLE OF INVENTION:

METHOD FOR INTERPRETING TANDEM MASS SPECTROMETRY DATA FOR
CLINICAL DIAGNOSIS

TECHNICAL FIELD:

5 Interpreting data used for clinical diagnostic purposes. In particular, a method for
interpreting electrospray tandem mass spectrometry data relating to the quantification of
metabolites used for diagnosing newborn babies is disclosed.

BACKGROUND ART:

Automated methods for assessing a patient's condition are known. Computerized
10 systems can be integrated to produce data that can be compared to a known result to allow for
proper diagnosing. Such data might be produced by a MRI or CAT scanner, which is used to
identify components within the human body.

One particular instrument used for identifying components of interest, whether they
are of medicinal or chemical interest, is the mass spectrometer. In reference to U.S. Patent
15 No. 5,453,613, compounds, when introduced to the electrospray tandem mass spectrometer,
are ionized and essentially fragmented. Each fragment produces a peak having local
maximums that are matched to reference spectra. A compound can be identified by its
associated fragments, each having a mass to charge ratio, which is then relative to the
concentrations of each fragment. All of the reference spectra and compound names can then
20 be stored in a library for correlation and determination. Thus, mass/charge ratios can be used
to identify components from known spectra stored in a database.

More recently, however, the use of spectrometry has been implemented in the field of
clinical diagnosis. See Chace, U.S. application serial number 09/277,119.

Inborn errors of metabolism usually result from defective enzymes or cofactors.
25 Resulting genetic disorders can be diagnosed by the metabolic profiling of amino acids and

acylcarnitines taken from blood spots subjected to a sampling protocol and thereafter introduced into an electrospray tandem mass spectrometer. An electrospray tandem mass spectrometer is very sensitive and specific and can detect a broad spectrum of disorders at the genetic level. With proper standards, data produced from the spectrometer includes values for particular metabolites. The metabolites that are of interest in detecting these disorders are, in particular, amino acids and acylcarnitines/carnitines and the derivatives thereof.

The spectra and resulting concentration values of each metabolite, as derived from mass spectrometry, are then compared to thresholds as a means for evaluating the contents of the blood sample. These thresholds determine the appropriate course of action necessary as a follow-up to the spectral analysis.

As seen in Wright et al., 5,545,895, spectrometry data is applied to a computerized search database for matching each component as a means of identification. In a clinical diagnostic setting, there must be further methods for evaluation beyond just that of the identification itself. The numbers must be quantified. Newborns can be born with metabolic disorders, which, if not treated within days, can result in death. Thus, after obtaining MS/MS (tandem mass spectrometer) data from blood samples from newborns, generally of the age of less than seven days old, there is a need for efficiently interpreting this data in relation to pre-determined metabolite concentration thresholds. This interpretation allows for proper decision-making necessary for the diagnosing and follow-up testing of newborns.

DISCLOSURE OF INVENTION:

The objective of the present invention is to provide a method for interpreting electrospray tandem mass spectrometry data from the steps following analysis to diagnosis. The method provides the next course of action necessary in determining the deficiency or elevation of a particular fragment directly proportional to a concentration of a metabolite that may cause a genetic defect. In accordance with U.S. application Ser. No. 09/277,119, when

an abnormal sample is flagged after being scanned, a recommended action is to be taken. The present method is a guideline for the necessary action following the preliminary analysis.

Internal standards are used to provide the quantitative information needed to detect specific components. Use of proper ratios of respective ions enables the detection of many metabolites at one time. Each particular metabolite is produced as a fragment yielding a concentration within the spectrometer after being quantified and derivatized from a blood spot. Each metabolite concentration is compared to a flag concentration, which is a quality assurance indicator used to identify a proper sampling quantification and analysis, and which is a diagnostic limit in determining whether or not the concentration of the metabolite is significant. The flag is pre-determined based on a standard deviation from what a normal concentration of a particular metabolite should be. This concentration threshold, or flag, must be appropriated for each scan done and for each type of metabolite reviewed. The concentration values produced will be above or below this threshold flag, which allows for the determination of the next course of action, whether it be a re-analysis or the interpretation that the baby is normal.

As an example, medium chain acyl-CoA dehydrogenase (MCAD) deficiency could be a result of an increased concentration of octanoylcarnitine (Chace et al.). Deficiency in the activity of MCAD presents with a Reye-like syndrome, mild hypoglycemia, or sudden death. The present method provides for numerical guidelines for determining just how significant the elevation is at the time of birth, and what the next step in the screening process would be, such as a follow-up and confirmatory DNA test. In this manner, decision trees for interpreting the concentrations for amino acids and acylcarnitine/carnitines are presented, some of which, if properly diagnosed, can lead to treatment. Each of these potentially fatal blood elevations or deficiencies is compared against the quantified concentration thresholds to allow for immediate attention and action. The comparison with the threshold flags also is

a determining factor for maintaining instrument quality and accuracy. This decision making process, coupled with the current method of screening newborns using electrospray tandem MS/MS, allows for a complete protocol for analyzing and diagnosing newborns with genetic disorders.

5 BRIEF DESCRIPTION OF THE DRAWINGS:

FIG. 1 is a flow diagram of the overall methodology representing the major steps involved from analysis to diagnosis.

FIG. 2 is a flow diagram showing the more detailed steps and decision tree involved in the re-analysis protocol.

10 FIG. 3 is a flow diagram showing the more detailed steps and decision tree involved in the follow-up protocol.

FIG. 4 is the decision tree for the implementation of the method for propionyl carnitine.

15 FIG. 5 is the decision tree for the implementation of the method for isovaleryl carnitine.

FIG. 6 is the decision tree for the implementation of the method for methionine.

FIG. 7 is the decision tree for the implementation of the method for glutaryl carnitine.

FIG. 8 is the decision tree for the implementation of the method for phenylalanine.

FIG. 9 is the decision tree for the implementation of the method for leucine.

20 FIG. 10 is the decision tree for the implementation of the method for citrulline.

FIG. 11 is the decision tree for the implementation of the method for octanoyl carnitine.

FIG. 12 is the decision tree for the implementation of the method for myristoyl carnitine.

25 FIG. 13 is the decision tree for the implementation of the method for hydroxy-C5.

BEST MODE FOR CARRYING OUT THE INVENTION:

The method will now be described in detail in relation to a preferred embodiment and implementation thereof which is exemplary in nature and descriptively specific as disclosed. As is customary, it will be understood that no limitation of the scope of the invention is
5 thereby intended. The invention encompasses such alterations and further modifications in the illustrated device, and such further applications of the principles of the invention illustrated herein, as would normally occur to persons skilled in the art to which the invention relates.

The steps in the overall methodology are shown in FIG. 1. After the blood samples
10 are scanned by the electrospray tandem mass spectrometer, the data is acquired **1**. This data can be presented on a monitor to allow for viewing and/or printing of the output. As part of this data acquiring **1**, the first values produced from the scan of the mass spectrometer are processed and printed into spreadsheet form to further allow checking of the calculations, a means of assuring accurate number production and quality. The acquired data **1** is then
15 examined by applying the first values obtained to the decision tree **2** particular to a metabolite. The first values to be interpreted **2** consist of mean metabolite concentrations and molar ratio concentrations produced from the fragmentation of the metabolites scanned. The concentration of the particular metabolite is read from the fragmentation of the butyl ester occurring at a specific mass to charge value upon derivatization of the metabolite. This
20 quantified number, normally in units of micromolarity, is compared to the flag threshold.

The metabolites, as further described, are grouped as carnitines/acylcarnitines or amino acids. Each particular metabolite is derivatized to enhance the detection of the fragment of concern, and would produce a peak upon scanning corresponding to a quantified concentration number, and compared to the decision tree **2** flag threshold, which is a
25 particular standard deviation away from a mean value for the particular metabolite fragment

concentration. The flag threshold particular for that metabolite is a diagnostic limit to these first values.

When the first values for the flags and metabolite fragment concentrations or molar ratio concentrations are applied to the decision tree **2**, it is identified whether or not there
5 needs to be a subsequent re-analysis **3**, or an immediate, stat re-analysis **4**. A subsequent analysis **3** may be necessarily performed if the scan revealed a mean concentration that is equal to or slightly greater than the pre-determined threshold for the flag setting. Each threshold is unique for a particular metabolite concentration, as further described. It should be understood that a concentration is deemed relevant if it exceeds a threshold when the
10 metabolite may cause defects if elevated, and the concentration is relevant if it is below the threshold when the metabolite of question may cause defects if deficient. Because of this fact, any elevation or deficiency can be called a deviation. For the purposes of clarification, an elevation will be discussed, whereby a deficiency should be inherently understood depending on the metabolite. Both upper and lower concentration flag thresholds and molar
15 ratio thresholds may be utilized in some cases where both an elevation and deficiency is significant in determining what defect may be present.

A stat re-analysis **4** may be performed if an initial concentration of the metabolite *significantly* deviates from the flag threshold, which may be evident of a genetic disorder.

An immediate preliminary follow-up **4a** would then follow. Whether or not the deviation is
20 deemed significant depends on the amount by which the metabolite concentration deviates from the flag threshold based on the interpretation guide for each disorder. In any effect, a subsequent analysis **3** or a stat re-analysis **4** requires the data to be re-acquired **1**. If the scan shows normal metabolite concentration levels as compared to the concentration flag thresholds and molar ratio thresholds, the interpretation may be that the blood component
25 levels are normal **5**.

Depending on which particular metabolite is being scanned, if the metabolite concentration is not significantly above the threshold, but still deviates in relation to the threshold, even after re-analysis, the next step would be to interpret **6** the sample as being evident of this elevation (or deficiency). In this case, a follow-up protocol **7** would be initiated, such that the detection process would be repeated and all attention would be focused on this sample. After any subsequent repeat, a diagnostic interpretation **8** may follow if the first concentration is consistent with any subsequent evaluation.

The criteria involved for deciding whether or not to subsequently re-analyze **3**, or to immediately (stat) re-analyze **4** with an immediate preliminary follow-up **4a**, are further described in relation to FIG. 2. The pre-determined threshold flags for each particular metabolite are compared to the initial concentrations of the metabolites **20** after being scanned. The threshold flag settings have been developed based upon many factors such as published reports, the clinical screening of individuals who are already sick, autopsy reports, and experience through repetition of genetic data analysis. If the initial concentration of the metabolite of interest is greater than the flag threshold **21**, then a determination is made as to how significant the elevation is **23**. This is determined by comparing each flag threshold to the initial concentration of the metabolite and identifying whether or not the elevation (or deficiency) exceeds the flag threshold by reference to criteria for each metabolite. If this were to occur, an immediate stat re-analysis **4** and preliminary follow-up **4a** would be performed. This is achieved by prioritizing the sample to allow for an immediate preliminary follow-up and re-running the sample from the same filter card to acquire a set of prioritized values. These values are then averaged as a mean with the first values to form priority mean values because they are more significant for implementation into the follow-up protocol.

If the initial concentration of the metabolite is not greater than the flag threshold **21**, the molar ratio concentrations are identified and compared to their respective concentration

flag thresholds and molar ratio thresholds **22**. The molar ratios are important because they account for variability of the blood spot on the filter card. Because the sample is originally dry, the variability of the thickness, number of cells, and change in volume must be accounted for. Thus, as the concentration of the metabolite may go up or down, the ratio of two analytes in one sample is a more sensitive indicator because they both change relative to one another.

If the molar ratio concentrations are not greater than the concentration flag thresholds and molar ratio thresholds **22**, then the sample may be labeled as normal. However, if the molar ratio concentrations are found to be greater **22** by reference to the criteria particular for that metabolite, the elevation significance is then compared as above **23**. Thus, if either the initial concentration of the metabolite or the molar ratios are significantly elevated by reference to the criteria, an immediate stat re-analysis **4** with preliminary follow-up **4a** is performed. And in both cases, if the elevation is present by comparison to the flag threshold **21** but not significant **23**, a sub-sequent re-analysis **3** is performed. This is achieved by acquiring a new set of data to obtain second values from the sample, by re-testing the sample and averaging the results to form mean values of the concentrations, which can then be implemented into the follow-up protocol, as follows.

A follow-up protocol is then initiated **7** following any re-analysis for determining any possible interpretation for diagnosis.

FIG. 3 lays out the criteria for broadly initiating a follow-up **7**. Any mean values or priority mean values are identified from the subsequent re-analysis **3** or the immediate re-analysis **4**, respectively. These values that are elevated to some degree (dependent on decision matrix) above the flag threshold are identified **30**. This follow-up **7** may also follow any re-analysis. The level of elevation (or deficiency, if required) is interpreted **31** as being mild **32**, moderate **33**, or significant **34** based on the criteria in the interpretation guide for

each metabolite, as further described. If the elevation is considered mild **32** based on the difference from the threshold, a routine repeat **35** is performed. This involves performing a less prioritized repeat of the testing without an alert to a parent or physician.

If the elevation is considered moderate **33** based on the criteria, an urgent repeat **36** is performed, which is different from a routine repeat because the elevation is reported to a physician. Accordingly, the physician can suggest to see the baby and possibly get another sample or confirm the results.

If the elevation is considered significant **34** based on the criteria, an urgent repeat plus additional testing to obtain a third set of values, such as for propionic acidemia, or other organic acidemias, is performed along with a referral to a specialist particular to a disorder to ascertain an expertise for clinical evaluation.

The present method can be further understood by referencing the tables and criteria in figures 4 -10. Each figure represents the decision tree presented for each particular metabolite, which is necessary because each metabolite ionizes a butyl ester fragment at a different mass to charge (m/z) value, and each metabolite concentration (in units micromolarity [μM]) must be compared to a different threshold flag. The butyl ester fragment is directly proportional to the concentration of the metabolite. The preferred values of the threshold flags, or automated interpretation flag settings, are seen in tabulated form for each metabolite.

FIG. 4 is used in the method for assisting in the diagnosis of propionic acidemia after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **40** for all concentrations and molar ratios used for determining the level of elevation of propionyl carnitine. The flag thresholds **40** are preferably set at about 5.0 μM for the propionyl carnitine (C3) concentration, about 0.3 for the mrm scan of propionyl carnitine with acetyl

carnitine (C3mm/C2), and about 1.75 for the molar ratio of the propionyl carnitine with palmitoyl carnitine (C3/C16).

The criteria for re-analysis **41** and criteria for an immediate, or STAT re-analysis **42** in relation to the flag thresholds **40** are also shown. A subsequent re-analysis to obtain mean values is performed provided any of the following occurs:

- i. C3 is equal to or greater than the C3 flag threshold;
- ii. C3/C16 is greater than or equal to the C3/C16 flag threshold and C3/C2 is greater than the C3/C2 flag threshold and C3 is greater than about 2.5 uM;
- iii. C3 is greater than about 4 uM, and either said C3/C16 is greater than said C3/C16 flag threshold or said C3/C2 is greater than said C3/C2 flag threshold.

An immediate, or STAT re-analysis with a preliminary follow-up to obtain priority mean values is performed provided any of the following occurs:

- i. C3 is greater than about 9.0 uM;
- ii. C3 is greater than about 7.0 uM and the C3/C2 is greater than the C3/C2 flag threshold, or the C3/C16 is greater than the C3/C16 flag threshold.

The procedure is repeated to get mean values for implementation into a specific follow-up protocol **43**, which ultimately leads to the diagnostic assistance for propionic acidemia.

FIG. 5 is used in the method for assisting in the diagnosis of isovaleric acidemia after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag threshold **50** for the concentration used for determining the level of elevation of isovaleryl carnitine. The flag threshold **50** for isovaleryl carnitine is preferably set at about 0.8 uM.

Criteria for re-analysis **51** and criteria for an immediate, or STAT re-analysis **52** in relation to the flag threshold **50** for isovaleryl carnitine (C5) are also shown. A subsequent re-

analysis **51** to obtain a mean value of C5 is performed provided the isovaleryl carnitine concentration (C5) is greater than or equal to the C5 flag threshold **50**.

An immediate re-analysis **52** with a preliminary follow-up to obtain a priority mean value of an isovaleryl carnitine concentration (C5) is performed provided any of the

5 following occurs:

- i. the C5 is greater than about 2.0 uM;
- ii. the C5 is greater than about 1.0 uM and the propionyl carnitine concentration (C3) [FIG. 4] is greater than about 2.5 uM.

The procedure is repeated to get mean values for implementation into a specific
10 follow-up protocol **53**, which ultimately leads to the diagnostic assistance for isovaleric acidemia.

FIG. 6 is used in the method for assisting in the diagnosis of hypermethionemia after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer.

The method as previously discussed is applied using the flag thresholds **60** for all
15 concentrations and molar ratios used for determining the level of elevation of methionine. The flag thresholds **60** are preferably set at 60 uM for the methionine concentration (met) and 1 for the molar ratio of methionine and phenylalanine (met/phe).

The criteria for re-analysis **61** and criteria for an immediate, or STAT re-analysis **62** in relation to the flag thresholds are also shown. A subsequent re-analysis to obtain mean
20 values is performed provided any of the following occur:

- i. met is greater than the met flag threshold;
- ii. met is greater than about 50 uM and the met/phe is greater than the met/phe flag threshold.

An immediate re-analysis with a preliminary follow-up to obtain priority mean values
25 is performed provided any of the following occur:

- i. met is greater than about 150 uM;
- ii. met is greater than about 125 uM and the met/phe is greater than about 1.25.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **63**, which ultimately leads to the diagnostic assistance for hypermethionemia.

FIG. 7 is used in the method for assisting in the diagnosis of glutaric acidemia after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **70** for all concentrations and molar ratios used for determining the level of elevation of glutaryl carnitine. The flag thresholds **70** are preferably set at 0.17 uM for the glutaryl carnitine concentration (C5DC) and 0.12 for the molar ratio of glutaryl carnitine with palmitoyl carnitine (C5DC:C16).

Criteria for re-analysis **71** and criteria for an immediate, or STAT re-analysis **72** in relation to the flag thresholds are also shown. A subsequent re-analysis **71** to obtain mean values is performed provided any of the following occur:

- i. C5DC is greater than the C5DC flag threshold;
- ii. C5DC:C16 is greater than the C5DC:C16 flag threshold, and the C5DC is greater than about 0.14 uM.

An immediate re-analysis **72** with a preliminary follow-up to obtain priority mean values is performed provided any of the following occur:

- i. C5DC is greater than about 0.4 uM;
- ii. C5DC is greater than about 0.2 uM, and the C5DC:C16 is greater than about 0.2 uM.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **73**, which ultimately leads to the diagnostic assistance for glutaric acidemia.

FIG. 8 is used in the method for assisting in the diagnosis of phenylketonuria (PKU) after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **80** for all concentrations and molar ratios used for determining the level of elevation of phenylalanine (phe) or tyrosine (tyr). The flag thresholds **80** are preferably set at 130 uM for the phenylalanine concentration (phe), 350 uM for the tyrosine concentration (tyr), and 2.5 for the molar ratio of phenylalanine with tyrosine (phe/tyr).

The criteria for re-analysis **81** and criteria for an immediate, or STAT re-analysis **82** in relation to the flag thresholds are also shown. A subsequent re-analysis **81** to obtain mean values is performed provided any of the following occur:

- i. phe is greater than the phe flag threshold;
- ii. tyr is greater than the tyr flag threshold;
- iii. phe/tyr is greater than the phe/tyr flag threshold and the phe is greater than about 100 uM.

An immediate re-analysis **82** with a preliminary follow-up to obtain priority mean values is performed provided any of the following occur:

- i. phe is greater than about 240 uM;
- ii. phe is greater than about 180 uM and the phe/tyr is greater than the phe/tyr flag threshold.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **83**, which ultimately leads to the diagnostic assistance for PKU.

FIG. 9 is used in the method for assisting in the diagnosis of Maple Syrup Urine Disease (MSUD) after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **90** for all concentrations and molar ratios used for determining the level of elevation of leucine. The flag thresholds are preferably set at 325 uM for the concentration of a combination of leucine and isoleucine (leu+ile); 300 uM for a concentration of valine (val); 8.0 for the molar ratio of leucine with phenylalanine (leu/phe); and 2.25 for the molar ratio of leucine with alanine (leu/ala).

The criteria for re-analysis **91** and criteria for an immediate, or STAT re-analysis **92** in relation to the flag thresholds are also shown. A subsequent re-analysis **91** to obtain mean values is performed provided any of the following occur:

- i. leu+ile is greater than about 400 uM;
- ii. leu+ile is greater than about 350 uM and val is greater than the val flag threshold;
- iii. leu+ile is greater than the leu+ile flag threshold and the leu/ala is greater than the leu/ala flag threshold, or the leu/phe is greater than the leu/phe flag threshold and the val is greater than the val flag threshold;

An immediate re-analysis **92** with a preliminary follow-up to obtain priority mean values is performed provided any of the following occur:

- i. leu+ile is greater than about 500 uM;
- ii. leu+ile is greater than about 400 uM and the leu/phe is greater than the leu/phe flag threshold and the leu/ala is greater than the leu/ala flag threshold.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **93**, which ultimately leads to the diagnostic assistance for MSUD.

FIG. 10 is used in the method for assisting in the diagnosis of citrullinemia after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag threshold **100** for the concentrations used for determining the level of elevation of citrulline. The flag threshold **100** is preferably set at 55 uM for a concentration of citrulline determined after a full scan (cit), and 55 uM for a concentration of citrulline determined after an mrm scan (cit[mrm]).

The criteria for re-analysis **101** and criteria for an immediate, or STAT re-analysis **102** in relation to the flag thresholds are also shown. A subsequent re-analysis **101** to obtain mean values is performed provided the following occurs:

- i. cit or cit(mrm) is greater than the cit flag threshold or the cit(mrm) flag threshold.

An immediate re-analysis **102** with a preliminary follow-up to obtain priority mean values is performed provided the following occurs:

- i. cit is greater than about 100 uM.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **103**, which ultimately leads to the diagnostic assistance for citrullinemia.

FIG. 11 is used in the method for assisting in the diagnosis of medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **110** for all concentrations and molar ratios used for determining the level of elevation of octanoyl carnitine. The flag thresholds **110** are preferably set at 0.35 uM for an octanoyl carnitine concentration (C8); 0.28 for a molar ratio of octanoyl carnitine with palmitoyl carnitine; 0.16 uM for a hexanoyl carnitine concentration (C6); 0.32 uM for a decenoyl carnitine concentration (C10:1); and 0.42 uM for a decanoyl carnitine concentration (C10).

The criteria for re-analysis **111** and criteria for an immediate, or STAT re-analysis **112** in relation to the flag thresholds **110** are also shown. A subsequent re-analysis **111** to obtain mean values is performed provided any of the following occur:

- i. C8 is greater than or equal to about 0.4 uM;
- 5 ii. C8 is greater than about 0.3 uM and the C8/C16 is greater than about 0.15;
- iii. C8 is greater than about 0.3 uM and the C6 is greater than about 0.2 uM, or the C10:1 is greater than about 0.2 uM and the C10 is greater than about 0.3 uM with a low acetyl flag;

An immediate re-analysis **112** with a preliminary follow-up to obtain priority mean
10 values is performed provided any of the following occur:

- i. C8 is greater than about 1.0 uM;
- ii. C8 is greater than about 0.5 uM and the C8/C16 is greater than about 0.35, or the C6 is greater than about 0.3 uM and the C10:1 is greater than about 0.3 uM.

The procedure is repeated to get mean values as previously discussed for
15 implementation into a specific follow-up protocol **113**, which ultimately leads to the diagnostic assistance for MCAD deficiency.

FIG. 12 is used in the method for assisting in the diagnosis of very long chain acylCoA dehydrogenase (VLCAD) deficiency after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously
20 discussed is applied using the flag thresholds **120** for all concentrations and molar ratios used for determining the level of elevation of the myristoylcarnitines. The flag thresholds **120** are preferably set at 0.85 uM for a saturated myristoyl carnitine concentration (C14); 0.70 for an unsaturated myristoyl carnitine concentration (C14:1); and 0.24 for a molar ratio of the unsaturated (C14:1) with palmitoyl carnitine.

The criteria for re-analysis **121** and criteria for an immediate, or STAT re-analysis **122** in relation to the flag thresholds are also shown. A subsequent re-analysis **121** to obtain mean values is performed provided any of the following occur:

i. C14 is greater than the C14 flag threshold, or said C14:1 is greater than the C14:1
5 flag threshold;

ii. C14 is greater than about 0.75 uM, or the C14:1 is greater than about 0.65 uM and the C14:1/C16 is greater than about 0.24.

An immediate re-analysis **122** with a preliminary follow-up to obtain priority mean values is performed provided any of the following occur:

i. C14 is greater than about 2.0 uM, or the C14:1 is greater than about 1.5 uM;

ii. C14 is greater than about 1.5 uM, and the C14:1 is greater than about 1.0 uM, and the C14:1/C16 is greater than about 0.3.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **123**, which ultimately leads to the
15 diagnostic assistance for VLCAD deficiency.

FIG. 13 is used in the method for assisting in the diagnosis of crotonyl co-A carboxylase deficiency after a dry blood spot on filter paper is derivatized and scanned using a tandem mass spectrometer. The method as previously discussed is applied using the flag thresholds **130** for all concentrations used for determining the level of elevation of the
20 hydroxy C5. The flag thresholds 130 are preferably set at 0.85 uM for a hydroxy-C5 concentration (C5OH), and 0.35 for a C5:1 concentration.

The criteria for re-analysis **131** and criteria for an immediate, or STAT re-analysis **132** in relation to the flag thresholds are also shown. A subsequent re-analysis **131** to obtain mean values is performed provided any of the following occur:

i. C5OH is greater than or equal to the C5OH flag threshold;

ii. C5:1 is greater than or equal to the C5:1 flag threshold;

An immediate re-analysis **132** with a preliminary follow-up to obtain priority mean values is performed provided any of the following occur:

i. C5OH is greater than about 3.0 uM;

5 ii. C5:1 is greater than about 1.0 uM.

The procedure is repeated to get mean values as previously discussed for implementation into a specific follow-up protocol **133**, which ultimately leads to the diagnostic assistance for crotonyl coA carboxylase deficiency.

C5OH represents a hydroxy-C5, which is an abbreviated form for
10 hydroxyisovalerylcarnitine and/or hydroxylmethylbutyrcarnitine. The C5:1 is the unsaturated form known as tiglylcarnitine.

In conclusion, according to each decision tree, or interpretation guide particular for each metabolite, the present method allows the data that is acquired to be quantified and reported to a physician to assist in a diagnosis of a genetic disease resulting from the
15 deviation (elevation or deficiency) of a blood metabolite. The method allows one to provide information to a physician that additional tests and/or clinical assessments (check up, examination, etc) are necessary because of the resulting elevation or deficiency of the metabolite, which then results in the confirmed final diagnosis. The values of the flag thresholds for a particular concentration and the molar ratio flag thresholds must be
20 quantified for a consistent and accurate interpretation of acylcarnitine and amino acid data.

TERMS

It should be understood that “about” as used in relation to Figs 4 – 13 means $\pm 15\%$ of the value shown in the criteria for each figure, which values are dependent upon the flag threshold. Any change in the value of the flag threshold, which might result from filter paper
25 characteristics, new insights into disease characteristics, new diseases found, methods of

sample collection, changes in methodology, or corrections from quality assurance programs, would necessitate the relative change of each value in the criteria for all follow-ups. Thus, all values shown in the drawings are the preferred values, which might fluctuate then by no more than $\pm 15\%$.

5 INDUSTRIAL APPLICABILITY:

The present method quantifies measurements for detecting inborn errors of metabolism usually resulting from defective enzymes or cofactors. Genetic disorders can ultimately be diagnosed by the metabolic profiling of the amino acids and acylcarnitines taken from the blood spots. Newborn babies born with certain metabolic disorders can die if
10 not treated within days. Thus, after obtaining MS/MS (tandem mass spectrometer) data from blood samples from newborns, there is a need for efficiently interpreting this data in relation to a variety of pre-determined metabolite concentration thresholds. This interpretation allows for proper decision-making necessary for the diagnosing and follow-up testing of newborns over a wide range of genetic disorders.

15

20

25

CLAIMS IN THE INVENTION:

I claim:

1. A method for interpreting data produced after a plurality of blood metabolites in a
5 plurality of blood samples are derivatized from dry blood spots on filter paper and
scanned by a tandem mass spectrometer for clinically diagnosing newborns, comprising
the steps of:

(a) acquiring said data, wherein said data includes values comprised of a plurality of
metabolite concentrations and a plurality of molar ratio concentrations;

10 (b) applying said values to a plurality of decision trees, wherein each of said decision
trees are referenced for a particular metabolite, and wherein each of said decision trees further
comprise a plurality of concentration flag thresholds and a plurality of molar ratio flag
thresholds as diagnostic limits to said metabolite concentrations and said molar ratio
concentrations respectively for each said metabolite;

15 (c) comparing said values with said concentration flag thresholds and molar ratio flag
thresholds;

(d) identifying whether or not there is a deviation of said values from said
concentration flag thresholds or said molar ratio flag thresholds;

(e) interpreting said sample as being normal for said metabolite, provided there is no
20 said deviation of any of said values from said concentration flag thresholds and said molar
ratio flag thresholds; and,

(f) interpreting said sample as being evident of said deviation, provided any of said
values deviate from said concentration flag thresholds or said molar ratio flag thresholds.

2. The method of claim 1, further comprising:

identifying whether or not said deviation is significant as opposed to slight, based on re-analysis criteria, wherein said re-analysis criteria are specific for each of said metabolites; performing a subsequent re-analysis, provided said deviation is slight, said subsequent re-analysis, further comprising the steps of:

- 5 repeating step (a);
- taking a mean of said values to form mean values;
- repeating steps (b) through (c) utilizing said mean values; and,
- implementing said mean values into a follow-up protocol.

3. The method of claim 1, further comprising:

- 10 performing an immediate re-analysis, provided said deviation is significant; said immediate re-analysis, further comprising the steps of:
 - prioritizing said sample to allow for an immediate preliminary follow-up;
 - repeating step (a);
 - taking a mean of said values to form priority mean values;
 - 15 repeating steps (b) through (c) utilizing said priority mean values; and,
 - implementing said priority mean values immediately into said follow-up protocol.

4. The method of claim 3, further comprising:

- initiating said follow-up protocol for clinically evaluating said newborn, further
- 20 comprising the steps of:
 - interpreting a level of said deviation as mild, moderate, or significant for diagnostic purposes based on follow-up criteria, wherein said follow-up criteria is specific for each of said metabolites and labeled with a code for said follow-up protocol;
 - repeating step (a) to form third values on a routine basis, provided said level is mild
 - 25 based on said follow-up criteria;

repeating step (a) to form third values on an urgent basis with a report to a physician, provided said level is moderate based on said follow-up criteria;

repeating step (a) to form third values on an urgent basis with additional test and an alert and immediate referral to a specialist, provided said level is significant based on said follow-up criteria; and,

reporting said data to a physician to assist in a diagnosis of a genetic disorder resulting from said deviation.

5. The method of Claim 1, wherein each of said metabolites are selected from the group comprised of propionyl carnitine, isovaleryl carnitine, octanoyl carnitine, glutaryl carnitine, myristoyl carnitine, hydroxy-C5, leucine, methionine, phenylalanine, and citrulline.

6. The method of Claim 4, wherein said genetic disorders include: propionic acidemia, MCAD deficiency, citrullinemia, MSUD, PKU, VLCAD deficiency, hypermethionemia, isovaleric acidemia, glutaric acidemia, and crotonyl-CoA carboxylase elevation.

7. A method for assisting in a diagnosis of propionic acidemia by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a propionyl carnitine concentration (C3), a molar ratio of an mrm scan of propionyl carnitine and acetyl carnitine (C3mrm/C2), and a molar ratio of propionyl carnitine and palmitoyl carnitine (C3/C16);

applying said values to a propionyl carnitine (C3) decision tree, wherein said decision tree further comprises a C3 flag threshold, a C3mrm/C2 flag threshold, and a C3/C16 flag threshold as diagnostic limits to said C3, said C3mrm/C2, and said C3/C16 respectively;

comparing said values, respectively, with each of said flag thresholds;

identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said propionyl carnitine, provided there is no said elevation of any of said values;

5 performing a subsequent re-analysis to obtain mean values, provided any of the following occurs:

i. said C3 is equal to or greater than said C3 flag threshold;

ii. said C3/C16 is greater than or equal to said C3/C16 flag threshold and said C3/C2 is greater than said C3/C2 flag threshold and said C3 is greater than about 2.5 uM;

10 iii. said C3 is greater than about 4 uM, and either said C3/C16 is greater than said C3/C16 flag threshold or said C3/C2 is greater than said C3/C2 flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occurs:

i. said C3 is greater than about 9.0 uM;

15 ii. said C3 is greater than about 7.0 uM and said C3/C2 is greater than said C3/C2 flag threshold or said C3/C16 is greater than said C3/C16 flag threshold; and,

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed;

20 reporting said data to a physician to assist in said diagnosis of propionic acidemia.

8. The method of Claim 7, wherein said C3 flag threshold, said C3mrm/C2 flag threshold, and said C3/C16 flag threshold are, respectively, 5.0 uM, 0.3 uM, and 1.75.

9. A method for assisting in a diagnosis of isovaleric acidemia by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass

25 spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of an isovaleryl carnitine concentration (C5) and a propionyl carnitine concentration (C3);

applying said isovaleryl carnitine concentration (C5) to an isovaleryl carnitine (C5) decision tree, wherein said decision tree further comprises a C5 flag threshold as

5 diagnostic limit to said isovaleryl carnitine concentration (C5);

comparing said C5 to said flag threshold;

identifying whether or not there is an elevation of C5 above said flag threshold;

interpreting said sample as being normal for said isovaleryl carnitine, provided there is no said elevation of said C5;

10 performing a subsequent re-analysis to obtain a mean value of said C5, provided said C5 is greater than or equal to said C5 flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain a priority mean value of said C5, provided any of the following occurs:

i. said C5 is greater than about 2.0 uM;

15 ii. said C5 is greater than about 1.0 uM and said C3 is greater than about 2.5 uM;

initiating a follow-up protocol based on follow up criteria utilizing said mean value or said priority mean value for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of isovaleric acidemia.

20 10. The method of Claim 9, wherein said C5 flag threshold is 0.8 uM.

11. A method for assisting in a diagnosis of hypermethionemia by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values consisting essentially of a
25 methionine concentration (met) and a molar ratio of methionine and phenylalanine (met/phe);

applying said values to a methionine (met) decision tree, wherein said decision tree further comprises a methionine flag threshold and a met/phe flag threshold as a diagnostic limit to said methionine concentration (met);

comparing said values, respectively, to each said flag threshold;

5 identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said hypermethionemia, provided there is no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the
10 following occur:

i. said met is greater than said met flag threshold;

ii. said met is greater than about 50 uM and said met/phe is greater than said met/phe flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain priority
15 mean values, provided any of the following occur:

i. said met is greater than about 150 uM;

ii. said met is greater than about 125 uM and said met/phe is greater than about 1.25;

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-
20 analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of hypermethionemia.

12. The method of Claim 11, wherein said methionine flag threshold and said met/phe flag threshold are, respectively, 60 uM and 1.0.

13. A method for assisting in a diagnosis of glutaric acidemia by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a glutaryl
5 carnitine concentration (C5DC) and a molar ratio of glutaryl carnitine and palmitoyl carnitine (C5DC:C16);

applying said values to a glutaryl carnitine (C5DC) decision tree, wherein said decision tree further comprises a C5DC flag threshold and a C5DC:C16 flag threshold as diagnostic limits to said glutaryl carnitine concentration (C5DC) and said C5DC:C16

10 respectively;

comparing said values, respectively, to each of said flag thresholds;

identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said glutaric acidemia, provided there is

15 no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the following occur:

i. said C5DC is greater than said C5DC flag threshold;

ii. said C5DC:C16 is greater than said C5DC:C16 flag threshold and said C5DC is

20 greater than about 0.14 uM;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

i. said C5DC is greater than about 0.4 uM;

ii. said C5DC is greater than about 0.2 uM and said C5DC:C16 is greater than about

25 0.2 uM;

initiating a follow-up protocol based on follow up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of glutaric acidemia.

5 14. The method of Claim 13, wherein said C5DC flag threshold and said C5DC:C16 flag threshold are, respectively, 0.17 uM and 0.12.

15. A method for assisting in a diagnosis of phenylketonuria by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

10 acquiring said data, wherein said data includes values consisting essentially of a phenylalanine concentration (phe), a tyrosine concentration (tyr), and a molar ratio of phenylalanine and tyrosine (phe/tyr).

applying said values to a phenylalanine (phe) decision tree, wherein said decision tree further comprises a phe flag threshold, a tyr flag threshold, and a phe/tyr flag threshold as

15 diagnostic limits to said phe, said tyr, and said phe/tyr molar ratio respectively;

comparing said values, respectively, to each of said flag thresholds;

identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said phenylketonuria, provided there is

20 no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the following occur:

- i. said phe is greater than said phe flag threshold;
- ii. said tyr is greater than said tyr flag threshold;

iii. said phe/tyr is greater than said phe/tyr flag threshold and said phe is greater than 100 uM;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

5 i. said phe is greater than about 240 uM;

ii. said phe is greater than about 180 uM and said phe/tyr is greater than said phe/tyr flag threshold;

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-
10 analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of phenylketonuria.

16. The method of Claim 15, wherein said phe flag threshold, said tyr flag threshold, and said phe/tyr flag threshold are, respectively, 130 uM, 350 uM, and 2.5.

17. A method for assisting in a diagnosis of Maple Syrup Urine Disease (MSUD) by
15 interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a leucine+isoleucine concentration (leu+ile), a valine concentration (val), a molar ratio of leucine and phenylalanine (leu/phe), and a molar ratio of leucine and alanine (leu/ala).

20 applying said values to a leucine (leu) decision tree, wherein said decision tree further comprises a leu+ile flag threshold, a val flag threshold, a leu/phe molar ratio flag threshold, and a leu/ala molar ratio flag threshold as diagnostic limits to said leu+ile, said val, said leu/phe, and said leu/ala respectively;

comparing said values, respectively, to each of said flag thresholds;

identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said MSUD, provided there is no said elevation of any of said values;

5 performing a subsequent re-analysis to obtain mean values, provided any of the following occur:

i. said leu+ile is greater than about 400 uM;

ii. said leu+ile is greater than about 350 uM and said val is greater than said val flag threshold;

10 iii. said leu+ile is greater than said leu+ile flag threshold and said leu/ala is greater than said leu/ala flag threshold, or said leu/phe is greater than said leu/phe flag threshold and said val is greater than said val flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

15 i. said leu+ile is greater than about 500 uM;

ii. said leu+ile is greater than about 400 uM and said leu/phe is greater than said leu/phe flag threshold and said leu/ala is greater than said leu/ala flag threshold;

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

20

reporting said data to a physician to assist in said diagnosis of MSUD.

18. The method Claim 17, wherein said leu+ile flag threshold, said val flag threshold, said leu/phe flag threshold, and said leu/ala flag threshold are, respectively, 325 uM, 300 uM, 8.0, and 2.25.

19. A method for assisting in a diagnosis of citrullinemia by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a citrulline
5 concentration (cit) and a citrulline concentration after an mrm scan (cit[mrm]);

applying said values to a citrulline (cit) decision tree, wherein said decision tree further comprises a cit flag threshold and a cit(mrm) flag threshold as diagnostic limits to said citrulline concentration (cit) and said cit(mrm) concentration respectively;

comparing said values, respectively, to each of said flag thresholds;

10 identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said citrullinemia, provided there is no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the
15 following occur:

i. said cit or said cit(mrm) is greater than said cit flag threshold or said cit(mrm) flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

20 i. said cit is greater than about 100 uM;

initiating a follow-up protocol based on follow up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of citrullinemia.

20. The method of Claim 19, wherein said cit flag threshold and said cit(mrm) flag threshold are both 55 uM.

21. A method for assisting in a diagnosis of medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency by interpreting data produced after a dry blood spot on filter paper is

5 derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of an octanoyl carnitine concentration (C8), a molar ratio of octanoyl carnitine and palmitoyl carnitine (C8/C16), a hexanoyl carnitine concentration (C6), a decenoyl carnitine concentration (C10:1), and a decanoyl carnitine concentration (C10);

10 applying said values to an octanoyl carnitine (C8) decision tree, wherein said decision tree further comprises a C8 flag threshold, a C8/C16 molar ratio flag threshold, a C6 flag threshold, a C10:1 flag threshold, and a C10 flag threshold as diagnostic limits to said C8, said C8/C16, said C6, said C10:1, and said C10 respectively;

comparing said values, respectively, to each of said flag thresholds;

15 identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said MCAD deficiency, provided there is no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the

20 following occur:

- i. said C8 is greater than or equal to about 0.4 uM;
- ii. said C8 is greater than about 0.3 uM and said C8/C16 is greater than about 0.15;
- iii. said C8 is greater than about 0.3 uM and said C6 is greater than about 0.2 uM, or
said C10:1 is greater than about 0.2 uM and said C10 is greater than about 0.3
25 uM with a low acetyl flag;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

- i. said C8 is greater than about 1.0 uM;
- ii. said C8 is greater than about 0.5 uM and said C8/C16 is greater than about 0.35, or
5 said C6 is greater than about 0.3 uM and said C10:1 is greater than about 0.3 uM;

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of MCAD deficiency.

10 22. The method of Claim 21, wherein said C8 flag threshold, said C8/C16 flag threshold, said C6 flag threshold, said C10:1 flag threshold, and said C10 flag threshold are, respectively, 0.35 uM, 0.28, 0.16 uM, 0.32 uM, and 0.42 uM.

23. A method for assisting in a diagnosis of VLCAD deficiency by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass
15 spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a saturated myristoyl carnitine concentration (C14), an unsaturated myristoyl carnitine concentration (C14:1), and a molar ratio of C14:1 and palmitoyl carnitine (C16);

20 applying said values to a myristoyl carnitine (C14) decision tree, wherein said decision tree further comprises a C14 flag threshold, a C14:1 flag threshold, and a C14:1/C16 flag threshold as diagnostic limits to said C14, said C14:1, and said C14:1/C16 respectively;

comparing said values, respectively, to each of said flag thresholds;

identifying whether or not there is an elevation of any of said values above any of said
25 flag thresholds;

interpreting said sample as being normal for said MSUD, provided there is no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the following occur:

- 5 i. said C14 is greater than said C14 flag threshold or said C14:1 is greater than said C14:1 flag threshold;

said C14 is greater than about 0.75 uM or said C14:1 is greater than about 0.65 uM and said C14:1/C16 is greater than about 0.24;

- performing an immediate re-analysis with a preliminary follow-up to obtain priority
10 mean values, provided any of the following occur:

- i. said C14 is greater than about 2.0 uM or said C14:1 is greater than about 1.5 uM;
ii. said C14 is greater than about 1.5 uM and said C14:1 is greater than about 1.0 uM
and said C14:1/C16 is greater than about 0.3;

- initiating a follow-up protocol based on follow-up criteria utilizing said mean values
15 or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

reporting said data to a physician to assist in said diagnosis of VLCAD deficiency.

24. The method of Claim 23, wherein said C14 flag threshold, said C14:1 flag threshold, and said C14:1/C16 flag threshold are, respectively, 0.85 uM, 0.70 uM, and 0.24.

- 20 25. A method for assisting in a diagnosis of a Crotonyl co-A carboxylase deficiency elevation by interpreting data produced after a dry blood spot on filter paper is derivatized and scanned by a tandem mass spectrometer, comprising the steps of:

acquiring said data, wherein said data includes values comprised of a C5-OH and C5:1;

applying said values to a hydroxy-C5 (C5-OH) decision tree, wherein said decision tree further comprises a C5OH flag threshold and a C5:1 flag threshold as diagnostic limits to said C5OH and said C5:1 respectively;

comparing said values, respectively, to each of said flag thresholds;

5 identifying whether or not there is an elevation of any of said values above any of said flag thresholds;

interpreting said sample as being normal for said crotonyl co-A carboxylase deficiency, provided there is no said elevation of any of said values;

performing a subsequent re-analysis to obtain mean values, provided any of the
10 following occur:

said C5OH is greater than or equal to said C5OH flag threshold;

said C5:1 is greater than or equal to said C5:1 flag threshold;

performing an immediate re-analysis with a preliminary follow-up to obtain priority mean values, provided any of the following occur:

15 i. said C5OH is greater than about 3.0 uM;

ii. said C5:1 is greater than about 1.0 uM;

initiating a follow-up protocol based on follow-up criteria utilizing said mean values or said priority mean values for diagnostic purposes provided either said subsequent re-analysis or said immediate re-analysis is performed; and,

20 reporting said data to a physician to assist in said diagnosis of crotonyl co-A carboxylase deficiency.

26. The method of Claim 25, wherein said C5OH flag threshold and said C5:1 flag threshold are, respectively, 0.85 uM and 0.35 uM.