



Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Ms. Cindy Lloyd, RAC
Manager, Regulatory Affairs
PerkinElmer Life and Analytical Sciences
3985 Eastern Road
Norton, OH 44203

AUG 24 2004

Re: k031878
Evaluation of Automatic Class III Designation
NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970
Regulation Number: 21 CFR 862.1055
Classification: Class II
Product Code: NQL

Dear Ms. Lloyd:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your petition for classification of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970 that is intended for use in the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism. FDA concludes that this device, and substantially equivalent devices of this generic type, should be classified into class II. This order, therefore, classifies the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970, and substantially equivalent devices of this generic type into class II under the generic name, Newborn Screening Test System for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry. This order also identifies the special controls applicable to this device.

FDA identifies this generic type of device as:

21 CFR 862.1055 - Newborn Screening Test System for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry

A newborn screening test system for amino acids, free carnitine and acylcarnitines by tandem mass spectrometry is a device that consists of stable isotope internal standards, control materials, extraction solutions, flow solvents, instrumentation, software packages, and other reagents and materials. The quantitative analysis of amino acids, free carnitines and acylcarnitines and their relationship with each other is intended to provide analyte concentration profiles that may aid in screening newborns for one or more inborn errors of amino acid, free carnitine, and acylcarnitine metabolism.

2004N-0482

BKG-1

In accordance with section 513(f)(1) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360c(f)(1)) (the act), devices that were not in commercial distribution prior to May 28, 1976 (the date of enactment of the Medical Device Amendments of 1976 (the amendments)), generally referred to as postamendments devices, are classified automatically by statute into class III without any FDA rulemaking process. These devices remain in class III and require premarket approval, unless and until the device is classified or reclassified into class I or II or FDA issues an order finding the device to be substantially equivalent, in accordance with section 513(i) of the act (21 U.S.C. 360c(i)), to a predicate device that does not require premarket approval. The agency determines whether new devices are substantially equivalent to previously marketed devices by means of premarket notification procedures in section 510(k) of the act (21 U.S.C. 360(k)) and Part 807 of the FDA regulations (21 CFR 807).

Section 513(f)(2) of the act provides that any person who submits a premarket notification under section 510(k) for a device may, within 30 days after receiving an order classifying the device in class III under section 513(f)(1), request FDA to classify the device under the criteria set forth in section 513(a)(1). FDA shall, within 60 days of receiving such a request classify the device. This classification shall be the initial classification of the device type. Within 30 days after the issuance of an order classifying the device, FDA must publish a notice in the Federal Register classifying the device type.

On July 02, 2004, FDA filed your petition requesting classification of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970 into class II. The petition was submitted under section 513(f)(2) of the act. In accordance with section 513(f)(1) of the act, FDA issued an order on June 09, 2004, automatically classifying the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit Model MS-8970 in class III, because it was not within a type of device which was introduced or delivered for introduction into interstate commerce for commercial distribution before May 28, 1976, which was subsequently reclassified into class I or class II. In order to classify the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970 into class I or II, it is necessary that the proposed class have sufficient regulatory controls to provide reasonable assurance of the safety and effectiveness of the device for its intended use.

After review of the information submitted in the petition, FDA has determined that the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970, intended for use in the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper, to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism can be classified in class II with the establishment of special controls. FDA believes that class II special controls provide reasonable assurance of the safety and effectiveness of the device.

FDA has identified no direct risks to health related to use of newborn screening test systems for amino acids, free carnitine and acylcarnitines by tandem mass spectrometry. However, failure of the test to perform as indicated or error in interpretation of results may lead to improper medical management of patients with inborn errors of metabolism. A falsely low (e.g. false negative / false normal) measurement could contribute to failure to detect a possible inborn error of metabolism, which could lead to functional impairment or death. A falsely high (e.g. false positive / false abnormal) measurement could contribute to unnecessary additional patient testing and added concern and apprehension of parents and physicians. The measures FDA recommends to mitigate these risks are described in the guidance document, "Class II Special Controls Guidance Document: Newborn Screening Test Systems for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry", which includes recommendations for performance validation and labeling.

In addition to the general controls of the act, Newborn Screening Test Systems for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry are subject to the following special controls: "Class II Special Controls Guidance Document: Newborn Screening Test Systems for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry". Section 510(m) of the act provides that FDA may exempt a class II device from the premarket notification requirements under section 510(k) of the act, if FDA determines that premarket notification is not necessary to provide reasonable assurance of the safety and effectiveness of the device. FDA has determined premarket notification is necessary to provide reasonable assurance of the safety and effectiveness of the device and, therefore, the device is not exempt from the premarket notification requirements. Thus, persons who intend to market this device must submit to FDA a premarket notification submission containing information on the newborn screening test system for amino acids, free carnitine and acylcarnitines by tandem mass spectrometry they intend to market prior to marketing the device.

A notice announcing this classification order will be published in the Federal Register. A copy of this order and supporting documentation are on file in the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852 and are available for inspection between 9 a.m. and 4 p.m., Monday through Friday.

As a result of this order, you may immediately market this device, subject to the general control provisions of the act and the special controls identified in this order.

If you have any questions concerning this classification order, please contact Carol C. Benson at (301) 594-1243.

Sincerely yours,

Steven Gutman, M.D.

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

AUG 24 2004

Ms. Cindy Lloyd, RAC
Manager, Regulatory Affairs
PerkinElmer Life and Analytical Sciences
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As a result of this order, you may immediately market this device, subject to the general control provisions of the act and the special controls identified in this order.

If you have any questions concerning this classification order, please contact Carol C. Benson at (301) 594-1243.

Sincerely yours,

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Page 4 – Ms. Cindy Lloyd

Prepared by: Carol Benson :OIVD/DCTD:HFZ-440:8/5/04
 Revised by Heather Rosecrans: 8-6-04
 Reviewed by Ethan Hausman and Carol Benson 8-13-04
 Finalized: Carol Benson: 8-13-04
 T/final: JRitchwood: OIVD/DCTD: HFZ-440: 8/17/04

cc: DMC (HFZ-401)
 510(k) Staff (HFZ-404)
 OIVD (HFZ-440)
 District Office (D.O.)

FILE
 COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE
OIVD HFZ-440	Ritchwood	8/17/04	401	Rosecrans	8/24			
	Benson	8-17-04						
	Hausman	8/23/04						

Memorandum

Date: August 17, 2004
From: Carol C. Benson, MA, MT (ASCP), OIVD/DCTD/HFZ-440
To: The Record of de novo petition k031878
Device: NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry
Kit, Model MS-8970
Subject: Evaluation of Automatic Class III Designation
Applicant: PerkinElmer Life and Analytical Sciences
Phone: 9-1-330-825-4525, ext. 170; Fax: 9-1-330-825-8520

I have completed a review of this petition for classification of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, Model MS-8970. I conclude that it can be classified in class II with the establishment of special controls. The special controls guidance document, "Class II Special Controls Guidance Document: Newborn Screening Test Systems for Amino Acids, Free Carnitine and Acylcarnitines by Tandem Mass Spectrometry" includes recommendations for performance validation and labeling.

Signature: Carol Benson
Carol C. Benson

K031818/A2



PerkinElmer Life and Analytical Sciences
3985 Eastern Road
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June 30, 2004

Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Office of Device Evaluation
Food and Drug Administration
9200 Corporate Blvd.
Rockville, MD 20850

RECEIVED
2004 JUL -2 A 10: 10
FDA/CDRH/ODE/PMO

Re: 510(k) Submission: K031878, NeoGram Amino Acids and Acylcarnitines Tandem
Mass Spectrometry Kit, Model MS-8970
Holder: PerkinElmer Life and Analytical Sciences, Inc.
3985 Eastern Road
Norton, OH 44203

Attn. Ms. Carol Benson

Dear Ms. Benson:

Attached please find our Request for Evaluation of Automatic Class III Designation Section 513(f)(2) which also includes the revisions you requested to the labeling, 510(k) summary, and indications for use statement.

Additionally, we have recently modified the labeling to remove Proline and Oxoproline from our measurement claims. Due to the poor reproducibility of these amino acids in controls manufactured over three pilot lots, we have chosen not to include them in the current product submission.

Should you have any questions or need additional information, please contact me at 330-825-4525 x 170 or cindy.lloyd@perkinelmer.com. Thank you for your time and attention.

Sincerely,

Cindy Lloyd, RAC
Manager, Regulatory Affair

SK6

**PerkinElmer Life and Analytical Sciences
NeoGram Amino Acids and Acylcarnitines
Tandem Mass Spectrometry Kit**

**Request for Evaluation of Automatic
Class III Designation Section 513(f)(2)**

6/30/04

**PerkinElmer Life and Analytical Sciences
NeoGram Amino Acids and Acylcarnitines
Tandem Mass Spectrometry Kit**

**Request for Evaluation of Automatic
Class III Designation Section 513(f)(2)**

6/30/04

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510 (k) Summary Revisions Requested.....	Appendix 3
Indications for Use Statement Revisions Requested.....	Appendix 4



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June 30, 2004

Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Office of Device Evaluation
Food and Drug Administration
9200 Corporate Blvd.
Rockville, MD 20850

Re: K031878
Trade Name: NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry
Kit, Model MS-8970
Regulatory Class: II
Product Code: NQL, JIT, JJY
Date of NSE Letter: June 9, 2004

Attn. Ms. Carol Benson

Dear Ms. Benson

This document is in response to the letter from Office of Device Evaluation (ODE) dated June 9, 2004, replying to our intent to market the above referenced kit, and the determination by ODE that the product is not substantially equivalent (NSE) to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to any device which has been reclassified into class I (General Controls) or class II (Special Controls). Based on the Food and Drug Administration Modernization Act of 1997, Section 207, we are requesting that the FDA make a risk-based classification determination of this in-vitro diagnostic device.

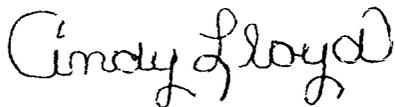
The information provided is consistent with the Guidance Document for Industry and CDRH Staff, entitled "New Section 513 (f) (2)- Evaluation of Automatic Class III Designation..." issued on February 19, 1998 [Section 207 (FDAMA); Section 513 (f) (2)]

of the FDCA; 21USC 360c (f) (2)]. This document provides guidance for the recommendations to be employed by industry when requesting this determination. The recommendations for information to be submitted should address the following items:

1. A coversheet clearly identifying the submission as "Request for Evaluation of Automatic Class III Designation under 513 (f) (2).
2. The 510(k) number under which the device was found not substantially equivalent.
3. A statement of cross reference to the information contained in the 510(k).
4. The classification being recommended under section 513 of the act.
5. A discussion of the potential benefits of the device when compared to the potential or anticipated risk when the device is used as intended.
6. A complete discussion of the proposed general and/or special controls to ensure reasonable assurance of the safety and effectiveness of the device, including whether the product should be exempt from pre-market review under section 510(k), whether design control should be applicable, and what special controls would allow the Agency to conclude the device is reasonably likely to be safe and effective for its intended use.
7. Any clinical or pre-clinical data not included in the 510(k) that are relevant to the request.

The additional sections of this document address the above items as appropriate as well as labeling changes requested on the June, 8 2004. Should there be need for additional discussion, I can be contacted at 330-825-4525 x 170. Thank you for your time and attention.

Sincerely,

A handwritten signature in cursive script that reads "Cindy Lloyd".

Cindy Lloyd, RAC
Manager, Regulatory Affairs

STATEMENT OF CROSS REFERENCE

The information contained in the original submission, dated May 19, 2003, and supplemental information submitted on November 6, 2003 and April 19, 2004, are the basis for the cross reference in this request. The corresponding coversheets to provide positive identification of the aforementioned submitted documents are in Appendix 1. References in this request should be made to the previously submitted documents.

RECOMMENDED CLASSIFICATION UNDER SECTION 513 OF THE ACT

Based on a review of products similar in nature in the in-vitro diagnostic area, we are requesting that the product be identified as Class II, requiring special controls. The request is based on the belief that general controls in and of themselves are not sufficient to provide reasonable assurance of safety and effectiveness. These special controls are identified in the remaining sections of this request.

POTENTIAL BENEFITS / RISKS REVIEW OF THE DEVICE

Potential Benefits: This kit is intended for the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper. Quantitative analysis of amino acids, free carnitine and acylcarnitines and their relationship with each other is intended to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism. The assay thus provides a means for early detection of several amino acidopathies, organic acidemias, and fatty acid oxidation disorders. Tables 1, 27, 28, 29, and 30 of the product insert detail the analytes measured by the kit and the disorders for which the assay will provide early detection in newborns.

The inborn errors of metabolism (IEM) detected by the assay, can cause severe metabolic distress including biochemical disturbances such as hyperammonemia in patients with urea-cycle disorders (amino acid disorders), severe metabolic acidosis in patients with disorders of organic acids, or hypoketotic hyperglycemia, cardiomyopathy, or rhabdomyolysis in patients with disorders of fatty acid oxidation. If left untreated, these disorders may lead to brain damage, liver damage, other organ damage, or death. Individuals suffering from one of these disorders may develop severe illness with irreversible effects or even fatality within the first few days of life.

Early identification through a neonatal screening program is, therefore, key to the reduction or prevention of disease associated with IEM because it provides the means for early intervention. Effective treatments such as supplementation with thymine for Maple syrup urine disease, Vitamin B12 for Methylmalonic aciduria, Riboflavin for Glutaric acidemia Type I and II, low-phenylalanine diet for Phenylketonuria, infusion of arginine hydrochloride for hyperammonemia, and fast avoidance combined with a low-fat high-carbohydrate diet for fatty acid oxidation disorders can prevent most of the metabolic problems described above. However, in order to realize the full benefit of the treatment, early administration (before symptoms or irreversible effects have manifested) is essential.

Early intervention through early diagnosis not only provides for improvement in quality of life, but also provides a more cost-effective method of treatment. Early administration of treatment can preclude problems associated with long-term care of individuals afflicted with neurological or other chronic and debilitating disease states. With a combined incidence of approximately 1 in 5000 individuals, early detection and intervention of these inborn errors of metabolism can significantly reduce the associated morbidity and mortality and thus have a very significant impact in public health in general.

In addition to general public health benefits, the methodology associated with the kit is an improvement over current practices employed for the screening of IEM by tandem mass spectrometry (MS/MS). At the present time, there is no standardized MS/MS

methodology to perform expanded IEM screening, thus newborn screening programs that have adopted or want to incorporate MS/MS IEM expanded screening are forced to develop their own assays, reagents, and procedures (homebrew assays). The development of MS/MS IEM assays for newborn screening is not only labor intensive but also complicated. Programs adopting this technology have to prepare complex mixtures of internal standards and control material and fully characterize their reagents and ancillary materials (microplates, solvents, plate covers, etc). The extent with which each of these homemade reagents and procedures is characterized varies from program to program and therefore, no formal provisions for reagent acceptability and, more importantly, stability are documented or defined on a consistent basis. The NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit will reduce the labor requirements for adopting this technology and provide a standardized means (reagents and technology) for performing the screening. This is particularly important for the organic acid and fatty acid oxidation disorders that can currently only be screened by MS/MS.

Potential Risks: The potential risks associated with the assay are anticipated to be from misdiagnosis or misuse:

1. **Misdiagnosis:** In the event that the kit does not perform as described, the risk is either that a negative newborn is identified as a positive (false positive), or that a positive newborn is not identified as positive (false negative). During the development of the product, several actions were taken to mitigate the above referenced risks; these include:
 - a. In order to prevent and detect performance-related problems of either type, control material that is similar to what might be expected in the screening laboratory setting is provided in the kit to assist in the consistent monitoring of the assay performance.
 - b. A detail training program has been developed in which the user will be instructed in the necessary theoretical mass spectrometry background, preventive maintenance procedures, the proper execution of the assays, proper reagent handling, and accepted practices for setting up a MS/MS screening program. The training material will aid the user in the identification and prevention of performance-related issues. The requirements and directions for performing the assays are also described in detail in the kit insert. This information together with the training course will provide clarity and consistency in performing the test.
 - c. A detailed interference study was performed and the substances identified as possible interfering agents that may affect test results are indicated in the product insert. This will aid in the proper monitoring and troubleshooting of performance-related issues while performing the test routinely.

- d. The performance characteristics of the assay (linearity, precision, sensitivity, recovery, carry over, and drift) have been determined and are detailed in the direction insert to provide a clear explanation and expectation of the assay function.
 - e. An extensive section about the clinical application of the assays (pilot study performed at the California Department of Hygiene) is included in the kit insert. Recommendations and instructions are provided for the determination of the decision values (cutoffs) and includes a discussion about establishing of borderline zones. This material should aid the user in understanding the statistical processes employed for setting up appropriate cutoffs and the consequence of doing so. This section clearly describes the results of the pilot study and thus provides the proper expectations for clinical specificity, sensitivity, repeat rates and false positive rates when the assay is used as intended. In addition, the product insert includes a series of appendices that describe commonly used and accepted algorithms and profiles for the detection of the disorders. This information will aid the user to properly interpret the assay results in the determination of preliminary presumptive positives and presumptive negatives.
2. Misuse: Misuse of the product could occur by those not trained in the use of the product, or by procurement of the assay by individuals not authorized to perform the assay.

To mitigate the risk of misuse, a detailed training program on the use of the assay has been developed. As indicated above, the training material and experience will qualify the user for performing the assay as intended. Additionally, only PerkinElmer distributors or PerkinElmer authorized distributors will convey the product to the market. Furthermore, screening laboratories are typically operated by state governments and must meet the requirements of CLIA (Clinical Laboratories Improvement Act) for proficiency and training.

PROPOSED SPECIAL CONTROLS FOR THE IN-VITRO DIAGNOSTIC DEVICE

We are proposing that this product should not be exempt from pre-market review under section 510(k) of the act. In reviewing similar products submitted to the Food and Drug Administration by PerkinElmer Life and Analytical Sciences, we believe that the proposed product is essentially the same broad type of screening assay as those we have developed in the past. Previous assays produced by PerkinElmer Life and Analytical Sciences used in neonatal screening, such as the NeoGram PKU Tandem Mass Spectrometry Assay (k021541), the Neonatal Phenylalanine Test kit (k943547), and the Neonatal Leucine assay (k982307) were identified as Class II devices. Because of the similar intended use of these products and, in particular, because of the similarities between the NeoGram PKU and the proposed assay (both are tandem mass spectrometry assays), we feel it is appropriate to classify the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit as a Class II device with the requirement of pre-market notification.

During the design phase of this product, **design controls** as identified in the guidance document "Design Control Guidance for Medical Device Manufacturers" (March 11, 1997, referencing FDA 21CFR 820.30 and Sub-clause 4.4 of ISO 9001), were employed to guide the development of the product. Design inputs, design outputs, design reviews, design verification and validation, and transfer to production activities were executed following the design control guidance and internal design control procedures. Documentation is contained in the design history file.

The use of recognized standards was also employed during the development of this product. The standards that were employed or referenced were typically those suggested by the NCCLS (National Committee for Clinical Laboratory Standards) and the other organizations identified below. The following is a list of the standards referenced in the product insert (or in the development of the product):

1. Blood collection on filter Paper for Neonatal Screening Programs; Approved Standard- Third Edition (1997). NCCLS Document LA4-A3. Vol. 17, No16.
2. Preliminary Evaluation of Quantitative Clinical Laboratory Methods; NCCLS Approved Guideline, 2002 EP10-A2 Vol. 22 No. 29.
3. Evaluation of Precision Performance of Clinical Chemistry Devices; NCCLS Approved Guideline, 1999 EP5A Vol.19 No.2.
4. How to define and determine reference intervals in the clinical laboratory; NCCLS Approved Guideline, 1995 C28-A, Vol. 15 No 4.
5. Method Comparison and Bias Estimation Using Patient Samples; NCCLS Approved Guideline (IVD) 1995 EP9-A.

6. Evaluation of Linearity of Quantitative Analytical Methods. NCCLS Approved Guideline EP-6 Vol. 6, No 18.
7. Interference Testing in Clinical Chemistry Proposed Guideline. NCCLS Document, 2002 EP-7A, Vol. 22, No. 17.
8. Procedures for Collection of Diagnostic Blood Specimens by Skin Puncture (1991), Section L-4, NCCLS.
9. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids and Tissue: Second Edition; Tentative Guideline. NCCLS M29-T2, Vol. 11, No 14.

In addition to employing or referencing the above recognized external standards, PerkinElmer Life and Analytical Sciences participates in the CDC/ APHL Newborn Screening Quality Assurance and Proficiency Testing programs. These programs specifically relate to inborn errors of metabolism and provide feedback from various participating laboratories on several metabolic disorders. Tandem mass spectrometry testing has been added to these programs. Our participation helps to ensure that the product is consistent with other methodologies in the field and provides a benchmark for the effectiveness of the assay. Results from the participation in the proficiency testing program are included in the original submission.

In addition to the aforementioned controls, PerkinElmer Life and Analytical Sciences follows the requirements of FDA's Quality Systems Regulations (QSR) for the production and development of clinical products. Compliance with QSR during product development and manufacture, also helps ensure the safety and effectiveness of the proposed product.

Clinical data and summary information were submitted previously with the Original 510(k) dated May 19, 2003, and supplemental information submitted on November 6, 2003 and April 19, 2004. The information contained in those submissions demonstrated that the assay was safe and effective during a large-scale pilot study in which 212,000 patients were screened. This information met the requirements of the agency at that time. If there are *any* additional documents required, these can be conveyed as necessary.



JUN - 9 2004

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Ms. Cindy Lloyd, RAC
Regulatory Affairs Manager
PerkinElmer Life and Analytical Sciences
3985 Eastern Road
Norton, OH 44203

Re: K031878

Trade Name: NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit
Regulatory Class: III
Product Code:NQL, JIT, JJY
Dated: April 19, 2004
Received: April 20, 2004

Dear Ms. Lloyd,

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. We have determined the device is not substantially equivalent to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to any device which has been reclassified into class I (General Controls) or class II (Special Controls. This decision is based on the fact that we are not aware of legally marketed preamendments device labeled or promoted for the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper using tandem mass spectrometry. (This relates to the indication of any type device for the use- - not the technology.)

Therefore, this device is classified by statute into class III (Premarket Approval), under Section 513(f) of the Federal Food, Drug, and Cosmetic Act (Act).

Section 515(a)(2) of the Act requires a class III device to have an approved premarket approval application (PMA) before it can be legally marketed, unless the device is reclassified.

Any commercial distribution of this device prior to approval of a PMA, Product Development Protocol (PDP), or the effective date of any order by the Food and Drug Administration re-classifying this device into class I or II, would be a violation of the Act. Clinical investigations of this device must be conducted in accordance with the investigational device exemptions (IDE) regulations.

Page 2 –Ms. Cindy Lloyd

The Food and Drug Administration Modernization Act of 1997 (FDAMA), section 207, deals with the Evaluation of Automatic Class III Designation. Under this section a manufacturer, whose device is found to be not substantially equivalent to a predicate device, can request FDA to make a risk- based classification for their device. I believe that based on the review of your device, it may be a candidate for Evaluation of Automatic Class III Designation. Therefore, you may wish to make such a request for this agency. For additional information on your options under Section 207, please refer to our guidance entitled, "New Section 513(f)(2)-Evaluation of Automatic Class III Designation, Guidance for Industry and Staff." This document is available on the World Wide Web/ CDRH Home Page at: <http://www.fda.gov/cdrh/modact/clasiii.html>.

If you wish to pursue the marketing of this device and need information or assistance for preparing investigational or premarket submissions, please contact the Division of Small Manufacturers, International and Consumer Assistance at its toll free number (800) 638-2041 or (301) 443-6597, or at its Internet address "<http://www.fda.gov/cdrh/dsmamain.html>".

Sincerely yours,



Jean M. Cooper, MS, D.V.M.

Director

Division of Chemistry and Toxicology

Office of *In Vitro* Diagnostic Device

Evaluation and Safety

Center for Devices and

Radiological Health

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

April 20, 2004

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD

510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

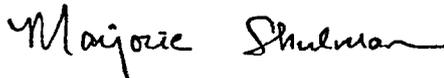
The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,



Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health



> PerkinElmer Life and Analytical Sciences
3985 Eastern Road
Norton OH 44203
Phone: 330-825-4525
Phone: 800-321-9632
Fax: 330-825-8520
<http://www.perkinelmer.com>

April 19, 2004

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850

Reference: 510(k) submission: K031878, Neogram Amino Acids and Acylcarnitines Tandem Mass
Holder: PerkinElmer Life Sciences, Inc.
3985 Eastern Rd
Norton, OH 44203

To: Clinical Chemistry Review Section, Carol Benson

Re: Request for Additional Information

Dear Ms. Benson:

Please find enclosed our response to the January 23, 2004 request for additional information needed to continue the review of our submission. If you have any further needs, please do not hesitate to contact us.

Sincerely,

Cindy Lloyd, RAC
Regulatory Affairs Manager
Phone: 330-825-4525, Ext. 170

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

February 19, 2004

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD

510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

Extended Until: 22-APR-2004

Based on your recent request, an extension of time has been granted for you to submit the additional information we requested.

If the additional information is not received by the "Extended Until" date shown above your premarket notification will be considered withdrawn.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,



Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health



PerkinElmer Life and Analytical Sciences
3985 Eastern Road
Norton OH 44203
Phone: 330-825-4525
Phone: 800-321-9632
Fax: 330-825-8520
<http://www.perkinelmer.com>

February 17, 2004

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850

Reference: 510(k) submission: K031878, Neogram Amino Acids and Acylcarnitines Tandem Mass
Holder: PerkinElmer Life Sciences, Inc.
3985 Eastern Rd
Norton, OH 44203

With regard to the above submission, PerkinElmer Life and Analytical Sciences, Inc., would like to request a 60-day extension to respond to the information requested by the reviewer(s).

Please contact me if you have any questions or require additional information to process this request.

Thank you,

A handwritten signature in cursive script that reads 'Cindy Lloyd'.

Cindy Lloyd, RAC
Regulatory Affairs Manager
PerkinElmer Life and Analytical Sciences, Inc.
Phone: 330-825-4525, Ext. 170

cc. Carol Benson

FEB-05-2004 THU 10:55 AM PERKINELMER LIFE SCI

FAX NO. 330 825 000

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

January 23, 2004

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Cabbage Run Blvd.
Rockville, Maryland 20850PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(1) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>

If after 30 days the requested information, or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

FEB-05-2004 THU 10:55 AM PERKALMER LIFE SCI

FAX NO. 330 825 0

P. 03

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Marjorie Shulman
Supervisor Consumer Safety Officer
Pre-market Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

November 07, 2003

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD

510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

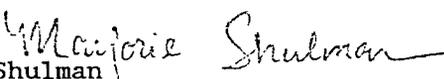
The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,


Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health



November 6, 2003

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850

Reference: 510(k) submission: K031878, Neogram Amino Acids and Acylcarnitines Tandem Mass
Holder: PerkinElmer Life Sciences, Inc.
3985 Eastern Rd
Norton, OH 44203

To: Clinical Chemistry Review Section, Carol Benson

Re: Request for Additional Information

Dear Ms. Benson:

Please find enclosed our response to the request for additional information. The enclosed document contains 179 pages and one CD-ROM in response to the seventeen (17) questions received on September 11, 2002. If you have any further needs, please do not hesitate to contact us.

Sincerely,

Cindy Lloyd, RAC
Regulatory Affairs Manager
Phone: 330-825-4525, Ext. 170

October 09, 2003

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD

510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

Extended Until: 10-NOV-2003

Based on your recent request, an extension of time has been granted for you to submit the additional information we requested.

If the additional information is not received by the "Extended Until" date shown above your premarket notification will be considered withdrawn.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,



Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health



> PerkinElmer Life and Analytical Science
3985 Eastern Road
Norton OH 44203
Phone: 330-825-4525
Phone: 800-321-9632
Fax: 330-825-8520
<http://www.perkinelmer.com>

October 8, 2003

Food and Drug Administration
Center for Devices and Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850

Reference: 510(k) submission: K031878, Neogram Amino Acids and Acylcarnitines Tandem Mass
Holder: PerkinElmer Life Sciences, Inc.
3985 Eastern Rd
Norton, OH 44203

With regard to the above submission, PerkinElmer would like to request a 30-day extension to respond to the information requested by the reviewer(s).

Please contact me if you have any questions or require additional information to process this request.

Thank you,

Cindy Lloyd, RAC
Regulatory Affairs Manager
Phone: 330-825-4525, Ext. 170

SEP 12 2003

September 12, 2003

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CINDY LLOYD

510(k) Number: K031878
Product: NEOGRAM AMINO
ACIDS AND
ACYLCARNITINES
TANDEM MASS

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>

If after 30 days the requested information, or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

If you have procedural or policy questions, please contact the
Division of Small Manufacturers International and Consumer Assistance (DSMICA)
at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me
at (301) 594-1190.

Sincerely yours,
Marjorie Shulman

Marjorie Shulman
Supervisor Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health



PerkinElmer Life Sciences, Inc.

3985 Eastern Road

Norton, OH 44203 USA

Phone: 330-825-4525

Fax: 330-825-8520

www.perkinelmer.com

June 20, 2003

Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Office of Device Evaluation
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850 USA

To: Clinical Chemistry Review Section

Re: Notification of Change in Contact Person for PerkinElmer Life Science's NeoGram
Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit, K031878

This letter is to notify you of a change in the contact person for this 510(k) submission.
The old contact was:

Carroll L. Martin
3985 Eastern Road
Norton, Ohio 44203
330-825-4525 x170

The new contact is:

Cindy Lloyd
3985 Eastern Road
Norton, Ohio 44203
330-825-4525

or

549 Albany Street
Boston, Massachusetts 02118
617-350 9305

As official correspondent for this submission, I would request that all communication and
correspondence be sent to her attention at one of the above addresses. Thank you for your
assistance.

Respectfully submitted,


Carroll L. Martin
Manager, Regulatory Affairs

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

June 19, 2003

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CARROLL L. MARTIN

510(k) Number: K031878
Received: 18-JUN-2003
Product: NEOGRAM AMINO ACIDS
AND ACYLCARNITINES
TANDEM MASS
SPECTROMETRY KIT,

The Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification has been completed or if any additional information is required. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA)(Public Law 107-250), authorizes FDA to collect user fees for premarket notification submissions. (For more information on MDUFMA, you may refer to our website at <http://www.fda.gov/oc/mdufma>).

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC)(HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review". Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

You should be familiar with the manual entitled, "Premarket Notification 510(k) Regulatory Requirements for Medical Devices" available from DSMICA. If you have other procedural or policy questions, or want information on how to check on the status of your submission, please contact DSMICA at (301) 443-6597 or its toll-free number (800) 638-2041, or at their Internet address <http://www.fda.gov/cdrh/dsmamain.html> or me at (301)594-1190.

Sincerely,
Marjorie Shulman

Marjorie Shulman
Marjorie Shulman
Supervisory Consumer Safety Officer
Office of Device Evaluation
Center for Devices and Radiological Health

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

June 18, 2003

PERKINELMER LIFE SCIENCES, INC.
3985 EASTERN ROAD
NORTON, OH 44203
ATTN: CARROLL L. MARTIN

510(k) Number: K031878
Received: 17-JUN-2003
Product: NEOGRAM AMINO ACIDS
User Fee ID Number: 6906ARNITINES
TANDEM MASS

The Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA) (Public Law 107-250), specifies that a submission shall be considered incomplete and shall not be accepted for filing until fees have been paid (Section 738(f)). Our records indicate that you have not submitted the user fee payment information and therefore your 510(k) cannot be filed and has been placed on hold. The payment information we need in order to begin the review of your 510(k) includes, the user fees cover sheet with the payment ID faxed to the Office of Financial Management at (301) 827-9213 and a check mailed to:

By Regular Mail

Food and Drug Administration
P.O. Box 956733
St. Louis, MO 63195-6733.

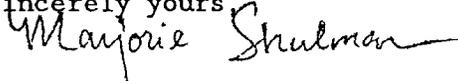
By Private Courier (e.g., Fed Ex, UPS, etc.)

U.S. Bank
956733
1005 Convention Plaza
St. Louis, MO 63101
(314) 418-4983

The check should be made out to the Food and Drug Administration referencing the payment identification number, and a copy of the User Fee Cover sheet should be included with the check. A copy of the Medical Device User Fee Cover Sheet should also be faxed to CDRH at (301) 594-2977 referencing the 510(k) number if you have not already sent it in with your 510(k) submission. After the FDA has been notified of the receipt of your user fee payment, your 510(k) will be filed and the review will begin. If payment has not been received within 30 days, your 510(k) will be deleted from the system. Additional information on user fees and how to submit your user fee payment may be found at <http://www.fda.gov/oc/mdufma>.

Please note that since your 510(k) has not been reviewed, additional information may be required during the review process and the file may be placed on hold once again. If you are unsure as to whether or not you need to file an application with FDA or what type of application to file, you should first telephone the Division of Small Manufacturers, International and Consumer Assistance (DSMICA), for guidance at (301)443-6597 or its toll-free number (800)638-2041, or contact them at their Internet address <http://www.fda.gov/cdrh/dsmamain.html>, or you may submit a 513(g) request to the Document Mail Center at the address above. If you have any questions concerning the contents of this letter, you may contact me at (301) 594-1190.

Sincerely yours



Marjorie Shulman
Consumer Safety Officer
Office of Device Evaluation
Center for Devices and
Radiological Health



May 19, 2003

Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Office of Device Evaluation
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850 USA

To: Clinical Chemistry Review Section

Re: 510(k) Notification for PerkinElmer Life Science's NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit

Accompanying, please find a traditional 510(k) submission for the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit. The information contained in the coversheet identifies the appropriate product code and CFR citation for this product. This product is a system comprised of the actual kit components (see direction insert), the software used on the instrument and a tandem mass spectrometer. The manufacturer of the tandem mass spectrometer is identified under Section H of the coversheet. The tandem mass spectrometer, as a standalone device, is identified in the CFR under Subpart C-Clinical Laboratory Instruments, Section 862.2860 (product code DOP). It is a Class I device, exempted from pre-market notification when used as described (see copy of CFR citation for this device). The device is not exempted as identified in 862.9(c)(2) when used "for use in screening or diagnosis of familial or acquired genetic disorders, including inborn errors of metabolism." The tandem mass spectrometer has been found to be safe and effective for use in screening of genetic disorders, including inborn errors of metabolism via a 510(k) filed by PerkinElmer Life Sciences, Inc. for its NeoGram PKU Test Kit, K021541. This submission is requesting that this kit and its components be considered as a system and the components used as a system are substantially equivalent to the Wallac Neonatal Leucine assay, the Isolab Neonatal Phenylalanine Test Kit, the NeoGram PKU by Tandem Mass Spectrometry assay, the Astoria Pacific Phenylalanine Test Kit and the Astoria Pacific Tyrosine Test Kit identified in the coversheet. In addition, substantial equivalence is claimed to home-brew tandem mass spectrometry assays that represent the current accepted practice for screening of amino acid metabolic disorders, fatty acid disorders and organic acid disorders.

As official correspondent for this submission, I would request that all communication and correspondence be sent to my attention at the above address. I can be reached by telephone at 330-825-4525 x170. Thank you for your assistance.

Respectfully submitted,

Carroll L. Martin
Manager, Regulatory Affairs

NeoGram[®] Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit

Directions for use. Reagents for **1920** assays including QC tests

Manufactured by:
PerkinElmer Life and Analytical Sciences Inc., Norton, OH, USA

FOR IN VITRO DIAGNOSTIC USE

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INTENDED USE

Intended use: This kit is intended for the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper. Table 1 details the analytes measured by the kit. Quantitative analysis of amino acids, free carnitine and acylcarnitines and their relationship with each other is intended to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism. This kit is to be used for **In Vitro diagnostic** use only, by trained, qualified laboratory personnel.

PerkinElmer Life and Analytical Sciences offers an instruction course on the use of this assay and required equipment. It is strongly recommended for users to attend this course prior to implementation of this assay in their laboratories.

Table 1. Analytes Measured by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit.

ANALYTE NAME	ABBREVIATION
Amino Acids	
Alanine	Ala
Arginine	Arg
Citruline	Cit
Glycine	Gly
Leucine	Leu
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Tyrosine	Tyr
Valine	Val
Carnitines	
Free carnitine	C0
Acetylcarnitine	C2
Propionylcarnitine	C3
Malonylcarnitine	C3DC
Butyrylcarnitine	C4
3-Hydroxy-butyrylcarnitine	C4OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutarylcarnitine	C5DC
3-Hydroxy-isovalerylcarnitine	C5OH
Hexanoylcarnitine	C6
Adipylcarnitine	C6DC
Octanoylcarnitine	C8
Octenoylcarnitine	C8:1
Decanoylcarnitine	C10
Decenoylcarnitine	C10:1

ANALYTE NAME	ABBREVIATION
Carnitines	
Decadienoylcarnitine	C10:2
Dodecanoylcarnitine	C12
Dodecenoylcarnitine	C12:1
Tetradecanoylcarnitine (Myristoylcarnitine)	C14
Tetradecenoylcarnitine	C14:1
Tetradecadienoylcarnitine	C14:2
3-Hydroxy-tetradecanoylcarnitine	C14OH
Hexadecanoylcarnitine (palmitoylcarnitine)	C16
Hexadecenoylcarnitine	C16:1
3-Hydroxy-hexadecanoylcarnitine	C16OH
3-Hydroxy-hexadecenoylcarnitine	C16:1OH
Octadecanoylcarnitine (Stearoylcarnitine)	C18
Octadecenoylcarnitine (Oleylcarnitine)	C18:1
Octadecadienoylcarnitine (Linoleylcarnitine)	C18:2
3-Hydroxy-octadecanoylcarnitine	C18OH
3-Hydroxy-octadecenoylcarnitine	C18:1OH

SUMMARY AND EXPLANATION OF THE ASSAY

Elevated amino acids, free carnitine and acylcarnitine levels in newborn blood can be indicative of one or more of several metabolic disorders. Free carnitine and acylcarnitines are markers for disorders that are classified as fatty acid oxidation (FAO) disorders and organic aciduria disorders (OAD)¹⁻⁵. Similarly, amino acids are used as markers for several metabolic disorders collectively known as amino acidopathies. These disorders are inborn errors of metabolism (or genetic metabolic deficiencies)¹⁻⁶.

The kit has the capability of measuring over 40 analytes, even though it is supplied with 23 internal standards and 23 controls. This is possible because analytes of the same chemical class and molecular structure will have similar performance characteristics. For example, C18, C18:1, C18:2 and C18:OH have very similar molecular structures and thus have similar performance characteristics. Therefore, one can use the C18 internal standard to estimate the concentrations of the above-mentioned analytes. What is assumed is that accuracy may be affected but not precision. Additionally, because of this similarity unlabeled C18 can be used as a surrogate external control for the entire C18 series or analytes. Thus as long as the internal standard and surrogate external control chosen to quantitate a particular analyte are kept constant for the analysis of the patient samples, one can distinguish between presumptive normal vs presumptive elevated samples. A similar argument can be applied to the use of amino acids internal standards and controls for the quantitation of amino acids not included in the amino acids internal standard and control sets. Tables 2 and 3 describe how the internal standards and controls included in the NeoGram Amino Acids and Acylcarnitines Tandem mass spectrometry kit should be used to quantitate the analytes measured by this assay.

Table 2. Acylcarnitines Measured by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit and their Corresponding Internal Standards and Controls.

ACYLCARNITINES		
<i>Analytes Measured vs. their Internal Standards and Controls</i>		
Analyte	Internal Standard	Control
C0	d ₉ -C0	C0
C2	d ₃ -C2	C2
C3	d ₃ -C3	C3
C3DC	d ₆ -C5DC	C5DC
C4	d ₃ -C4	C4
C4OH	d ₃ -C4	C4
C5	d ₉ -C5	C5
C5:1	d ₉ -C5	C5
C5DC	d ₆ -C5DC	C5DC
C5OH	d ₉ -C5	C5
C6	d ₃ -C6	C6
C6DC	d ₆ -C5DC	C5DC
C8	d ₃ -C8	C8
C8:1	d ₃ -C8	C8
C10	d ₃ -C10	C10
C10:1	d ₃ -C10	C10
C10:2	d ₃ -C10	C10
C12	d ₃ -C12	C12
C12:1	d ₃ -C12	C12
C14	d ₃ -C14	C14
C14:1	d ₃ -C14	C14
C14:2	d ₃ -C14	C14
C14OH	d ₃ -C14	C14
C16	d ₃ -C16	C16
C16:1	d ₃ -C16	C16
C16O:1	d ₃ -C16	C16
C16:1OH	d ₃ -C16	C16
C18	d ₃ -C18	C18
C18:1	d ₃ -C18	C18
C18:2	d ₃ -C18	C18
C18OH	d ₃ -C18	C18
C18:1OH	d ₃ -C18	C18

Table 3. Amino Acids Measured by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit and their Corresponding Internal Standards and Controls.

AMINO ACIDS		
Analytes Measured vs. their Internal Standards and Controls		
Analyte	Internal Standard	Control
Ala	d ₄ -Ala	Ala
Arg	d ₄ , ¹³ C-Arg	Cit
Cit	d ₂ -Cit	Cit
Gly	¹⁵ N, 2- ¹³ C-Gly	Gly
Leu/Ile	d ₃ -Leu	Leu
Met	d ₃ -Met	Met
Orn	d ₂ -Orn	Cit
Phe	d ₅ -Phe	Phe
Tyr	¹³ C ₆ -Tyr	Tyr
Val	d ₈ -Val	Val

Disorders of Amino Acid Metabolism

In amino acidopathies, enzymes necessary for the metabolism of certain amino acids are unavailable or have reduced activity. As a result, the concentration of the affected amino acids and alternate metabolites increases in the infant's body. These excesses can have deleterious effects on the infant's health including death. Some commonly studied amino acidopathies are:

Phenylketonuria (PKU) is a disorder of aromatic amino acid metabolism in which phenylalanine cannot be converted to tyrosine. If untreated, PKU leads to various degrees of mental retardation. *Hyperphenylalaninemia* leads to mental retardation and muscular rigidity. *Homocystinuria* leads to vascular occlusive disease, osteoporosis, accumulation of homocystine and methionine, and variable developmental delays. *Maple Syrup Urine Disease (MSUD)* is caused by a disorder of branched-chain amino acid metabolism resulting in elevated levels of leucine, isoleucine and valine in the blood. If untreated, lethargy progressive to coma, developmental delay, and convulsions will develop. *Tyrosinemia type I* (hereditary tyrosinemia), leads to acute hepatic failure or chronic cirrhosis and hepatocellular carcinoma. *Citrullinemia* leads to convulsions, anorexia, vomiting and lethargy, followed rapidly by potentially lethal coma.¹⁻⁶

Fatty Acid Oxidation (FAO) Disorders

In FAO disorders, enzymes necessary for fatty acid breakdown are unavailable or have reduced activity. Breakdown, or oxidation, of fatty acids is necessary for energy production when glucose levels, the body's main source of energy, are low. Without this energy supply some individuals may have recurring incidences of low blood sugar levels. In cases of fasting, often caused by illnesses such as ear infections or flu, there may be metabolic crisis. Affected individuals may show vomiting, diarrhea, lethargy, seizures and coma. Failure to diagnose FAO disorders may result in excessive fat buildup in the liver, heart and kidneys. This buildup can cause a variety of symptoms, ranging from hepatic failure, encephalopathy, and heart and eye complications to general problems with muscle development. Many of these clinical symptoms can lead to death. Many deaths due to FAO disorders have been misdiagnosed as SIDS or Reye's Syndrome¹⁻⁶.

Organic Aciduria (OA) Disorders

The metabolic pathways of organic acids are disrupted in OA disorders and thus accumulation of the acids in blood and urine alters the acid-base balance of the body. Resulting modifications or adaptations to intermediary metabolic pathways may cause numerous clinical symptoms, including metabolic acidosis, ketosis, hyperammonemia, failure to thrive, sepsis or coma¹⁻⁶.

PRINCIPLES OF THE ASSAY

The measurement of amino acids, free carnitine, and acylcarnitines involves extraction of dried blood spots from newborns with a solution containing stable-isotope labeled internal standards and analysis using a tandem mass spectrometry (MS/MS) system. The response of each analyte relative to their corresponding stable-isotope labeled internal standard is proportional to analyte concentration. In the NeoGram Amino Acid and Acylcarnitines tandem mass spectrometry kit, data is acquired in the Neutral Loss of 102, Precursor Ion of 85, and Multiple Reaction Monitoring (MRM) modes. During these scan modes, a collisionally induced product of each analyte is measured for a set time period. Data acquisition and processing is performed by the NeoGram and Analyst software packages included with the assay system.

The Wallac MS² triple-quadrupole mass spectrometer that is used for these measurements is a computer-controlled device that separates and quantitates ions based on their mass to charge (m/z) ratio. Each quadrupole is a mass filter comprised of 4 horizontal metal rods. The magnetic field around the rods is controlled by varying an applied radio frequency (rf) potential, allowing the ions to be filtered based on their mass to charge (m/z) ratio. The extracted sample is delivered to the ion source of the mass spectrometer by the liquid handling (LC) system consisting of the autosampler, micro pump(s) and solvent vacuum degasser. The Turbolon spray (ion source) generates a fine spray of charged droplets from which ions are emitted as the solvent evaporates. These ions are introduced into the quadrupole area for mass analysis (Figure 1).

Sample Delivery

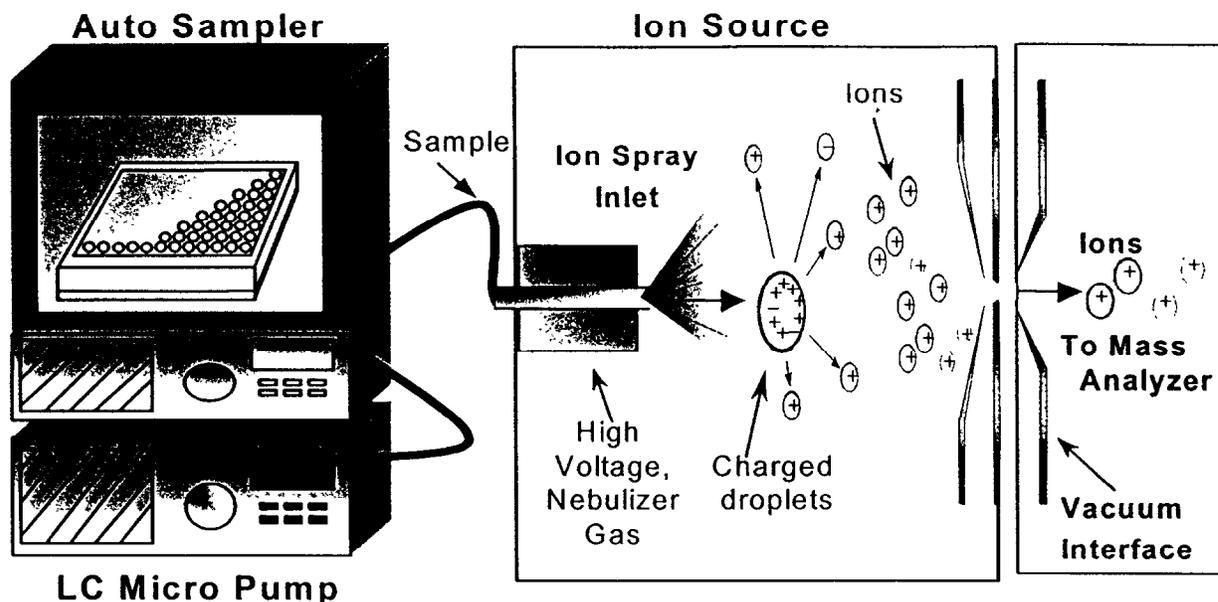


Figure 1

In the Wallac MS² system, the ions pass through a vacuum interface. An orthogonal flow of nitrogen along the interface creates what is called a curtain gas interface. This interface excludes non-charged materials and aids in removing solvent molecules from the ions. The ions are transported through the curtain gas and through a narrow opening into the mass filtering and analysis portion of the instrument. Non-charged molecules are pumped away by the turbo pump, and the ions are focused in the first quadrupole, Q0. The focused ion beam is then introduced into the first mass filtering quadrupole, Q1. In the Q1 region, the ions are separated by their m/z ratio and are moved along into the collision cell. The collision cell is a non-mass filtering quadrupole. A slight pressure of nitrogen gas is introduced into the collision cell. The ions collide with the gas molecules and fragment into smaller ions. This process is called Collisionally Activated Dissociation (CAD). The collision energies have been optimized to induce the fragmentation of amino acids, free carnitine and acylcarnitines to preferentially generate the product ions that are appropriate for each scan mode employed in this assay (Figures 1 to 3 and schemes 1 to 3).

In the Precursor Ion of 85 mode, Q1 is scanned from low to high mass while Q3 is fixed to only allow ions at m/z 85 to pass through. As Q1 is scanned, each precursor ion at a particular m/z is sent to the collision cell. If that precursor ion produces a fragment ion at m/z 85 that fragment ion will pass through Q3 and is detected. An algorithm relates when an m/z 85 ion is detected and the m/z at which Q1 was at that moment. What is thus, reported in the MS/MS spectrum is the m/z values of all the precursor ions that generated a product at m/z 85 (Figure 2).

Mass Analysis

Precursor Ion Scan

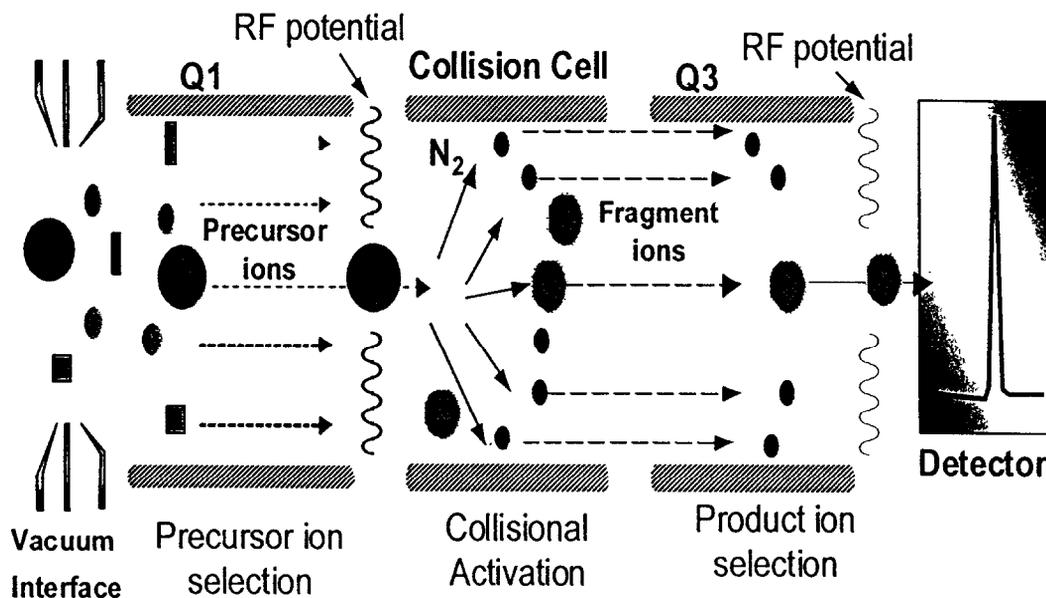


Figure 2

In the Neutral Loss of 102 scan, Q1 scans a particular mass range while Q3 is set to scan the same mass range but with a constant mass offset from Q1. This mass offset corresponds to a common neutral loss from the parent ions upon fragmentation. In the case of butyl esters of amino acids (see schemes 1 and 3), this loss is equivalent to 102 mass units. Q1 and Q3 are linked to scan with this constant offset (Q1 minus 102) so that as precursor ions are sorted and directed to the collision cell by Q1, only product ions that result from the loss of 102 mass units will be allowed to pass through Q3 and be detected (Figure 3).

The third type of scan employed, the Multiple Reaction Monitoring (MRM) scan, adheres to the same principles as stated above for the Neutral Loss and Precursor Ion scans. The only difference is that in the MRM mode, instead of scanning Q1, Q1 is set to select a particular parent ion. The selected parent ion is sent to the collision cell and the desired product is specified in Q3.

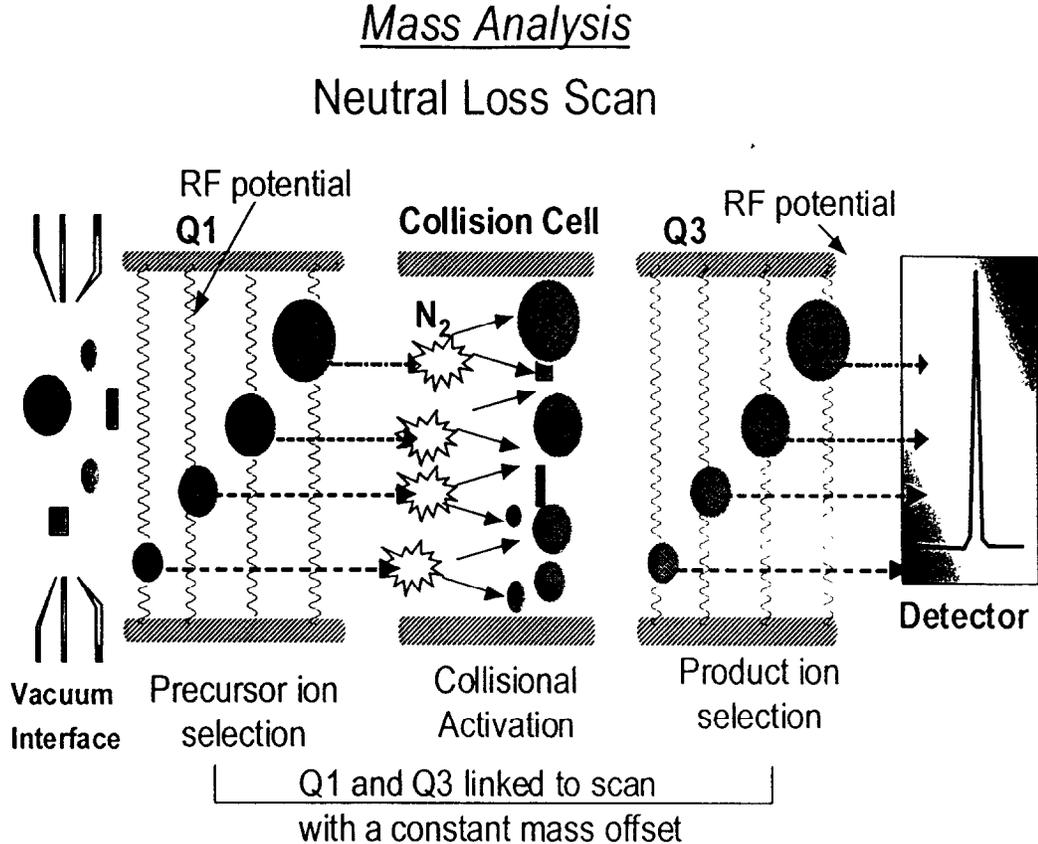
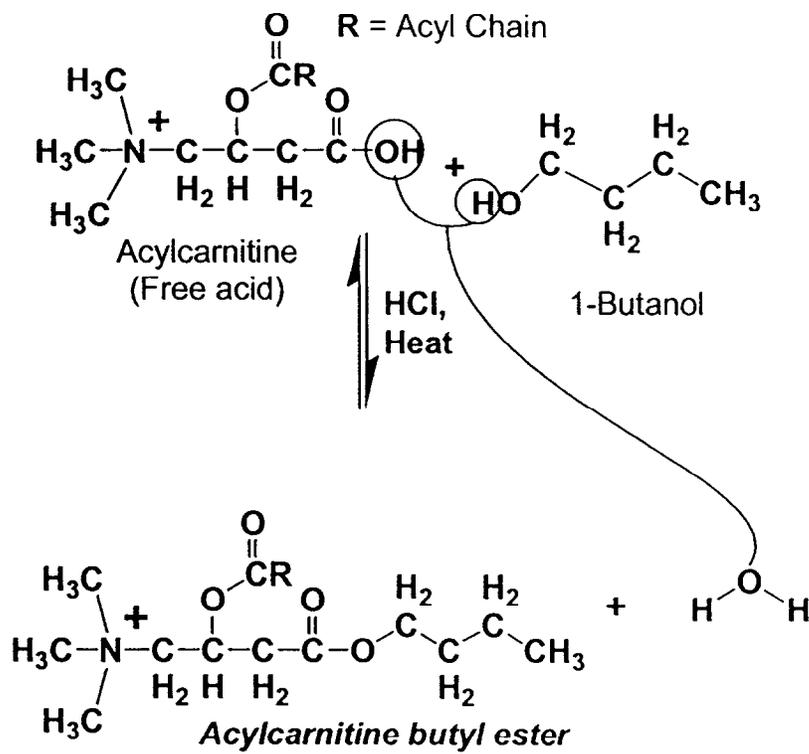


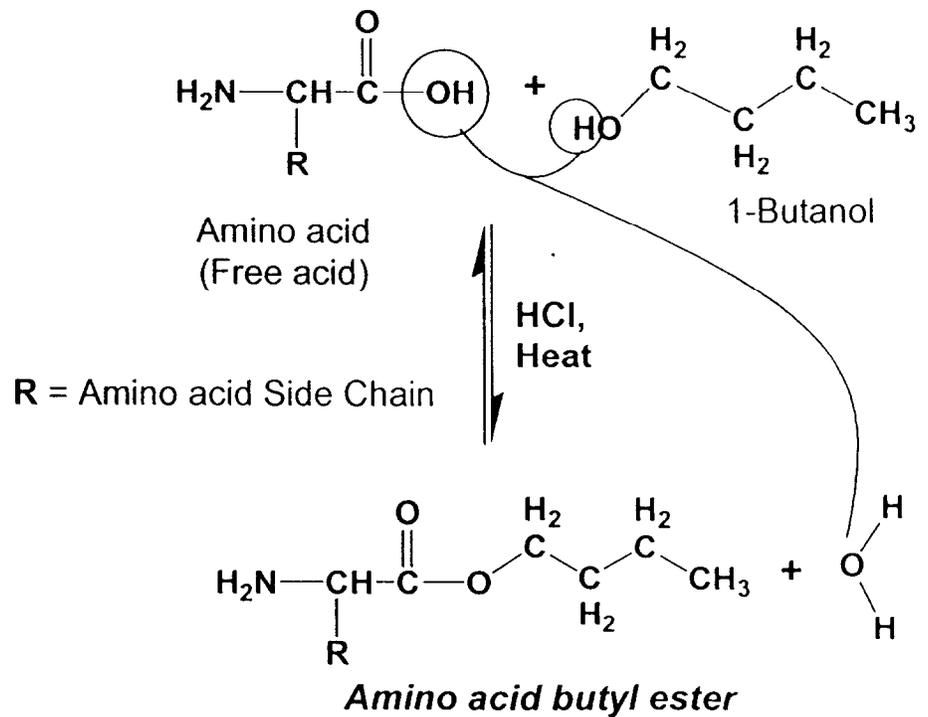
Figure 3

As part of sample preparation, the NeoGram Amino Acids and Acylcarnitine assay involves a chemical derivatization step. Analyte chemical derivatization generally involves the chemical modification of the analyte by the addition of a protective group. In this particular assay, the derivatization involves the addition of a butyl group to the carboxylate functionality of the amino acids, free carnitine, and acylcarnitines to produce butyl esters (Scheme 1).

ACYLCARNITINES

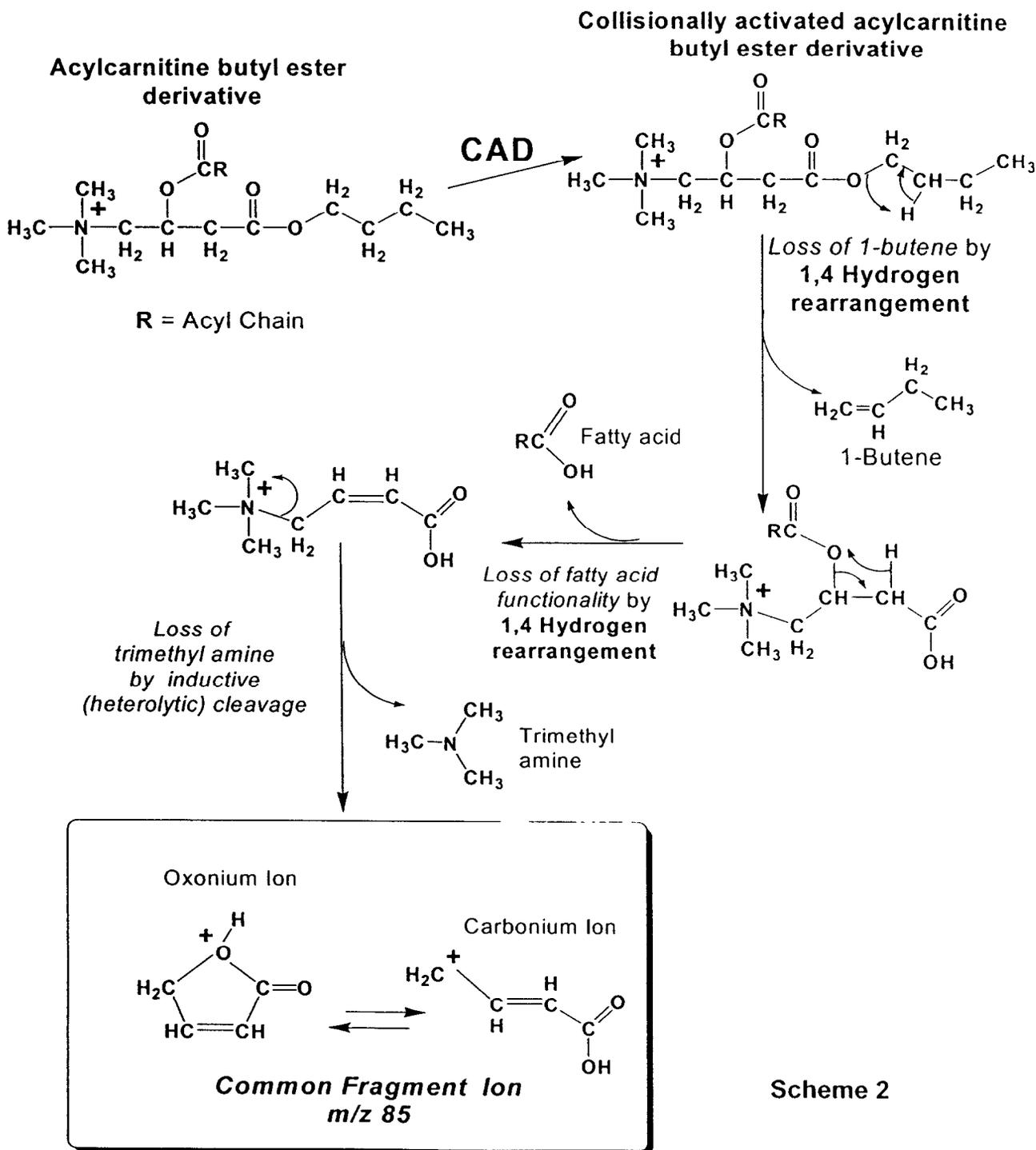


AMINO ACIDS

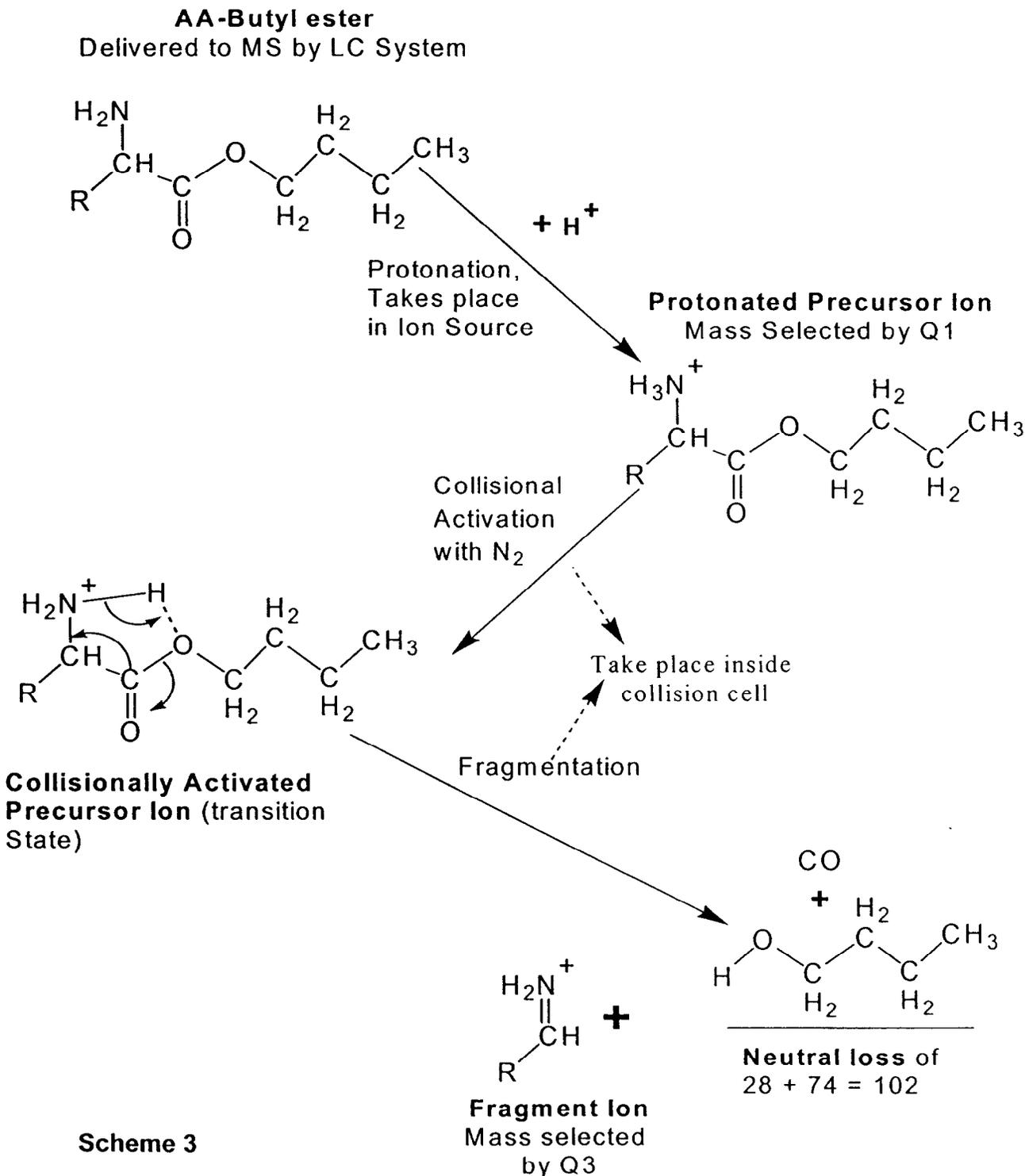


Scheme 1

Once the sample is derivatized, it is sent by an LC system to the mass spectrometer for mass analysis. As stated above, mass analysis for the acylcarnitines is performed in the Precursor ion of 85 mode. Below is the reaction mechanism for the formation of the common fragment ion at m/z 85 from collisionally activated butylated acylcarnitines. The entire fragmentation reaction takes place inside the collision Cell (Scheme 2).



Similarly to the acylcarnitines, the derivatized amino acids are sent by the LC system to the mass spectrometer for mass analysis. In the case of most of the amino acids present in the sample, mass analysis is performed in the Neutral Loss of 102 mode. Below is the reaction mechanism for the formation of the common neutral loss of 102 mass units from butylated amino acid precursor ions resulting from collisional activated dissociation (CAD). The entire fragmentation reaction takes place inside the collision Cell (Scheme 3).



Finally, not all the acylcarnitines are measured in the Precursor Ion of 85 full scan mode. Free carnitine is measured as an MRM using the Precursor Ion of 103 mass units from the butylated free carnitine precursor ion. Acetyl carnitine and propionyl carnitine are measured as MRM using the conventional Precursor Ion of 85 mass transition. Similarly, not all of the amino acids are measured in the full scan based on the Neutral Loss of 102. Arginine is measured as an MRM using a neutral loss of 161 mass units, glycine is measured in the MRM mode as a neutral loss of 56 mass units, and citruline and ornithine are measured as MRM using a neutral loss of 119 mass units. We have separated these analytes for analysis as MRM transitions because they exhibited much better sensitivities when measured as the transitions stated above.

NOTE: The NeoGram Amino acids and Acylcarnitines kit has two associated acquisition methods. In one, most of the acylcarnitines and amino acids are measured using the full scan methods mentioned above. In addition, the kit has an acquisition method in which all of the analytes are measured in the MRM mode. Both of these methods perform equally well. The advantage of the full scan method is that more information may be available per scan as all potential analytes may be recorded. The advantage of the MRM method is that it is more specific and thus more precise. In addition, the MRM method may allow for the selective measurement of particular analytes. The intention of this assay is to provide both capabilities to support particular laboratory needs.

KIT CONTENTS

Each NeoGram Amino acids and Acylcarnitines kit contains reagents for 1920 assays, including QC tests. The expiry date of the complete package is stated on the outer label.

Component	Quantity	Storage
Stable-isotope Amino Acids Internal standards	1 vial dried	20-30°C
Stable-isotope Carnitine and Acylcarnitines Internal standards	1 vial dried	20-30°C

The labeled amino acids as well as the carnitine and acylcarnitines concentrations per vial of Internal Standards are stated on the corresponding inserts (see assay procedure for instructions).

Stable-isotope Standard	CAS #	Approximate Amount per vial (µmoles)
CARNITINE STANDARDS		
d ₉ -Free carnitine (d ₉ -C0)	6645-46-1	0.152
d ₃ -Acetyl carnitine (d ₃ -C2)	5080-50-2	0.019
d ₃ -Propionyl carnitine (d ₃ -C3)	17298-37-2	0.0114
d ₃ -Butyrylcarnitine (d ₃ -C4)	NA	0.0076
d ₉ -Isovalerylcarnitine (d ₉ -C5)	NA	0.0076
d ₆ -Glutarylcarnitine (d ₆ -C5DC)	NA	0.0076
d ₃ -Hexanoylcarnitine (d ₃ -C6)	6920-35-0	0.0076
d ₃ -Octanoylcarnitine (d ₃ -C8)	18822-86-1	0.0076
d ₃ -Decanoylcarnitine (d ₃ -C10)	18822-87-2	0.0076
d ₃ -Lauroylcarnitine (d ₃ -C12)	7023-03-2	0.0152
d ₃ -Myristoylcarnitine (d ₃ -C14)	1822-89-4	0.0152
d ₃ -Palmitoylcarnitine (d ₃ -C16)	1887-64-0	0.0152
d ₃ -Octadecanoylcarnitine (d ₃ -C18)	1822-91-8	0.0152

Stable-isotope Standard	CAS #	Approximate Amount per vial (µmoles)
AMINO ACID STANDARDS		
¹⁵ N, ²⁻¹³ C-Glycine	91795-59-42	2.5
² H ₄ -Alanine	56-41-7	0.5
² H ₈ -Valine	72-18-4	0.5
² H ₃ -Leucine	87828-86-2	0.5
² H ₃ -Methionine	13010-53-2	0.5
² H ₅ -Phenylalanine	63-91-2	0.5
¹³ C ₆ - Tyrosine	60-18-4	0.5
² H ₂ -Ornithine.2HCl	3184-13-2	0.5
² H ₂ -Citruline	372-75-8	0.5
² H ₄ , ¹³ C-Arginine.HCl	1119-34-2	0.5

Component	Quantity	Storage and shelf life
Dried Blood Spot Controls:	2 filter paper cards (Schleicher & Schuell, no. 903) containing 5 spots of each level per card.	2 - 8°C until expiry date stated on the label. Desiccated package should remain sealed during storage.
Low Level		
High Level		

Analytes included in the Controls and **approximate mean concentrations** (µmol/L):

Analyte	Low Control	High Control
Free carnitine (C0)	163.3	394.6
Acetyl carnitine (C2)	41.2	82.9
Propionyl carnitine (C3)	5.5	14.3
Butyryl carnitine (C4)	4.2	11.3
Isovaleryl carnitine (C5)	1.5	3.0
Glutaryl carnitine (C5DC)	0.5	1.0
Hexanoyl carnitine (C6)	0.8	2.2
Octanoyl carnitine (C8)	1.2	3.1
Decanoyl carnitine (C10)	0.6	1.5
Lauroyl carnitine (C12)	2.7	8.0
Myristoyl carnitine (C14)	1.7	4.8
Palmitoyl carnitine (C16)	10.7	30.5
Octadecanoyl carnitine (C18)	1.9	4.5
Alanine	580	1336
Citrulline	189	572
Glycine	578	1468
Leucine	578	1466
Methionine	120	369
Phenylalanine	283	827
Tyrosine	329	1033
Valine	329	770

* The assigned concentrations (mean and standard deviation) for each analyte are given on the individual control bags. The controls are prepared in whole human blood. The hemoglobin concentration has been adjusted to 17 ± 0.5 g/dL prior to analyte addition and dispensing onto Schleicher & Schuell, no. 903 paper. The hematocrit level is approximately equal to that of a newborn.

Component	Quantity	Storage and shelf life
Flow Solvent (a mixture of HPLC Grade Acetonitrile and 18 MΩ water).	3 bottles 473 mL (each)	20 - 30°C until expiry date stated on the bottle label. Note: Store out of direct sunlight.
Extraction Solution (a mixture of HPLC Grade Methanol and 18 MΩ water).	1 bottle 237 mL	20 - 30°C until expiry date stated on the bottle label. Note: Store out of direct sunlight.
Reconstitution Solution (a mixture of HPLC Grade Acetonitrile and 18 MΩ Water with ACS Grade Acetic Acid).	1 bottle 180 mL	20 - 30°C until expiry date stated on the bottle label. Note: Store out of direct sunlight.
3N HCL in n-Butanol	2x100 mL	20 - 25°C
Adhesive plastic covers	20 covers	20 - 25°C
Heat sealing film	20 sheets	20 - 25°C
Aluminum foil sheets	20 sheets	20 - 25°C
NUNC V-bottom heat-resistant microtiter plates	20 plates	20 - 25°C
Truncated V-bottomed clear microtiter plates	20 plates	20 - 25°C
Bar codes	20 codes	20 - 25°C

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

The NeoGram Amino Acids and Acylcarnitines Kit requires the following items, which are available from PerkinElmer Life and Analytical Sciences Inc. or its distributors:

Wallac MS² Tandem Mass spectrometer: #1445-001

NeoGram software: #MS-110S

PerkinElmer Vacuum Degasser Series 200: #MS-1110 or equivalent

PerkinElmer Autosampler Series 200: #MS-1250 or Gilson Autosampler 215: #MS-1250

PerkinElmer Series 200 Micropump: #MS-1030 or equivalent

SPE Dry Dual sample plate concentrator

Automatic puncher - DELFIA[®] Plate Punch (#1296-031), WALLAC DBS Puncher (#1296-071) or a manual puncher to cut out filter paper disks with a diameter of 1/8-inch (3 mm).

Labsystems OY Incubator/Shaker Model 1410: #NC-1034 or equivalent

Labsystems OY Incubator/Shaker Model 1415; #NC-1033 or equivalent

Abgene Plate Sealer: #MS-2500 (110v) or #MS-2501 (240v)

Hand pipettor (8-channel), 30-300 µL: #NC-3360

Additional items required:

- Reagent reservoirs (for example, Solution Basins from Labcor products, Inc) Fume Exhaust Hood.
- A source of compressed air for plate drying. In the absence of compressed air, high purity nitrogen gas cylinders can be used.

Note:

- *The device is intended to be used with the controls and internal standards recommended here in (Tables 2 and 3). The use of other standards and control materials with this device has not been validated.*
- *Additional QC materials may be necessary to meet federal, state and local guidelines for QC testing.*
- *Arginine and Ornithine are commercially available amino acids, however, they are not included in the NeoGram AAAC kit control material because of their shorter stability in blood spots. It is recommended that laboratories using the NeoGram AAAC kit source additional QC material containing these analytes.*

SPECIMEN COLLECTION AND HANDLING

The NeoGram Amino Acids and Acylcarnitines kit is intended for use with blood samples (neonatal heel prick) collected and dried on filter paper. We recommend the use of an FDA approved filter paper for sample collection (such as Schleicher & Schuell no. 903 filter paper). A method based on dried blood samples requires skillful collecting, handling and transport of samples⁷. Strictly adhere to the collection technique as described in detail in NCCLS document LA4-A3⁸.

1. Clean the skin with an alcohol swab and allow to air dry.

2. Puncture the infant's heel with a sterile lancet or with an automated lancet device to the depth of approximately 2.0 mm. Puncturing deeper than 2.0 mm on small infants may cause bone damage.
3. Wipe away the first drop of blood. Gently touch the filter paper against a large drop of blood and, in one step, allow a sufficient quantity of blood to soak through to completely fill a preprinted circle on the filter paper. Examine both sides of the filter paper to make sure that the blood penetrated and saturated the paper. Milking or squeezing the puncture may cause hemolysis of the specimen and admixture of tissue fluids with the specimen. Do not layer successive drops of blood in the collection circle (this causes caking).
4. Allow the blood specimen to air-dry in a horizontal position for at least 3 hours at room temperature, not in direct light.
5. Be sure that the required information on the specimen card has been completed, including the name, address, patient identification number and sex of the infant; the name and address of the hospital, the name, address and phone number of the physician who should be notified. Record the time and date of birth and also the time and date of collection. Indicate whether the infant was pre-term or postdate and if so, to what degree. Record the infant's birth weight and indicate whether he or she was or was not a twin.
6. Place each specimen in its own paper envelope and transport or mail to the laboratory within 24 hours after collecting the specimen.

In order to avoid faulty assay results, sample spots not uniformly saturated with blood as well as sample disks punched too close to the edge of the blood spot should be rejected. No special storage conditions are necessary when samples are analyzed within 21 days of collection, as demonstrated in validation studies.

WARNINGS AND PRECAUTIONS

The kit is for In Vitro diagnostic use only. This kit contains reagents manufactured from human blood components. The source materials have been tested by FDA approved immunoassay for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies and found to be negative. Nevertheless all recommended precautions for the handling of blood derivatives should be observed. Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.

- Handle all patient specimens as potentially infectious.
- Follow local, federal and state regulations for handling of methanol, acetonitrile and butanol solutions.
- Disposal of all waste should be in accordance with local, federal and state regulations.

ASSAY PROCEDURE

1. Preparation of internal standards reagents:
 - (a) Reconstitute the vial of dried Amino Acids internal standards with 1.0 mL of the Extraction Solution until completely dissolved (may take up to 2 hours). **This solution is stable for 30 days at 2-8°C.**
 - (b) Reconstitute the vial of dried Acylcarnitines internal standards with 1.0 mL of the Extraction Solution. *Do not invert the vial during reconstitution. Always keep the vial upright to prevent leaking.* **This solution is stable for 30 days at 2-8°C.**
 - (c) Dilute the internal standards from steps (a) and (b) by a factor of 1:200 using the Extraction Solution. An example of a 1:200 dilution would be the dispensing a 0.5 mL aliquot from the vial of each reconstituted isotopic standards into a 100 mL volumetric flask and QS'ing to 100 mL final volume with extraction solution.

This will be the daily working extraction solution containing amino acids and acylcarnitines internal standards. **This solution is stable for 24 hours and should be prepared daily.**

2. Punch out one filter paper disk from the dried blood spot, using an automatic or a manual puncher, into each well of the provided truncated V-bottomed, clear microtiter plate. The diameter of the disks should be approximately 1/8 inch (3 mm). It is recommended to use the first 2-4 wells of each plate as blanks (add only extraction solvent containing internal standards) to allow the LC system and mass spectrometer to achieve synchronization.
3. Using a multichannel pipette and reverse pipetting, add 90 μ L of the daily working extraction solution from step 1 to each well containing a filter paper disk. Cover the plate with adhesive plastic covering ensuring a good seal to minimize evaporation.
4. Immediately after covering, shake the plate for 30 minutes at 30°C (plate can be shaken at speeds ranging from 650 to 750 rpm).
5. Remove plate from incubator/shaker and remove cover from plate. Transfer 60 μ L of extracted solution to a NUNC heat resistant microtiter plate.
6. Evaporate each sample well to dryness.
7. Using the reverse pipetting technique, pipette 50 μ L of 3N HCL in n-Butanol reagent into each test well of the microtiter plate from step 6.
8. Cover the plate with a heat sealing film (see Instructions for use of the Abgene plate sealer) and incubate for 30 minutes at 60 °C.
9. Uncover plate and evaporate each sample well to dryness.

10. Using the reverse pipetting technique, add 75 μ L of Reconstitution Solution to each test well of the microplate. Cover plate with aluminum foil and incubate at 27 °C for 10 minutes with shaking (plate can be shaken at speeds ranging from 400 to 750 rpm).
11. Place the plate into the Autosampler.
12. To run the assay, start the NeoGram program, create worklists, and use the appropriate data acquisition method. Refer to the MS² NeoGram Database User Guide for details.

Note:

- ***During method installation, qualified personnel will optimize the spray height for your particular instrument. This optimal position will be indicated in the site visit report. Make sure you adjust the spray height to its optimal position before running the assay.***
- ***Use only the concentrations indicated in the inserts for the stable-isotope amino acids internal standards and stable-isotope carnitine and acylcarnitines internal standards.***

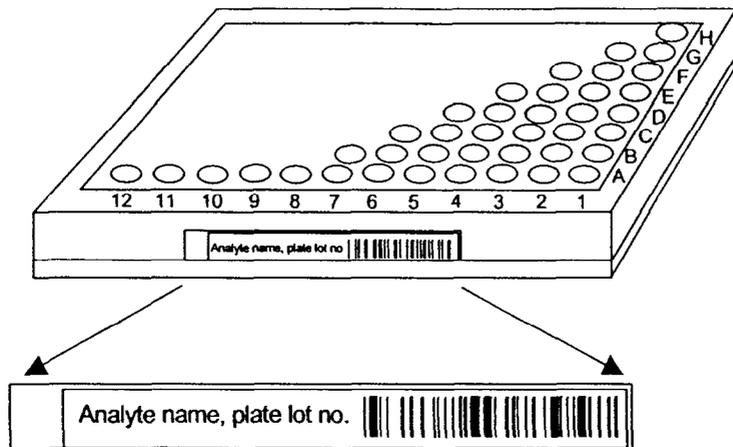
Warning: Data acquisition files should only be adjusted by qualified personnel.

INSTRUCTIONS FOR USE OF THE ABGENE PLATE SEALER

1. Turn on the instrument with power switch located on the upper left side of the cabinet. The green power lamp should be illuminated and amber heater lamp should flicker on and off when unit is ready. (When starting from cold, it is preferable to warm up the heater for 15 minutes prior to sealing).
2. Slide microplate to be sealed onto platform of sealer, making sure it is flat between the two centralized springs.
3. Place sealing film over plate, ensuring that the sealing surface is face down.
4. Holding handle of top of unit, push heater down to plate.
5. Once heater is resting on top of plate, continue to push down to compress the floating springs that are located behind the plate.
6. Once these springs are fully compressed (distance approximately 5 mm), hold the heater in position for **2-5 seconds**.
7. Return heater to top of track.
8. Remove the plate.
9. Turn power switch off when finished sealing.

INSTRUCTIONS FOR USE OF BAR CODE LABELS

The barcode labels included with this kit are designed so that they can be scanned to associate a worklist with each plate. The barcode label can be placed on any side surface of the plate. The position of the label on the plate is not critical (see diagram below).



PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for the successful use of the NeoGram Amino Acids and Acylcarnitines kit.
 - The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed on the kit label.
2. The plate covers used during the extraction and derivatization steps must cover all wells containing the reagents to minimize evaporation.
3. The aluminum foil used to cover the microtiter plate must be tight at the plate surface and folded under on all sides to minimize evaporation.
4. Use reverse pipetting for addition of Extraction Solution, 3N HCL in n-Butanol and Reconstitution Solution into the wells. Reverse pipette by drawing up more volume than is to be dispensed. Push the plunger all the way down before withdrawing, but go only to the first stop when expelling. Reverse pipette is generally more accurate when dispensing small volumes.
5. Re-use of solutions that are already poured out of the reagent bottles is not recommended.

CALCULATION OF RESULTS

The NeoGram Amino Acids and Acylcarnitines assay system incorporates programs for calculation of the concentrations of amino acids, carnitine and all types of acylcarnitines (saturated, unsaturated, hydroxylated and dicarboxylated), as well as programs for the

calculation of concentration ratios. The values are calculated by comparing the measured analyte intensities to those of the internal standards in the extraction solution. The results are obtained as printouts of these values (refer to NeoGram User Manual).

Quality Control:

The Low and High blood spot controls included in the kit should be run in duplicate in each plate. The mean values and one standard deviation (SD) for each analyte in each of the two controls are given on the label of the control bag. In addition to the included controls, it is good practice to run outside quality control samples that bridge several lot numbers of kits. Participation in quality control programs is recommended.

NOTE:

The mean values for the controls are obtained by running replicate measurements using the NeoGram Amino Acids and Acylcarnitines Kit. The values are assigned by taking the mean of all runs.

LIMITATIONS OF THE PROCEDURE

The NeoGram Amino acids and Acylcarnitines kit is a screening assay, **not a diagnostic test**. The data acquired from the use of this kit should aid other medically established procedures and should be interpreted in conjunction with other clinical data available to the physician. **A diagnostic procedure should be used for confirmation of presumptive abnormal amino acid and acylcarnitine profiles. Users should follow state and local guidelines for follow up and confirmation testing.**

1. Sample qualities that may cause faulty assay results are:
 - Storage of blood spots.
 - Sample spots not uniformly saturated with blood.
 - Sample disks punched too close to the edge of the blood spot.
 - Variables such as hematocrit, prematurity and age of the infant may affect the interpretation of values produced.
Also refer to NCCLS document LA4-A3⁸.
2. Incomplete butylation of acylcarnitines with m/z ranging from 260.2 (C6) to 428.2 (C18) may interfere with the smaller chain butylated acylcarnitines at the same mass range (C2 to C14). An excess of 3N HCl in n-Butanol is provided for in the method to minimize this effect. Dibutylated glutamate and butylated C2 share the same m/z value (260 amu). When collisionally activated, both dibutylated glutamate and butylated C2 will produce a fragment ion at m/z 85. Thus, in the precursor of 85 scan used to analyze the acylcarnitines there is, in fact, a potential interference from glutamate for the quantitation of C2. However, the effect of glutamate on C2 is most significant (outside a 2 SD reference range) at glutamate levels larger than 204 $\mu\text{mol/L}$ (3 mg/dL) in blood. Thus, in normal newborn patients, where the glutamate concentrations are expected to be below 204 $\mu\text{mol/L}$, no significant clinical interference is anticipated.
3. It has been reported that pivalic acid (an antibiotic that may be administered during pregnancy or to the infant) is the precursor of pivalylcarnitine. Pivalylcarnitine is an isomer of isovalerylcarnitine (diagnostic marker for isovaleric academia, IVA) and thus

the presence of elevated pivalylcarnitine in subjects administered pivalic acid may result in false positive cases for IVA⁹.

Please also refer to the "PROCEDURAL NOTES" section.

EXPECTED RESULTS AND INTERPRETATION OF RESULTS

Each laboratory should run a pilot study to determine the distribution of the concentrations for each analyte for their own population. From these distributions, means and cutoff values should be determined. It is recommended that laboratories should establish two levels of cutoffs, an abnormal and a borderline level. Cutoff values should also be compared to published ranges but should be individualized to the methodology used and patient population. Cutoff values for reporting abnormal and borderline results for each analyte should be established by using statistical measurements (e.g. percentiles, means, and standards deviations) in consultation with metabolic disease specialists who can provide additional guidance based on incidence rates, disease severity, and typical profiles of known positive patients. The determination of presumptive abnormal and borderline amino acid, carnitine and acylcarnitine concentration profiles should be based on predetermined cutoffs obtained from pilot studies in which at least 5000 samples have been analyzed with the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit. If available, samples from patients with known disorders (true positives) should be run to provide additional guidance in setting conservative abnormal and borderline cutoff levels. As larger numbers of samples and confirmation of presumptive positive results are obtained by each laboratory, it is recommended that this information be used for reviewing the cutoffs on a regular basis. The actions to be taken when specimens fall under either of three categories- Presumptive positive, borderline, and presumptive negative- are described below.

NOTE: *Supplementary information on nomenclature and analyte profiles related to particular disorders is provided in the Appendix at the end of the insert.*

Presumptive Positive, Borderline, and Presumptive Negative results

Presumptive Positives:

Sample results that are above (or below if it is a low cutoff) the abnormal cutoffs should be considered **presumptive positive**. For all specimens designated as presumptive positive for one or more disorders or analytes, the following actions are suggested:

- Immediate confirmation by retesting the original specimen with the original method in duplicate
- If confirmation testing cannot be completed the same day and the potential disorder requires immediate medical intervention, immediate notification of the appropriate physician or hospital of a possible presumptive positive (pending confirmation) should be made.

Borderline Specimens:

Samples that are below (or above if it is a low cutoff) the abnormal cutoff but above (or below if it is a low cutoff) the borderline cutoff should be considered **borderline specimens**. For specimens whose initial results are borderline the following actions are suggested:

- Immediate confirmation by retesting the original specimen with the original method in duplicate

Confirmation testing (repeat testing):

Regardless of whether confirmation testing was initiated because of initial borderline or presumptive positive status, if the **confirmation testing result** (average of the duplicate retest) **is above the abnormal cutoff** then the patient should be considered presumptive positive and the following actions are recommended:

- Immediate notification of the appropriate physician or hospital
- Patient results should be immediately faxed to the appropriate physician or hospital, with all abnormal results and disorder risks clearly highlighted and recommended actions indicated
- Immediate referral to the clinical management team for follow-up
- Request for a second specimen for retesting (unless the baby's health dictates otherwise)

Regardless of whether confirmation testing was initiated because of initial borderline or abnormal status, if the **confirmation testing result** (average of the duplicate retest) **is below the abnormal cutoff but above the borderline cutoff**, then these actions are recommended:

- All patient results (initial and confirmatory) should be **combined to obtain an overall average**.
- If the **overall average** is above the abnormal cutoff treat the patient as presumptive positive as indicated above.
- If the **overall average** is below the abnormal cutoff but above the borderline cutoff then treat the patient as borderline as follows:
 - Patient results should be immediately faxed to the physician or hospital, with all borderline results highlighted and an appropriate warning message to the medical provider that although this result is not indicative of the disorder that it is elevated and should be taken into consideration if the child is ill or in distress.
 - No further retesting or request for additional specimen unless initiated by the medical provider

Presumptive Negatives:

If all the initial results of any specimen, or all the averages of any confirmation results of any specimen are below all the borderline and abnormal cutoffs, the sample should be treated as normal (or low risk) and reported appropriately.

NeoGram AAAC Kit Cutoff Determination and Pilot Study

As an example of the implementation of the procedure described above we present the results of a pilot study performed with the NeoGram Amino Acids and Acylcarnitines (NeoGram AAAC) kit.

Notes:

- ***The following information is provided only as a guideline.*** It is strongly recommended that each laboratory perform a pilot study as described above.
- Confirmation by a diagnostic test procedure must be performed when abnormal profiles are suspected.
- Race was not known for the study subjects
- Follow local, state or federal requirements for follow-up testing.

Cutoff Determination

The NeoGram AAAC Kit cutoffs were determined from data acquired in a pilot study performed in collaboration with the California Department of Hygiene. This large-scale pilot study involved the screening of amino acid, fatty acid and organic acid inborn errors of metabolism by tandem mass spectrometry (MS/MS). The results from 212,345 patient samples whose amino acid and acylcarnitine concentrations were measured with the NeoGram AAAC kit were used in this population study.

An initial subset of the data consisting of 50,958 presumed normal samples and 17 specimens previously diagnosed to be true positive for inborn errors of metabolism was used to determine the cutoffs for the NeoGram AAAC kit. Statistical analysis of the results per individual analyte with emphasis on distribution tails and percentiles as well as projected false positive rates and the resulting impact on laboratory workload were taken into consideration for arriving to the cutoff levels. The values were set giving consideration to whether or not the analyte in question was a primary or a secondary marker and evaluation of published experiences by other programs regarding their cutoffs and their use of MS/MS for newborn screening. In addition, the results the 17 known true positive cases for inborn errors of metabolism were taken into consideration and thus the percentile analysis was performed with the initial goal of determining cutoffs values that would result in a 0.01%-0.03% false positive rate while at the same time correctly identifying all available known true positive samples.

In addition to determining abnormal cutoff levels, we performed an analysis of the data to establish a borderline zone. The borderline cutoffs were determined from the same set of 50,958 presumed normal samples. Based on experience from other laboratories in handling borderline results, an analysis was performed with emphasis in detecting all samples that

were up to 15% below (for high cutoffs) or above (for low cutoffs) the corresponding cutoff value. Subsequently, the predetermined borderline zone was adjusted so that no borderline cutoff would produce more warning flags than the corresponding cutoff flags. Such analysis resulted in a borderline zone that is in average 7% (1 SD= 6.6%) below or above the corresponding high or low cutoffs. The abnormal and borderline cutoffs determined here are presented in Table 4. Tables 5 and 6 present best estimates of physiological ranges for these analytes.

Table 4: NeoGram AAAC kit Abnormal and Borderline cutoffs*

Analyte	Abnormal Cutoff	Borderline Cutoff	Analyte	Abnormal Cutoff	Borderline Cutoff
C0	158.00	145.00	C16	12.00	11.30
C0 - low	10.00	12.00	C16 OH	0.75	0.65
C2	65.00	61.00	C16:1	1.55	1.47
C2-low	5.00	5.50	C16:1 OH	1.20	1.05
C3	11.00	10.40	C18	3.60	3.40
C3/C2	0.38	0.36	C18 OH	0.50	0.45
C3DC	1.10	0.95	C18:1	4.40	4.20
C4	2.50	2.46	C18:1 OH	0.50	0.45
C5	2.00	1.90	C18:2	2.30	2.20
C5 OH	1.55	1.47	Ala	1128.00	1075.00
C5:1	0.66	0.63	Arg	150.00	130.00
C5DC	0.70	0.65	Cit	85.00	70.00
C6	1.30	1.24	Gly	1450.00	1390.00
C6DC	2.00	1.85	Leu	300.00	280.00
C8	1.30	1.20	Leu/Ala	1.70	1.60
C8:1	1.40	1.32	Met	140.00	120.00
C10	1.10	1.06	Orn	390.00	370.00
C10:1	1.00	0.90	Tyr	575.00	530.00
C10:2	1.00	0.80	5-Oxo Pro	168.00	158.00
C12	2.15	2.00	Val	250.00	220.00
C12:1	1.50	1.35	Val/Ala	1.50	1.30
C14	1.30	1.20	Phe Phe/Tyr	(Phe >= 300) OR (Phe >= 212 and Phe/Tyr >= 2) OR (Phe >= 166 and Phe/Tyr >= 3)	(Phe >= 275) OR (Phe >= 200 and Phe/Tyr >= 1.8) OR (Phe >= 150 and Phe/Tyr >= 2.9)
C14 OH	0.65	0.60			
C14:1	1.10	1.04			
C14:2	0.80	0.70			

* Concentration cutoffs in $\mu\text{mol/L}$ Ratio cutoffs- No units

**Table 5: Published and Best Estimates Physiological Ranges in $\mu\text{mol/L}$:
Amino Acids**

Analyte	REFERENCE 1		REFERENCE 2		BEST ESTIMATE RANGE		CALIFORNIA PILOT MEAN [#]	
	Lower	Upper	Lower	Upper	Lower	Upper	Mean	Cutoff
ALA			239	345	239	345	332	1128
ARG			53	71	53	71	17	150
VAL	79.9	152.1	123	199	80	199	123	250
PHE	50.8	92.6	45	65	45	93	91	300
TYR	53.9	146.1	33	75	33	146	107	575
ORN			39	61	39	61	89	390
MET	18.18	37.22	15	21	15	37	32	140
LEU/ILE	70.3	135.7	87	145	70	145	142	300
GLY	291	513	178	248	178	513	505	1450
CIT	*	*	*	*	*	*	17	85

[#] Mean of 212292 normal patients tested with the NeoGram AAAC kit during the California Pilot Study.

* No published reference range for these analytes: see Pilot study from California.

**Table 6: Published and Best Estimates Physiological Ranges in $\mu\text{mol/L}$:
Acylcarnitines**

Analyte	REFERENCE 3*		REFERENCE 4		REFERENCE 5		BEST ESTIMATE RANGE		CALIFORNIA PILOT MEAN [#]	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Mean	Cutoff
C0	9.4	51.8	**	**	**	**	9.4	51.8	40.51	158
C2	4.4	20.8	5.94	32.78	3	42	3	42	19.08	65
C3	0.8	3.6	**	3.6	0.21	4.7	0.21	4.7	3.12	11
C4	0.21	0.73	**	0.29	0.05	1	0.05	1	0.58	2.5
C5	0.11	0.43	**	0.52	0.04	0.61	0.04	0.61	0.31	2.0
C5:1	0.03	0.23	**	**	**	**	0.03	0.23	0.10	0.66
C6	0.11	0.35	**	0.12	**	**	0.11	0.35	0.18	1.3
C8	0.02	0.26	**	0.15	0.01	0.36	0.01	0.36	0.23	1.3
C8:1	0.01	0.33	**	**	**	**	0.01	0.33	0.26	1.4
C10	0.02	0.22	**	0.24	**	**	0.02	0.24	0.17	1.1
C10:1	0.03	0.19	**	**	0.08	1.1	0.03	1.1	0.14	1.0
C12	0	0.36	**	0.26	**	**	0	0.36	0.41	2.15
C14	0.08	0.52	**	0.39	**	**	0.08	0.52	0.34	1.3
C14:1	0.01	0.25	**	**	**	**	0.01	0.25	0.23	1.1
C14OH	0.03	0.23	**	**	**	**	0.03	0.23	0.09	0.65
C16	1.2	6.4	1.06	7.54	0.25	9.7	0.25	9.7	3.46	12
C16:1	0.07	0.51	**	**	**	**	0.07	0.51	0.33	1.55

Analyte	REFERENCE 3*		REFERENCE 4		REFERENCE 5		BEST ESTIMATE RANGE		CALIFORNIA PILOT MEAN [#]	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Mean	Cutoff
C16OH	0.02	0.26	**	**	**	**	0.02	0.26	0.09	0.75
C16:1OH	0	0.32	**	**	**	**	0	0.32	0.09	1.2
C18	0.3	2.3	**	1.19	**	**	0.3	2.3	0.99	3.6
C18:1	0.7	3.1	**	**	**	**	0.7	3.1	1.38	4.4
C18:1OH	0	0.16	**	**	**	**	0	0.16	0.06	0.5
C10:2	**	**	**	**	**	**	**	**	0.10	1.0
C12:1	**	**	**	**	**	**	**	**	0.20	1.5
C14:2	**	**	**	**	**	**	**	**	0.09	0.8
C18 OH	**	**	**	**	**	**	**	**	0.07	0.5
C18:2	**	**	**	**	**	**	**	**	0.27	2.3
C3DC	**	**	**	**	**	**	**	**	0.12	1.1
C4DC	**	**	**	**	**	**	**	**	0.65	NA
C5 OH	**	**	**	**	**	**	**	**	0.31	1.55
C5DC	**	**	**	**	**	**	**	**	0.10	0.70
C6DC	**	**	**	**	**	**	**	**	0.20	2.0

*The reported values are means +/- 1SD. The best estimates from this reference were calculated as the reported mean +/- 2SD

[#] Mean of 212292 normal patients tested with the NeoGram AAAC kit during the California Pilot Study

** No published reference range for these analytes: see Pilot study from California.

Study Results

The study continued by applying the cutoffs presented in Table 4 to the entire data set of 212,345 neonatal screening results (including the 50,975 presumed negative and 17 true positive samples mentioned above). During this analysis, 1352 analyte measurements were flagged as having a value greater than a cutoff level. Of the 1352 measurements flagged, there were only 1141 unique specimens represented. In other words, during this study, 1141 infants were flagged for elevated amino acid and/or acylcarnitine levels. Of these 1141 infants 347 were flagged due to one or more amino acid only measurements, 783 were flagged for one or more acylcarnitine only measurements and 11 infants were flagged because of both amino acid and acylcarnitine measurements. Request of follow up information on these cases revealed that 53 of these 1141 infants were confirmed to be true positives for a metabolic disorder. Within this group of 53 confirmed cases, 10 disorders were represented (Table 7). Overall, with the cutoffs determined in this study the assay detected 53 true positive cases and resulted in 1089 false positive determinations for the data set used. In general, the number of falsely elevated flags per analyte ranged from 10 to 82, representing the false positive rates that were expected based on the cutoff evaluation. The false positive rates for individual analytes ranged from 0.005% to 0.039% of the entire data set (Table 8). The aggregate false positive rate (i.e. taking into consideration all 42

analytes evaluated) was 0.513% of the entire data set. In addition, there were 1110 measurements flagged as borderline representing 974 unique specimens providing an overall borderline specimen rate of 0.46%. None of the borderline specimens was diagnosed to be true positive for an inborn error of metabolism. The positive predictive values (PPV = Number of confirmed cases/number of flags) found in this study for each of the individual markers ranged from 1.8 to 45%; the PPV for all the amino acids was 5.3%, and the PPV for all acylcarnitines was 4.7%. The overall PPV for the entire data set was 5%. This included the screening of more than 30 disorders and the measurement of more than 40 analytes. The specificity of the NeoGram AAAC assay within the study for all of the analytes detected ranged from 99.96 to 99.99%. The sensitivity of the NeoGram AAAC kit observed was consistently 100%. In other words, no false negative cases were found throughout the study (Table 9). Finally, Table 10 shows the incidence rates for the disorders detected within this study.

Table 7: Disorders Represented by the Positive Specimens Detected by the NeoGram AAAC kit Study and their profiles (212,346 samples screened)

DISORDER	MARKERS WITH MEASUREMENTS EXCEEDING CUTOFF LEVELS							
3MCC	Val	C5 OH						
ARGD	Arg							
EMA	C4							
EMA	C4							
EMA	C4							
LCHAD	C16 OH C18 OH C18:1 OH							
LCHAD	C3/C2	C8:1	C10	C12	C12:1	C14	C14 OH	
	C14:1	C16 OH	C16:1	C16:1 OH	C18 OH	C18:1	C18:1 OH	
MCAD	C6	C8						
MCAD	C8							
MCAD	C6	C8						
MCAD	C6	C8						C10
MCAD	C6	C8						
MCAD	C6	C8						
MCAD	C6	C8						C10
MCAD	C6	C8	C10					C10:1
MCAD	C8							
MCAD	C6	C8						C10
MCAD	C4							
MMA	C3	C3/C2						
MMA	C3	C3/C2						
MMA	C3	C3/C2						
MMA	C3	C3/C2						
MMA	C3	C3/C2						
MSUD	Leu	Leu/Ala	Val					Val/Ala
MSUD	Leu	Leu/Ala	Val					Val/Ala
MSUD	Leu	Leu/Ala	Val					Val/Ala

Table 7 (continued): Disorders Represented by the Positive Specimens Detected by the NeoGram AAAC kit Study and their profiles (212,346 samples screened)

DISORDER	MARKERS WITH MEASUREMENTS EXCEEDING CUTOFF LEVELS			
PA	C3	C3/C2		
PA	C3	C3/C2		
PA	C3	C3/C2		
PKU	Phe	Phe/Tyr	Val	
MMA	C4			
MMA	C3	C3/C2		
MMA	C3	C3/C2		
MMA	C3	C3/C2		
MMA	C3	C3/C2		
MMA	C3	C3/C2		
MSUD	Leu	Leu/Ala	Val	Val/Ala
MSUD	Leu	Leu/Ala	Val	Val/Ala
MSUD	Leu	Leu/Ala	Val	Val/Ala
PA	C3	C3/C2		
PA	C3	C3/C2		
PA	C3	C3/C2		
PKU	Phe	Phe/Tyr	Val	
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
PKU	Phe	Phe/Tyr		
SCAD	C4			
SCAD	C4	C16		
SCAD	C4			

Table 8. False Positive Rates

Analyte	Number \geq Cutoff	% of 212,346 \geq Cutoff
C0	15	0.007
C2	11	0.005
C3 + C3/C3	82	0.039
C3DC	25	0.012
C4	81	0.038
C5	24	0.011
C5 OH	54	0.025
C5:1	25	0.012
C5DC	23	0.011
C6	11	0.005
C6DC	19	0.009
C8	43	0.020
C8:1	30	0.014
C10	32	0.015
C10:1	40	0.019
C10:2	16	0.008
C12	16	0.008
C12:1	23	0.001
C14	44	0.021
C14 OH	12	0.006
C14:1	47	0.022
C14:2	10	0.005
C16	19	0.009
C16 OH	15	0.007
C16:1	15	0.007
C16:1 OH	11	0.005
C18	18	0.008
C18 OH	31	0.015
C18:1	17	0.008
C18:1 OH	20	0.009
C18:2	23	0.011
Ala	21	0.010
Arg	21	0.010
Cit	15	0.007
Gly	31	0.015
Leu + Leu/Ala	47	0.022
Met	29	0.014
Orn	21	0.010
Phe + Phe/Tyr	70	0.033
Tyr	25	0.012
Val + Val/Ala	61	0.029

Table 9. Specificity and Positive Predictive Values Observed within the Study

Analyte	No. above Cutoff	No. Confirmed	Specificity	%PPV	Disorders Identified*
C0	15	0	99.993%		
C0 (low)	3	0	99.998%		
C2	11	0	99.995%		
C2 (low)	12	0	99.994%		
C3 + C3/C2	91	9	99.961%	9.9%	6 MMA, 3 PA
C3DC	25	0	99.988%		
C4	94	13	99.962%	13.8%	3 EMA, 1 MMA, 1 MCAD, 8 SCAD
C5	24	0	99.989%		
C5 OH	55	1	99.975%	1.8%	1 3MCC
C5:1	25	0	99.988%		
C5DC	23	0	99.989%		
C6	20	9	99.995%	45.0%	9 MCAD
C6DC	19	0	99.991%		
C8	54	11	99.980%	20.4%	11 MCAD
C8:1	31	1	99.986%	3.2%	1 LCHAD
C10	36	4	99.985%	11.1%	4 MCAD
C10:1	41	1	99.981%	2.4%	1 MCAD
C10:2	16	0	99.992%		
C12	17	1	99.992%	5.9%	1 LCHAD
C12:1	24	1	99.989%	4.2%	1 LCHAD
C14	45	1	99.979%	2.2%	1 LCHAD
C14 OH	13	1	99.994%	7.7%	1 LCHAD
C14:1	48	1	99.978%	2.1%	1 LCHAD
C14:2	10	0	99.995%		
C16	20	1	99.991%	5.0%	1 SCAD
C16 OH	17	2	99.993%	11.8%	2 LCHAD
C16:1	16	1	99.993%	6.3%	1 LCHAD
C16:1 OH	12	1	99.995%	8.3%	1 LCHAD
C18	18	0	99.992%		
C18 OH	33	2	99.985%	6.1%	2 LCHAD
C18:1	18	1	99.992%	5.6%	1 LCHAD
C18:1 OH	22	2	99.991%	9.1%	2 LCHAD
C18:2	23	0	99.989%		
Ala	21	0	99.990%		
Arg	22	1	99.990%	4.5%	1 ARGD
Cit	15	0	99.993%		
Gly	31	0	99.985%		
Leu + Leu/Ala	50	3	99.978%	6.0%	3 MSUD
Met	29	0	99.986%		
Orn	21	0	99.990%		
Phe + Phe/Tyr	84	14	99.967%	16.7%	14 PKU
Tyr	25	0	99.988%		
Val + Val/Ala	64	3	99.971%	4.7%	3 MSUD

* Some disorders are represented by elevations in multiple analyte markers. Thus, a single patient disorder may be listed more than once if it resulted in multiple marker elevations. Example: One LCHAD patient had elevated C16 OH, C18 OH and C18:1 OH. This one LCHAD case is listed three times in the table above, once for each of the three markers.

Table 10. Incidence Rates Observed and Comparisons to Literature

Disorder	No. Identified	Observed Incidence Rates
3MCC	1	1:212,346
ARGD	1	1:212,346
EMA	3	1:70,782
LCHAD	2	1:106,173
MCAD	11	1:19,304
MMA	7	1:30,335
MSUD	3	1:70,782
PA	3	1:70,782
PKU	14	1:15,167
SCAD	8	1:26,543
TOTAL	53	1:4007

PERFORMANCE CHARACTERISTICS

I. LINEARITY AND FUNCTIONAL SENSITIVITY

1. Linearity

The linearity of the analytes shown in Tables 11 and 12 was determined by testing dried blood spot samples containing a number of different of amino acid and acylcarnitine concentrations. Dried blood spots were prepared with high levels of both amino acids and acylcarnitines. Punches were then made from these enriched spots and extracted with extraction solution. Two-fold serial dilutions were performed on this extract with blank extraction solution. Neat extraction solution was used as a true zero. For each analyte the mean measured concentration of each level was plotted against the theoretical added amounts and linear regression analysis performed. The results of these plots are presented in Tables 11 and 12.

The analytes included in Tables 11 and 12 represent a fraction of all the analytes detected by the assay (refer to Table 1 in the Intended Use Section). Most of the remaining analytes are not available and thus their linearity limits cannot be determined using the procedure described above. However, because analytes of similar structure and chemical class will have similar responses within the assay, the linearity limits of these analytes were estimated based on those obtained by their closest analog from Tables 11 and 12 as well as on their lowest and highest concentrations successfully measured during the pilot study. In these cases, the most conservative of either the reported linearity limits in Tables 11 and 12 or the corresponding lowest and highest measured concentrations were used for determining the linearity limits of the analytes shown in Table 13.

Table 11. Upper and lower linearity Limits ($\mu\text{mol/L}$)-Acylcarnitines

LIMITS	C0	C2	C3	C4	C5DC	C6	C8	C10	C12	C14	C16	C18
UPPER	1062.5	127.5	57.18	27.03	2.50	10.03	8.22	5.4	15.97	24.5	61.4	10.25
LOWER	2.13	0.13	0.09	0.02	0.16	0.01	0.02	0.01	0.04	0.01	0.04	0.01
R ²	0.9986	0.9954	0.9990	0.9985	0.9964	0.9971	0.9984	0.9971	0.9989	0.9969	0.9982	

Table 12 Upper and lower linearity Limits ($\mu\text{mol/L}$) -Amino Acids

LIMITS	ALA	ARG	GLU	GLY	LEU	MET	ORN	PHE	TYR	VAL
UPPER	2397.5	404.3	1527.5	3805.0	1950.0	913.3	978.0	1657.5	2787.5	1840.0
LOWER	4.92	0.31	0.53	9.81	9.06	8.03	1.60	0.75	1.42	114.80
R ²	0.9980	0.9992	0.9954	0.9962	0.9929	0.9988	0.9996	0.9974	0.9986	0.9957

Table 13. Upper and lower linearity Limits ($\mu\text{mol/L}$) - Additional Analytes

LIMITS	C3DC	C4DC	C6DC	C5OH	C5	C5:1	C8:1	C10:1	C10:2	C12:1	C14:OH
UPPER	2.2	2.5	2.5	10	10	1.56	3.38	1.5	2.13	2.5	2.17
LOWER	0.2	0.2	0.2	0.02	0.02	0.02	0.02	0.01	0.01	0.04	0.01
LIMITS	C14:2	C16:OH	C16:1:OH	C16:2	C18:1:OH	C18:1	C18:OH				
UPPER	3.43	2.55	4.7	2.4	2.53	3.3	2.7	6.36			
LOWER	0.01	0.01	0.04	0.04	0.04	0.09	0.09	0.09			

2. Functional Sensitivity

The lower linearity limits from Tables 11 to 13 represent the functional sensitivities of the assay for each of the analytes tested.

In order to determine whether the functional sensitivity limits are adequate for use in newborn screening, the values presented were compared to average endogenous levels obtained from 212,292 normal newborns (Tables 14 to 16). All of these functional sensitivity limits are at or below the normal endogenous levels of these analytes, suggesting that these limits are adequate for use in screening amino acid and acylcarnitine blood levels in newborns.

Table 14. Comparison of Functional Sensitivity Limits to Normal Endogenous Levels in $\mu\text{mol/L}$ -Acylcarnitines.

LIMITS	C0	C2	C3	C4	C5DC	C6	C8	C10	C12	C14	C16	C18
Functional Sensitivity	2.13	0.13	0.09	0.02	0.16	0.01	0.02	0.01	0.04	0.01	0.04	0.01
Endogenous Level	40.5	19.1	3.12	0.58	0.10	0.19	0.23	0.17	0.41	0.34	3.46	0.99

Table 15. Comparison of Functional Sensitivity Limits to Normal Endogenous Levels in $\mu\text{mol/L}$ -Amino Acids.

LIMITS	ALA	ARG	ASP	GLY	LEU	MET	ORN	PHE	TYR	VAL
Functional Sensitivity	4.92	0.31	0.53	9.81	9.06	8.03	1.60	0.75	1.42	114.8
Endogenous Level	331.85	16.62	17.0	504.8	142.1	31.7	88.86	90.96	107.28	122.93

Table 16. Comparison of Functional Sensitivity Limits to Normal Endogenous Levels in $\mu\text{mol/L}$ -Additional Analytes.

LIMITS	C3DC	C4DC	C6DC	C5OH	C5	C5:1	C8:1	C10:1	C10:2	C12:1	C14OH
Functional sensitivity	0.2	0.2	0.2	0.02	0.02	0.02	0.02	0.01	0.01	0.04	0.01
Endogenous Level	0.12	0.65	0.20	0.31	0.31	0.10	0.26	0.14	0.10	0.20	0.09
LIMITS	C14:1	C14:2	C16OH	C16:1OH	C16:1	C18:1OH	C18:1	C18OH			
Functional sensitivity	0.01	0.01	0.04	0.04	0.04	0.09	0.09	0.09			
Endogenous Level	0.23	0.09	0.10	0.094	0.33	0.064	1.38	0.066			

II. REPRODUCIBILITY (Full Scan)

Dried blood spots were prepared using two concentrations of amino acids and acylcarnitines spiked into whole blood. These spots were designated "whole blood medium" (WBM) and "whole blood high" (WBH). In addition, non-spiked endogenous blood, "whole blood normal" (WBN) was also prepared. To test for precision, duplicates of the normal, medium and high samples were analyzed in two runs per day for a total of 20 days; this produced a total of 40 precision runs. Results are presented in Tables 17 and 18.

1. TOTAL IMPRECISION (Full Scan)

The total imprecision of the kit using the Full Scan method was determined by combining all the imprecision components, i.e., within run (WR), between run (BR), between day (BD) and inter-injection (I-I) according to the following formula:

$$\text{Total Imprecision} = [(WR)^2 + (BR)^2 + (BD)^2 + (I-I)^2]^{1/2}$$

Note: The Inter-injection percent CV for the low and high samples were averaged. This value was then applied to each of the samples (WBN, WBM, and WBH) presented in Tables 17 and 18. Finally, the values obtained for each level was averaged to obtain the total imprecision for the Full Scan. The data is presented in Tables 17 and 18.

Table 17. Full Scan Method Total Imprecision-Amino Acids

Amino Acids	Ala			Arg			Cit		
	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH
Mean (µmol/L)	315.56	391.60	558.75	35.21	68.46	154.38	84.79	145.89	265.23
Within-run %CV	10.0	8.2	9.2	8.6	6.3	8.7	5.1	4.3	4.6
Between-day %CV	7.4	8.1	7.0	18.6	23.2	17.5	8.8	9.5	8.1
Between-run-within-day %CV	2.5	1.2	1.2	5.0	3.9	6.8	3.5	1.5	3.6
Inter-injection %CV	1.1	1.1	1.1	1.5	1.5	1.5	1.3	1.3	1.3
TOTAL PRECISION %CV	12.7	11.7	11.7	21.4	25.7	20.8	10.8	10.6	10.0
AVERAGE TOTAL PRECISION %CV	12.0			22.6			10.5		
Amino Acids	Gly			Leu			Met		
	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH
Mean (µmol/L)	339.09	470.84	723.29	196.50	268.91	400.65	78.20	136.66	248.70
Within-run %CV	6.0	4.1	4.6	6.4	3.1	10.6	14.3	12.8	17.6
Between-day %CV	7.3	8.1	7.2	8.6	9.0	9.1	10.2	10.3	8.6
Between-run-within-day %CV	1.6	2.1	2.9	4.8	5.8	3.5	5.1	2.4	8.1
Inter-injection %CV	0.9	0.9	0.9	0.7	0.7	0.7	1.3	1.3	1.3
TOTAL PRECISION %CV	9.7	9.4	9.1	12.9	13.4	14.5	18.4	16.6	21.2
AVERAGE TOTAL PRECISION %CV	9.4			13.6			18.7		

Table 17. Continued

	Orn			Phe			Tyr			Val		
	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH
Mean ($\mu\text{mol/L}$)	166.88	237.01	365.16	105.29	155.65	251.45	91.05	137.61	229.33	184.61	242.78	355.19
Within-run %CV	8.6	9.1	11.1	8.9	6.4	7.1	10.2	8.2	8.6	9.7	9.3	16.0
Between-day %CV	6.1	7.4	4.9	9.3	9.6	8.8	10.2	9.0	8.0	8.2	8.6	13.8
Between-run-within-day %CV	2.0	1.9	3.6	4.5	1.6	3.4	2.8	1.8	1.5	4.2	7.7	8.7
Interinjection %CV	1.3	1.3	1.3	0.8	0.8	0.8	0.9	0.9	0.9	1.3	1.3	1.3
TOTAL PRECISION %CV	10.8	12.0	12.8	13.6	11.6	11.8	14.7	13.1	11.9	13.4	14.9	22.9
AVERAGE TOTAL PRECISION %CV	11.8			12.4			13.2			17.1		

Table 18. Full Scan Method Total Imprecision-Acylcarnitines

CARNITINES	C0			C2			C3			C4		
	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH
Mean ($\mu\text{mol/L}$)	68.79	96.80	149.25	21.48	25.02	31.60	3.65	5.23	8.37	2.84	5.05	9.37
Within-run %CV	5.6	4.0	4.8	6.2	4.5	5.1	23.8	32.6	28.3	21.5	18.8	19.0
Between-day %CV	5.0	5.2	4.6	13.5	14.1	13.5	19.7	16.4	16.7	1.7	5.2	5.0
Between-run-within-day %CV	2.1	2.8	3.4	9.1	10.8	9.1	5.6	4.5	5.7	20.1	11.3	7.4
Interinjection %CV	1.2	1.2	1.2	1.7	1.7	1.7	2.7	2.7	2.7	2.3	2.3	2.3
TOTAL PRECISION %CV	7.9	7.2	7.5	17.5	18.4	17.1	34.8	39.4	33.5	29.5	22.7	21.1
AVERAGE TOTAL PRECISION %CV	7.5			17.7			35.9			24.4		

	C6			C8			C10			C12		
	WBN	WBM	WBH									
Mean ($\mu\text{mol/L}$)	1.40	2.69	5.23	1.79	3.51	6.26	1.33	2.35	4.52	1.29	2.19	4.35
Within-run %CV	23.6	23.4	20.8	25.1	23.5	16.5	16.5	15.7	18.6	22.5	16.9	15.4
Between-day %CV	7.9	10.4	8.6	6.2	12.4	8.3	9.0	4.3	9.7	12.4	11.9	4.6
Between-run-within-day %CV	9.3	11.1	13.4	10.1	10.7	12.3	3.0	8.1	6.0	3.1	5.0	8.1
Interinjection %CV	3.6	3.6	3.6	2.9	2.9	2.9	2.8	2.8	2.8	2.7	2.7	2.7
TOTAL PRECISION %CV	26.8	25.9	26.5	27.9	28.8	22.3	19.3	18.4	22.0	26.0	21.4	18.2
AVERAGE TOTAL PRECISION %CV	26.4			26.3			19.9			21.9		

	C14			C16			C18		
	WBN	WBM	WBH	WBN	WBM	WBH	WBN	WBM	WBH
Mean ($\mu\text{mol/L}$)	1.16	2.17	4.13	1.26	2.39	4.62	2.08	3.03	5.0
Within-run %CV	26.7	14.3	12.8	11.0	16.3	12.9	20.2	13.9	13.6
Between-day %CV	4.3	2.3	9.0	10.8	8.4	6.2	6.7	8.6	4.9
Between-run-within-day %CV	8.6	6.9	12.8	4.0	7.5	4.2	10.1	5.9	5.6
Interinjection %CV	2.1	2.1	2.1	1.6	1.6	1.6	2.0	2.0	2.0
TOTAL PRECISION %CV	28.5	16.2	20.3	16.0	19.8	15.0	23.6	17.5	15.6
AVERAGE TOTAL PRECISION %CV	21.7			16.9			18.9		

2. MRM Method-Precision.

For the MRM method precision study, blood spots containing 3 different levels of amino acids and acylcarnitines were prepared and designated "Low, Medium and High". In

addition, non-spiked endogenous blood, designated "Endo" was also prepared. To test for precision, triplicates of the four sample levels were analyzed in two runs per day for a total of 5 days; this produced a total of 10 precision runs¹⁰. These studies produced a within-run precision component and a Total precision component. This total precision component did not include the inter-injection precision. To calculate the actual total precision of the All MRM Method, The "total precision" obtained in this study was designated as the "Sub-total precision". The inter-injection component was added to the sub-total precision according to the equation below and thus arriving to the "Total Imprecision" of the All MRM Method (Tables 19 and 20).

$$\text{Total Imprecision} = [(\text{sub-total})^2 + (\text{inter-injection})^2]^{1/2}$$

Table 19. All MRM Method Total Imprecision-Amino Acids

Amino Acids ALL MRM	Ala				Arg				Cit							
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High				
Mean (µmol/L)	223.3	388.2	595.1	993.1	24.6	73.0	142	255.1	47.0	175.5	349	662.2				
Within-run %CV	6.0	6.0	6.0	7.0	4.0	4.0	2.0	4.0	5.0	4.0	3.0	4.0				
Sub-Total %CV	6.0	6.0	8.0	7.0	7.4	4.0	4.0	5.0	5.0	5.0	4.0	5.0				
Inter-injection %CV	1.1	1.1	1.1	1.1	1.5	1.5	1.5	1.5	1.3	1.3	1.3	1.3				
TOTAL PRECISION %CV	6.1	6.1	8.1	7.1	7.6	4.3	4.3	5.2	5.2	5.2	4.2	5.2				
AVERAGE TOTAL PRECISION %CV	6.8				5.3				4.9							
	Gly				Leu				Met							
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High				
Mean (µmol/L)	226.1	505.5	843	1500	134.3	373.8	691	1221	16.6	87.5	187	367.1				
Within-run %CV	6.0	7.0	4.0	5.0	6.0	5.0	4.0	5.0	4.0	6.0	4.0	5.0				
Sub-Total %CV	6.0	7.0	6.0	5.0	9.0	7.0	4.0	5.0	4.0	7.0	6.0	6.0				
Inter-injection %CV	0.9	0.9	0.9	0.9	0.7	0.7	0.7	0.7	1.3	1.3	1.3	1.3				
TOTAL PRECISION %CV	6.1	7.1	6.1	5.1	9.0	7.0	4.1	5.0	4.2	7.1	6.1	6.1				
AVERAGE TOTAL PRECISION %CV	6.1				6.3				5.9							
	Orn				Phe				Tyr				Val			
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High
Mean (µmol/L)	91.1	194.7	311.9	512.7	56.4	202.1	402	730.6	50.6	250.5	521	970.7	119.4	253.5	425	753
Within-run %CV	4.0	5.0	4.0	4.0	6.0	5.0	4.0	5.0	6.0	5.0	4.0	4.0	5.0	4.0	5.0	5.0
Sub-Total %CV	4.0	5.0	4.0	5.0	6.0	5.0	6.0	6.0	6.0	6.0	4.0	6.0	7.0	4.0	5.0	5.0
Inter-injection %CV	1.3	1.3	1.3	1.3	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.9	1.3	1.3	1.3	1.3
TOTAL PRECISION %CV	4.2	5.2	4.2	5.2	6.1	5.1	6.1	6.1	6.1	6.1	4.1	6.1	7.1	4.2	5.2	5.2
AVERAGE TOTAL PRECISION %CV	4.7				5.8				5.6				5.4			

Table 20. All MRM Method Total Imprecision-Acylcarnitines

CARNITINES ALL MRM	C0				C2				C3				C4			
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High
Mean (µmol/L)	5.7	12.9	22.7	41.0	20.1	33.1	49	75.6	18	19.3	19	37.9	0.03	2.3	4.9	10.0
Within-run %CV	6.0	4.0	5.0	5.0	6.0	5.0	5.0	5.0	8.0	5.0	6.0	5.0	8.0	7.0	7.0	4.0
Sub-Total %CV	12.0	8.0	10.0	10.0	8.0	11.0	7.0	10.0	10.0	5.0	6.0	5.0	8.0	7.0	7.0	6.0
Inter-injection %CV	1.2	1.2	1.2	1.2	1.7	1.7	1.7	1.7	2.7	2.7	2.7	2.7	2.3	2.3	2.3	2.3
TOTAL PRECISION %CV	12.1	8.1	10.1	10.1	8.2	11.1	7.2	10.1	10.4	5.7	6.6	5.7	8.3	7.4	7.4	6.4
AVERAGE TOTAL PRECISION %CV	10.1				9.2				7.1				7.4			
	C6				C8				C10				C12			
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High
Mean (µmol/L)	0.04	0.57	1.31	2.74	0.12	0.74	1.59	3.24	0.08	0.48	1.1	2.22	0.17	1.42	3.1	6.59
Within-run %CV	12.0	7.0	6.0	5.0	8.0	6.0	5.0	7.0	9.0	6.0	4.0	5.0	9.0	6.0	5.0	6.0
Sub-Total %CV	14.0	10.0	6.0	5.0	9.0	9.0	7.0	7.0	9.0	12.0	6.0	8.0	11.0	6.0	5.0	9.0
Inter-injection %CV	3.6	3.6	3.6	3.6	2.9	2.9	2.9	2.9	2.8	2.8	2.8	2.8	2.7	2.7	2.7	2.7
TOTAL PRECISION %CV	14.5	10.6	7.0	6.2	9.5	9.5	7.6	7.6	9.4	12.3	6.6	8.5	11.3	6.6	5.7	9.4
AVERAGE TOTAL PRECISION %CV	9.6				8.5				9.2				8.2			
	C14				C16				C18							
	Endo	Low	Mid	High	Endo	Low	Mid	High	Endo	Low	Mid	High				
Mean (µmol/L)	0.12	1.26	2.88	6.06	0.92	3.54	18.6	37.8	0.59	1.41	2.6	4.9				
Within-run %CV	7.0	6.0	6.0	5.0	8.0	5.0	7.0	7.0	7.0	4.0	5.0	6.0				
Sub-Total %CV	11.0	9.0	11.0	9.0	8.0	8.0	7.0	7.0	7.0	7.0	5.0	7.0				
Inter-injection %CV	2.1	2.1	2.1	2.1	1.6	1.6	1.6	1.6	2.0	2.0	2.0	2.0				
TOTAL PRECISION %CV	11.2	9.2	11.2	9.2	8.2	8.2	7.2	7.2	7.3	7.3	5.4	7.3				
AVERAGE TOTAL PRECISION %CV	10.2				7.7				6.8							

The analytes included in Tables 19 to 20 represent a fraction of all the analytes detected by the assay (refer to Table 1 in the Intended Use Section). Most of the remaining analytes are not available and thus their precision can only be determined at their endogenous levels (Table 21). However, because analytes of similar structure and chemical class will have similar responses within the assay, the precision of these analytes at higher concentration levels (i.e. cutoffs levels) are estimated to be the precision obtained by their closest analogs from Tables 17-20. The compounds to be used as reference for estimating the precision of these additional analytes at higher concentration levels are indicated in table 22.

**Table 21: Within Run Imprecision at endogenous levels (MRM mode):
Additional Analytes**

ANALYTE	MEAN μmol/L	SD	%CV	ANALYTE	MEAN μmol/L	SD	%CV
C5	0.175	0.021	11.9	C14:2	0.045	0.009	19.39
C5:1	0.035	0.005	14.2	C14OH	0.020	0.004	18.43
C5-OH	0.309	0.022	7.1	C16:1	0.063	0.007	11.48
C8:1	0.059	0.007	11.4	C16OH	0.016	0.004	21.74
C10:1	0.033	0.005	16.0	C18:1	1.503	0.112	7.48
C10:2	0.016	0.003	19.3	C18:2	0.286	0.020	6.98
C3DC	0.034	0.005	13.50	C18:1OH	0.031	0.010	30.68
C4DC	0.490	0.036	7.25	C18OH	0.016	0.005	28.87
C6DC	0.027	0.005	16.67				
C12:1	0.027	0.004	14.6				
C14:1	0.073	0.010	13.01				

Table 22: Reference Guide for Precision Estimation

Precision Reference	Analyte	Precision Reference	Analyte
C6	C5	C16	C16:1
C6	C5:1	C16	C16OH
C6	C5OH	C16	C16:1OH
C8	C8:1	C18	C18:1
C10	C10:1	C18	C18:2
C10	C10:2	C18	C18:1OH
C5DC	C3DC	C18	C18OH
C5DC	C4DC		
C5DC	C6DC		
C12	C12:1		
C14	C14:1		
C14	C14:2		
C14	C14-OH		

III. RECOVERY

The recovery of the NeoGram Amino Acids and Acylcarnitines assay was determined from a series of studies in which whole blood samples containing several levels of amino acids and acylcarnitines (including endogenous samples) were analyzed. For several of these studies the recovery was calculated according to equation 1 below and for others the recovery was determined based on the slope of regressions lines in which measured amounts were plotted against the spike values. The summary of these results is presented in Table 23.

$$\text{Eq. (1) Recovery} = \frac{[\text{Measured amount} - \text{Measured endogenous amount}]}{\text{Added amount}}$$

Table 23. Recoveries by the NeoGram Amino Acids and Acylcarnitines Assay

Overall Recovery-Acylcarnitines				Overall Recovery-Amino Acids			
Analyte	Mean	SD	%CV	Analyte	Mean	SD	%CV
C0	139%	0.17	12.3	Alanine	71%	0.08	11.3
C2	67%	0.15	22.6	Arginine	91%	0.11	12.2
C3	97%	0.18	18.7	Citrilline	93%	0.08	9.0
C4	104%	0.12	11.4	Glycine	87%	0.1	11.3
C5DC	102%	0.14	13.6	Leucine	69%	0.06	9.3
C6	89%	0.09	9.8	Methionine	89%	0.16	18.0
C8	105%	0.22	20.7	Ornithine	72%	0.1	14.0
C10	83%	0.83	10.0	Phenylalanine	96%	0.21	22.3
C12	106%	0.35	33.4	Tyrosine	81%	0.09	11.3
C14	95%	0.17	17.7	Valine	68%	0.1	16.3
C16	98%	0.15	15.2				
C18	89%	0.16	18.1				
	Mean	SD	%CV		Mean	SD	%CV
Overall (AC)	99%	0.17	16.7	Overall (AA)	83%	0.11	13.4
	Overall Mean	SD	%CV				
	91%	0.16	17.6				

IV. COMPARISON OF METHODS

A sample set of dried blood spots was analyzed with the NeoGram AAAC kit and an established in-house tandem mass spectrometry-based derivatized assay currently used for routine newborn screening of amino acids metabolic disorders as well as fatty acid oxidation and organic acid metabolic defects. Results from the following analytes were compared: C0, C2, C3, C4, C6, C8, C10, C12, C14, C16, C18, Ala, Gly, Leu, Met, Phe, Tyr and Val. Overall, the NeoGram AAAC kit and the reference assay demonstrated comparable results. The mean difference between the two methods for the 18 analytes mentioned above was 17% (range: 3.0 – 36%), and was calculated as the difference in the linear relationship between the two methods. The correlation between analyte results was very good and any differences observed were primarily due to lower results from the NeoGram AAAC assay compared to the results reported by the reference method. The majority of bias, if observed at all, occurred in the elevated analyte ranges. The levels of bias around the cutoff levels

appear to be less significant or non-existent. Table 24 presents a summary of comparison results including the slope, intercept and R² values for each analyte.

Table 24. Comparison of Results: NeoGram AAAC Kit vs. Reference in-house Tandem Mass Spectrometry Assay.

ANALYTE	SLOPE	95 %CONFIDENCE INTERVAL		INTERCEPT	95 %CONFIDENCE INTERVAL		R ²	% BIAS (at cutoff)
		LOWER	UPPER		LOWER	UPPER		
ALA	0.72	0.685	0.757	-14.39	-51.481	22.696	0.98	-37
GLY	1.07	1.030	1.114	40.96	-1.567	83.488	0.98	12
LEU	0.94	0.904	0.980	22.95	7.015	38.880	0.98	5
MET	0.97	0.930	1.006	8.29	-3.748	20.333	0.98	18
PHE	0.87	0.821	0.912	4.75	-13.319	22.820	0.97	-15
TYR	0.67	0.651	0.689	6.95	-0.056	14.093	0.99	-25
VAL	0.72	0.658	0.777	23.67	-3.945	51.285	0.94	-31
CO	1.12	1.088	1.158	5.06	0.533	9.578	0.99	11
C2	0.72	0.685	0.761	4.94	2.926	6.952	0.97	-32
C3	0.8	0.742	0.856	-0.1	-0.921	0.726	0.95	-20
C4	1.04	0.989	1.096	0.38	0.177	0.581	0.97	32
C6	0.92	0.867	0.971	-0.23	-0.738	0.279	0.97	-2
C8	0.87	0.838	0.905	0.5	0.086	0.910	0.98	5
C10	0.64	0.610	0.666	-0.09	-0.450	0.271	0.98	-27
C12	1.12	1.050	1.180	0.04	-0.806	0.727	0.97	17
C14	0.83	0.794	0.872	0.21	-0.367	0.784	0.98	6
C16	0.7	0.644	0.746	-0.01	-1.129	1.102	0.95	-35
C18	0.64	0.603	0.668	-0.01	-0.805	0.794	0.97	-24

V. INTERFERENCE

Fifty-one compounds were considered to have the potential to be present in newborn blood or in the blood of pregnant mothers. Additionally, several compounds such as preservatives, buffers, and anticoagulants were considered to have the potential to be present or be introduced during sample collection and analytical processes. The overall effects of these 51 substances were unknown and thus were evaluated for both amino acids and acylcarnitines with the exception of glutamate, ampicillin, and gentamicin whose effects were evaluated for the acylcarnitines only. Blood spots containing known concentrations of amino acids and acylcarnitines as well as potential interferents were prepared according to NCCLS guidelines¹¹. Control Blood spots were prepared as well. To be identified as a potential interferent a compound must have had a significant effect on an analyte concentration when compared to control values. The criteria used were the following: a median value outside (+/-) 15% of the control, a median value outside (+/-) one standard

deviation of the control, and a significant Student's T-test (two-sample assuming equal variance, $p = 0.05$). After analysis, 5 analytes were considered to potentially result in clinically significant interference in newborn screening and were subjected to the follow up dose response study. These 5 potential interferents (and analytes affected) included:

1. Asparagine (ASN) interfering with ornithine
2. Hydroxyproline (HP) interfering with leucine.
3. Methionine sulfone (MSF) interfering with tyrosine.
4. Methionine sulfoxide (MSX) interfering with methionine.
5. Glutamate (GLU) interfering with acetylcarnitine (C2).

For the dose response study, whole blood was enriched with several levels of each identified potential interfering compound and spotted on filter paper¹¹. Control spots were also prepared. These spots were analyzed by MS/MS using the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit. Each interferent level of the Dose Response study was evaluated by the same criteria used for the initial screen described above. The results of these dose response tests are presented in Table 25. In addition, linear regression analysis was performed on the dose response data sets to estimate the lowest interferent concentration that would cause a 15% increase in the target analyte concentration (Table 26) This was necessary to obtain an estimation of the effect of intermediate interferent levels.

Table 25. Interference Dose Response Evaluations.

25a. Hydroxyproline (HP) interference on Leucine

HP level ($\mu\text{mol/L}$)	76.2	438.0	799.8	1161.7	1523.5
Median Intf	527.0	1033.0	1330.0	1765.	2165.0
Median Control	470.5	470.5	470.5	470.5	470.5
% Increase/Control	(+) 12.0	(+) 119.8	(+) 182.7	(+) 275.1	(+) 360.1

25b. Methionine Sulphone (MSF) interference on Tyrosine

MSF level ($\mu\text{mol/L}$)	61.3	352.7	644.0	935.3	1226.7
Median Intf	270.5	296.0	336.5	324.5	407.5
Median Control	256.0	256.0	256.0	256.0	256.0
% Increase/Control	(+) 5.7	(+) 15.6	(+) 31.4	(+) 26.6	(+) 59.2

25c. Methionine Sulfoxide (MSX) interference on Methionine

MSX level ($\mu\text{mol/L}$)	68	391	714	1037	1360
Median Intf	174.5	315	605.00	674.50	949.00
Median Control	134.00	134.00	134.00	134.00	134.00
% Increase/Control	(+) 13.0	(+) 135.1	(+) 351.5	(+) 403.4	(+) 608.2

Table 25. Continued

25d. Asparagine (ASN) interference on Ornithine

ASN level ($\mu\text{mol/L}$)	75.7	435.0	794.3	1153.7	1513.0
Median Intf	240.5	311.5	365.50	372.0	421.00
Median Control	267.50	267.50	267.50	267.50	267.50
% Increase/Control	(-)10.1	(+) 16.4	(+) 36.6	(+) 39.1	(+) 57.4

25e. Glutamate (GLU) interference on Acetylcarnitine

GLU level ($\mu\text{mol/L}$)	68.1	136.3	204.4	272.6	340.7	408.9	477.0	545.1	613.3	681.4
Median Intf	9.34	9.76	10.31	10.53	11.75	12.67	13.28	13.32	14.62	14.92
Median Control	8.93	8.93	8.93	8.93	8.93	8.93	8.93	8.93	8.93	8.93
% Increase/Control	(+)4.6	(+)9.3	(+)15. 5	(+)17. 9	(+)31. 6	(+)41. 9	(+)48. 7	(+)49. 2	(+)63. 7	(+)67. 1

Level ($\mu\text{mol/L}$) = Interferent spike level

Median Intf = Median concentration ($\mu\text{mol/L}$) of each analyte measured in the interferent-spiked samples.

Median Control = Median concentration ($\mu\text{mol/L}$) of each analyte measured in the control samples.

% Increase/Control = Percent increase over Median Control.

Table 26. Dose response experiments for interferents/analyte

Interferent/Analyte	Lowest Interferent Concentration Tested (%) ¹	Estimated Interferent Concentration (15% analyte Increase) ²	Lowest Interferent Concentration Tested that caused >15% analyte increase. ³
Methionine sulfoxide/methionine	68 $\mu\text{mol/L}$ (+13%)	34 $\mu\text{mol/L}$	391 $\mu\text{mol/L}$ (+135%)
Asparagine/ornithine	75.7 $\mu\text{mol/L}$ (-10.1%)	363 $\mu\text{mol/L}$	435 $\mu\text{mol/L}$ (+16.4%)
Hydroxyproline/leucine	76.2 $\mu\text{mol/L}$ (+12%)	64 $\mu\text{mol/L}$	438 $\mu\text{mol/L}$ (+119.8%)
Methionine sulfone/tyrosine	61.3 $\mu\text{mol/L}$ (+5.7%)	363 $\mu\text{mol/L}$	352.7 $\mu\text{mol/L}$ (+15.6%)
Glutamate/acetylcarnitine	68.1 $\mu\text{mol/L}$ (+4.6%)	143 $\mu\text{mol/L}$	204.4 $\mu\text{mol/L}$ (+15.5%)

¹Lowest Interferent concentration tested ($\mu\text{mol/L}$). Percent analyte concentration change over control expressed in parenthesis.

²Estimated interferent concentration ($\mu\text{mol/L}$) that would cause a 15 percent change over measured analyte control concentration.

³Lowest Interferent concentration ($\mu\text{mol/L}$) that caused at least a 15 percent change over measured analyte control concentration. Percent analyte concentration change over control value expressed in parenthesis.

1. **Hydroxyproline interference on leucine:** Hydroxyproline is an isomer of leucine; these two compounds share the same mass to charge ratio, m/z (188 amu) and produce the same MS/MS identifying fragment ion. As a result the peak at m/z 188 in the amino acid MS/MS scan contains not only leucine but also hydroxyproline. In addition to hydroxyproline, a third isomer, isoleucine, can also be found at this m/z 188

peak. Leucine/isoleucine levels are used as markers for Maple Urine Syrup Disease (MSUD), a disorder of branched chain amino acids metabolism including leucine, isoleucine, and valine. MSUD detection is not based on leucine/Isoleucine levels alone but also on the levels of valine as well concentration ratios, such as the leucine/alanine ratio, that are also used to mitigate this mass overlap. The reported normal levels of hydroxyproline in infants ages 0-2 years old is $< 32 \mu\text{mol/L}$ ¹². The lowest hydroxyproline concentration that caused a statistically significant increase in leucine levels was $438 \mu\text{mol/L}$ (Table 21a; 120% increase over control values) The lowest hydroxyproline concentration tested, $76.2 \mu\text{mol/L}$, only caused a 12% increase in leucine concentrations. Linear regression analysis predicts that the lowest concentration of hydroxyproline that would cause a 15% increase in the concentration of leucine is approximately $64 \mu\text{mol/L}$ and that $32 \mu\text{mol/L}$ of hydroxyproline should cause only a 5% increase in the concentration of Leucine (Table 26). This data suggests that 2 to 2.5 times the typical endogenous levels of hydroxyproline would be needed to cause a statistically significant increase (15% or more) in leucine concentrations. Therefore, it is unlikely that hydroxyproline will be a clinically significant interferent for this assay.

- 2. Glutamate interference with acetylcarnitine (C2):** Although glutamate and C2 are not isomers, they share the same m/z (260 amu). In addition, glutamate produces a fragment ion at m/z 85 amu, which is also a common fragment ion produced by all acylcarnitines and is used as the identifying mass transition in the MS/MS acylcarnitine scan. Therefore, although glutamate is an amino acid, the peak at m/z 260 could contain both glutamate and C2. The reported normal range for Glutamate is 27 – 77 $\mu\text{mol/L}$ for infants 0-2 years old¹³. This range is 3 to 7 times below the lowest measured and estimated glutamate concentrations (Table 25a, 240 and 143 $\mu\text{mol/L}$, respectively) where interference becomes significant. Therefore, our results indicate that glutamate should not be a clinically significant interferent for this assay.
- 3. Methionine sulfoxide interference on methionine and methionine sulfone interference on tyrosine:** Methionine is readily oxidized by oxygen reactive species (ROS) to form methionine sulfoxide. Conversion of the sulfoxide to sulfone requires stronger oxidative conditions not commonly observed *in vivo*. The enzyme methionine sulfoxide reductase (MSR) provides an intracellular repair mechanism for converting methionine sulfoxide back to methionine, but there is no biological mechanism for the conversion of methionine sulfone¹⁴. Thus, the mechanism of interference is an *invitro* reduction of the spiked methionine sulfoxide into methionine for the methionine sulfoxide/methionine pair and a molecular mass overlap for the methionine sulfone/tyrosine pair. Because methionine sulfoxide is the product of the oxidation of methionine and methionine sulfone is the product of the oxidation of methionine/sulfoxide, the highest concentration of either of these two compounds could not exceed the normal level of methionine in infants age 0-2 years old ($15\text{-}21 \mu\text{mol/L}$)¹². The spiked concentration at which methionine sulfoxide becomes an interferent for methionine is $391 \mu\text{mol/L}$ (Table 25c). The spiked concentration at which methionine sulfone becomes an interferent for tyrosine is $352 \mu\text{mol/L}$ (Table 25b). The lowest estimated methionine sulfoxide concentration that would cause a 15% increase in the concentration of methionine is $34 \mu\text{mol/L}$ (Table 26). The lowest estimated methionine sulfone concentration that would cause a 15% increase in the concentration of tyrosine

is 363 $\mu\text{mol/L}$ (Table 26). These levels are 3 to 4 times higher than the expected normal methionine sulfoxide ranges and 16 to 23 times the expected normal levels of methionine sulfone. Therefore, it is unlikely that methionine sulfoxide or methionine sulfone will be a clinically significant interference for this assay.

4. **Asparagine interference on ornithine:** Ornithine and asparagine share the same molecular mass and produce the same identifying product ion, thus in the MS/MS amino acid scan they are both recorded at m/z 189 amu. The normal expected asparagine levels in blood could not be found. The level at which asparagine caused a significant effect on the concentration of ornithine is 435 $\mu\text{mol/L}$ (Table 25d). The lowest estimated value at which asparagine could cause a 15% increase in the expected ornithine concentration is 363 $\mu\text{mol/L}$ (Table 26). Thus asparagine could be a potential interferent for ornithine at blood levels around 360–435 $\mu\text{mol/L}$.

In general, the levels at which the compounds discussed above cause a statistically significant change on a target analyte concentration are several times higher than their expected endogenous levels. These compounds are, therefore, not likely to cause clinical significant interference in the routine use of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry assay in newborn screening settings.

In addition to above findings, pivalic acid may interfere with the screening of Isovaleric academia (IVA). It has been reported that pivalic acid (antibiotic given during pregnancy or to the infant) is the precursor of pivalylcarnitine. Pivalylcarnitine is an isomer of isovalerylcarnitine (diagnostic marker for IVA) and thus the presence of elevated pivalylcarnitine in subjects administered pivalic acid may result in false positive cases for IVA⁹.

VI. CARRYOVER

Carryover was evaluated in a series of experiments using the triple quadrupole tandem mass spectrometer equipped with a Gilson 215 Autosampler. These experiments are described below ("Sample Sets").

Sample Sets-carryover:

Data was grouped into six sets:

1. Reference Low samples (24 replicates)
2. Reference Mid samples (24 replicates)
3. Low samples directly following High samples (22 replicates)
4. Low samples directly following Mid samples (22 replicates)
5. Low samples directly following Low samples (22 replicates)
6. Mid samples directly following High samples (22 replicates)

The means of each analyte in the above sample sets were then grouped into 4 data sets and analyzed by T-tests:

1. Reference Low vs. Low after High
2. Reference Low vs. Low after Mid
3. Low after High vs. Low after Low
4. Reference Mid vs. Mid after High

From a total of 92 T-tests, 73 demonstrated no statistical significant difference between the data sets. Therefore, there was no evidence of carry over in these particular comparisons. However, 19 data sets did returned significant T-tests. In 15 of these data sets, however, a **negative** difference was observed, i.e., the trend observed was opposite to that expected if carryover was present and thus no carryover was observed in these particular cases. The remaining 4 sets did show positive differences between the reference and test means. Closer inspection of these 4 cases revealed that the percent differences observed between these data sets were well within the imprecision values of the assays, suggesting that carryover is not present in these sets either. Thus it was concluded that there was no indication of carryover for the NeoGram Amino Acid and Acylcarnitines Tandem Mass Spectrometry kit.

VII. DRIFT

The drift evaluation for the kit was completed on the tandem mass spectrometer system equipped with the Gilson 215 Autosampler. Ten 96-well plates were prepared with dried blood spot samples and run consecutively and continuously for an assay time of approximately 20 hours (approx 2 hours/plate). Low and high amino acid and acylcarnitine controls as well as seven replicate low, mid and high samples were interspersed throughout these plates.

For each analyte, the mean concentrations of each of the samples described above after each plate analysis interval were inspected to determine if any clear trends over time were visible. In general, the mean measured concentrations are very consistent for each analyte at all levels throughout the entire 20-hour period. The assay variability over the 20-hour test period is well within the expected precision of the assay as demonstrated by percent CVs that ranged from 2.4 to 11.3% for the amino acids and from 4.2 to 24.0% for the acylcarnitines. In addition, there was only a slight change in concentration from the measurements performed at 2 hours vs. 20 hours. The average percent concentration change for the amino acids ranged from 1.7 to 10.7 %, for the acylcarnitines, 7.8 to 21.4 %. These changes were, again, within the expected precision of the assay. From this data it was concluded that no significant drift was observed in the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit over the 20-hour period tested.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event PerkinElmer Life and Analytical Sciences Inc. and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use. PerkinElmer Life and Analytical Sciences Inc., its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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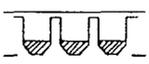
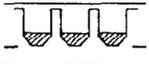
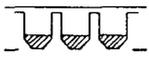
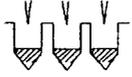
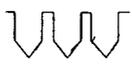
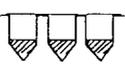
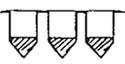
All equipment required for this kit must be serviced by qualified service technicians. Questions on the operation and performance of this test should be directed to the PerkinElmer Life and Analytical Sciences Technical Service Department. For immediate service call (800) 321-9632 or (330) 825-4525. Customers outside the U.S. should contact their Local Sales Office for Technical Assistance.

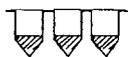
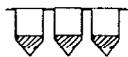
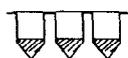
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**NeoGram Amino Acids and Acylcarnitines Kit MS -8970
Summary Protocol Sheet**

ASSAY PROCEDURE		
Reconstitute Amino Acids (AA) and Acylcarnitines (AC) Internal Standard vials		1.0 mL Extraction Solution per vial
Dilute AA and AC Internal Standards to working concentration		1:200 Dilution using Extraction Solution.
Punch out controls and unknowns		Use a Truncated V-Bottomed microtiter plate
Add Extraction Solution containing Internal Standards		90 μ L per well
Cover plate		Cover plate with adhesive plastic cover to minimize evaporation of Extraction Solution.
DBS Extraction / Shake		Shake at 650-750 rpm for 30 min. at 30°C.
Uncover		Remove cover from plate
Transfer		Transfer 60 μ L to NUNC plate
Evaporate		Evaporate to dryness
Add 3N HCl in n-Butanol		50 μ L per well
Cover plate		Cover plate with heat sealing film to minimize evaporation of Derivatization Solution.
Incubate		At 60°C for 30 min.
Uncover		Remove cover from plate

Evaporate		Evaporate to dryness
Add Sample Reconstitution Solution		75 μ L per well
Cover plate		Cover plate with foil to minimize evaporation of Reconstitution Solution.
Shake		Shake at any speed between 650-750 rpm for 10 min. at 27°C.
Load plate on auto sampler		Start NeoGram and create worklists. Use the appropriate internal standard concentrations and NeoGram Acquisition Method.
Measure		Start measurement in NeoGram

APPENDIX

This appendix can be used as a glossary. The information provided here is supplementary and should not be used for diagnosis.

Table 27: Analyte Names and their Most Common Abbreviation

Abbreviation	Name	Name	Abbreviation
C0	Free Carnitine	Alanine	Ala
C2	Acetylcarnitine	Arginine	Arg
C3	Propionylcarnitine	Asparagine	Asn
C3DC	Malonylcarnitine	Citrulline	Cit
C3-2M-DC	Methylmalonylcarnitine	Cysteine	Cys
C4	Butyrylcarnitine	Glutamine	Gln
C4OH	3-hydroxy(OH)butyrylcarnitine	Glycine	Gly
C4DC	Methylmalonylcarnitine	Histidine	His
C5	Isovalerylcarnitine	Homocysteine	Hcy
C5:1	Tiglylcarnitine	Hydroxylysine	H(O)-Lys
C5DC	Glutarylcarnitine	Hydroxyproline	H(O)-Pro
C5-CM-DC	Methylglutarylcarnitine	Isoleucine	Ile
C5OH	3-hydroxy(OH)isovalerylcarnitine	Leucine	Leu
C6	Hexanoylcarnitine	Lysine	Lys
C6:1	Hexenoylcarnitine	Methionine	Met
C7	Heptanoylcarnitine	L-Me-Histidine	L-Me-His
C6DC	Adipylcarnitine	3-Me-Histidine	3-Me-His
C8	Octanoylcarnitine	Norleucine	Nle
C8:1	Octenoylcarnitine	Ornithine	Orn
C9	Nonanoylcarnitine	5-Oxoproline	5-Oxo-Pro
C10	Decanoylcarnitine	Proline	Pro
C10:3	Decatrienoylcarnitine	Phenylalanine	Phe
C10:2	Decadienoylcarnitine	Serine	Ser
C10:1	Decenoylcarnitine	Threonine	Thr
C10OH	3-hydroxy(OH)decanoylcarnitine	Tryptophan	Trp
C12	Dodecanoylcarnitine (Lauroyl)	Tyrosine	Tyr
C12:1	Dodecenoylcarnitine	Valine	Val
C12OH	3-hydroxy(OH)dodecanoylcarnitine		
C14	Myristoylcarnitine (Tetradecanoyl)		
C14:1	Myristoleylcarnitine (Tetradecenoyl)		
C14:1OH	3-hydroxy(OH)tetradecenoylcarnitine		
C14:2	Tetradecadienoylcarnitine		
C14OH	3-hydroxy(OH)myristoylcarnitine		
C16	Palmitoylcarnitine		
C16:1	Hexadecenoylcarnitine		
C16:1OH	3-hydroxy(OH)palmitoleylcarnitine		
C16OH			
C18	Octadecanoylcarnitine (Stearoyl)		
C18:1	Octadecenoylcarnitine (Oleyl)		
C18:2OH	3-hydroxy(OH)linoleylcarnitine		
C18:1OH	3-hydroxy(OH)octadecenoylcarnitine (3-OH-oleyl)		
C18:2	Linoleylcarnitine		
C18OH	3-hydroxy(OH)octadecanoylcarnitine (3-OH-stearoyl)		

Table 28: Internal Standard Names and their Most Common Abbreviation

Stable-isotope Standards			
Abbreviation Name		Alternate Name	Alternate Abbreviation
² H ₉ -C0	² H ₉ -Free Carnitine	d ₉ -Free Carnitine	d ₉ -C0
² H ₃ -C2	² H ₃ -Acetylcarnitine	d ₃ -Acetylcarnitine	d ₃ -C2
² H ₃ -C3	² H ₃ -Propionylcarnitine	d ₃ -Propionylcarnitine	d ₃ -C3
² H ₃ -C4	² H ₃ -Butyrylcarnitine	d ₃ -Butyrylcarnitine	d ₃ -C4
² H ₉ -C5	² H ₉ -Isovalerylcarnitine	d ₉ -Isovalerylcarnitine	d ₉ -C5
² H ₃ -C6	² H ₃ -Hexanoylcarnitine	d ₃ -Hexanoylcarnitine	d ₃ -C6
² H ₃ -C8	² H ₃ -Octanoylcarnitine	d ₃ -Octanoylcarnitine	d ₃ -C8
² H ₃ -C10	² H ₃ -Decanoylcarnitine	d ₃ -Decanoylcarnitine	d ₃ -C10
² H ₃ -C12	² H ₃ -Dodecanoylcarnitine (Lauroyl)	d ₃ -Dodecanoylcarnitine (Lauroyl)	d ₃ -C12
² H ₃ -C14	² H ₃ -Myristoylcarnitine (Tetradecanoyl)	d ₃ -Myristoylcarnitine (Tetradecanoyl)	d ₃ -C14
² H ₃ -C16	² H ₃ -Palmitoylcarnitine	d ₃ -Palmitoylcarnitine	d ₃ -C16
² H ₃ -C18	² H ₃ -Octadecanoylcarnitine (Stearoyl)	d ₃ -Octadecanoylcarnitine (Stearoyl)	d ₃ -C18
² H ₄ -Ala	² H ₄ -Alanine	d ₄ -Alanine	d ₄ -Ala
² H ₄ - ¹³ C-Arg	² H ₄ - ¹³ C-Arginine.HCl	d ₄ - ¹³ C-Arginine.HCl	d ₄ - ¹³ C-Arg
² H ₂ -Cit	² H ₂ -Citrulline	d ₂ -Citrulline	d ₂ -Cit
¹⁵ N, ² - ¹³ C-Gly	¹⁵ N, ² - ¹³ C-Glycine		
² H ₃ -Leu	² H ₃ -Leucine	d ₃ -Leucine	d ₃ -Leu
² H ₃ -Met	² H ₃ -Methionine	d ₃ -Methionine	d ₃ -Met
² H ₂ -Orn	² H ₂ -Ornithine.2HCl	d ₂ -Ornithine.2HCl	d ₂ -Orn
² H ₅ -Phe	² H ₅ -Phenylalanine	d ₅ -Phenylalanine	d ₅ -Phe
¹³ C ₆ -Tyr	¹³ C ₆ -Tyrosine		
² H ₈ -Val	² H ₈ -Valine	d ₈ -Valine	d ₈ -Val

Table 29: Disorder Names and their Most Common Abbreviation

Amino Acid Disorders	
ARGD	Argininemia
ASA, ASL, ALD	Argininosuccinic aciduria (Argininosuccinate lyase deficiency, Argininosuccinase deficiency)
ASD, ASS	Citrullinemia (Argininosuccinic acid synthetase deficiency, Argininosuccinate synthetase deficiency)
HCU, HCYS	Homocystinuria (Cystathione synthase deficiency)
Hmet	Hypermethioninemia
HHH	Hyperornithinemia, Hyperammonemia, Hyperhomocitrullinuria syndrome (Ornithine translocase deficiency)
PRO	Hyperprolinemia
MSUD, BCKA	Maple Syrup Urine Disease (Branched chain ketoaciduria)

Table 29: Disorder Names and their Most Common Abbreviation

Amino Acid Disorders (continued)	
NKG, NKHI	Nonketotic hyperglycinemia
PKU	Phenylketonuria
PYP/PIP	Pyroglutamic/pipecolic acidemia
	Tyrosenemia, Type I
	Tyrosenemia, Type II
	5-oxoprolinuria
	(Pyroglutamic aciduria)
Fatty Acid and Organic Acid Disorders	
2-MBCD	2-methylbutyryl CoA dehydrogenase deficiency
	2,4-Dienoyl-CoA reductase deficiency
HMG	3-hydroxy-3-methylglutaryl CoA lyase deficiency
	(hydroxymethylglutaric acidemia)
3MCC, 3-MMC	3-methylcrotonyl CoA carboxylase deficiency
	(3-methylcrotonylglycinemia)
CPT I	Carnitine palmitoyltransferase, type I deficiency
CPT II	Carnitine palmitoyltransferase, type II deficiency
CTD	Carnitine transporter defect
CATR, CACT	Carnitine/acylcarnitine translocase defect
EMA	Ethylmalonic acidemia
GA I	Glutaric acidemia, type I
	(Glutaryl CoA dehydrogenase deficiency)
IBCD	Isobutyryl CoA dehydrogenase deficiency
IVA	Isovaleric acidemia
LCAD	Long-chain acyl-CoA dehydrogenase deficiency
LCHAD	Long-chain hydroxyacyl-CoA dehydrogenase deficiency
MA	Malonic aciduria
MCAD	Medium-chain acyl-CoA dehydrogenase deficiency
MMA	Methylmalonic acidemia
BKT	Mitochondrial acetoacetyl CoA thiolase deficiency
	(Beta-Ketothiolase deficiency)
MADD, GA II	Multiple acyl-CoA dehydrogenase deficiency
	(Glutaric acidemia, type II)
MCD	Multiple Co-A carboxylase deficiency
	(Holocarboxylase synthetase deficiency)
PA, PPA	Propionic acidemia
SCAD	Short-chain acyl-CoA dehydrogenase deficiency
SCHAD	Short-chain hydroxyacyl-CoA dehydrogenase deficiency
TFP	Trifunctional protein deficiency
VLCAD	Very-long-chain acyl-CoA dehydrogenase deficiency

Table 30: Diseases and Markers; Known Analyte Profiles

Fatty Acid Oxidation Disorders	
<p>SCAD C4, C6¹ C4^{2,5,7} C4, C5, C4/C2, C5/C2⁴ C4, C4/C2, C4/C3⁶</p> <p>MCAD C8, C10:1¹ C8, C10, C10:1, C6² C6, C8, C10, C10:1, C8/C10³ C6, C8, C10:1^{4,7} C6, C8, C10:1, C10, C8/C2, C8/C10, C8/C12⁵ C6, C8, C10:1, C8/C10⁶</p> <p>Glutaric acidemia Type II C5DC¹ C4, C5, C8:1, C8, C12, C14, C16, C5DC² C4, C5DC, C5, C6, C8, C10³ C6, C8, C10, C5/C2⁴ C4, C5, C6, C8, C10⁶ C8, C10, C5DC⁷</p> <p>Carnitine transporter defect C0, C2⁵</p> <p>CPT I C16, C18:1, C18² C0, C16, C18, C0/(C16+C18)⁵</p> <p>Ethylmalonic acidemia C4, C5, C4/C2, C5/C2⁴</p> <p>2,4 Dienoyl CoA reductase deficiency⁶ C10:2</p>	<p>LCAD ⁴ C14:2, C14:1, C14:1/C16</p> <p>VLCAD ¹ C14, C14:1, C16, C18 ² C14:1, C14, C16 ³ C14:1, C14, C16:1, C16, C18:1, C18, C14:1/C12:1 ⁴ C14:2, C14:1, C14:1/C16 ⁵ C14:1 or C14 ⁶ C14, C14:1, C14:2, C16:1, C14:1/C16 ⁷ C14:1</p> <p>LCHAD ¹ C14, C14:1, C16, C18, C14OH, C14:1OH, C16OH, C18OH ² C16OH, C18:1OH, C18OH ³ C16OH, C18:1OH, C18OH, C16OH/C16 ⁵ C14OH, C16:1OH, C16OH, C18:1OH, C18:OH, C14:1, C14 ⁶ C16OH, C18:1, C18:1OH, C18:2, C18:2OH ⁷ C16OH</p> <p>Trifunctional protein deficiency ² C16OH, C18:1OH, C18OH ⁶ C16OH, C18:1OH, C18OH, C16OH/C16 ⁵ C14OH, C16:1OH, C16OH, C18:1OH, C18:OH, C14:1, C14</p> <p>Carnitine/acylcarnitine translocase deficiency ² C16, C18:1, C18 ⁵ C0, C16, C18, C0/(C16+C18) ⁷ C16</p> <p>CPT II ¹ C14, C14:1, C16, C16:1 ³ C16, C18, C18:1, C16/C14:1 ⁶ C16, C18:1, C18:2 ⁷ C16</p>

Table 30: Continued

Aminoacidopathies	
<p>Phenylketonuria Phe^{2,4,6} Phe, Phe/Tyr⁷ Phe, Tyr^{1,5}</p> <p>Maple syrup urine disease Leu+Ile¹ Leu+Ile, Val^{2,4,6} Leu, Val⁵ Leu, Leu/Phe⁷</p> <p>Homocystinuria Met^{1,2,4,6} Met, Met/Phe, Met/Leu⁵ Met, Met/Phe⁷</p> <p>Hypermethioninemia Met² Met, Met/Phe⁷</p> <p>Citrullinemia Cit^{1,2,6} Cit/Phe, Cit/Tyr⁴ Cit, Orn/Cit, Cit/Arg⁵ Cit, Cit/Arg⁷</p> <p>Nonketotic hyperglycinemia Gly^{2,4,5}</p>	<p>Tyrosinemia Type I ^{1,4} Met, Tyr ^{2,6} Tyr ⁷ Tyr, Tyr/Phe</p> <p>Tyrosinemia II ² Tyr ⁷</p> <p>Argininemia Arg ² Arg, Arg/Orn</p> <p>PRO Hyperprolinemia ⁴ Pro/Phe</p> <p>HHH ² Orn, HomoCit ⁷ Orn, Orn/Cit</p> <p>5-oxoprolinuria ² 5-Oxopro ⁴ pyroGlu/Phe</p> <p>Argininosuccinic aciduria ^{1,6} Cit ⁴ Cit/Tyr ⁵ Asa ⁷ Cit, Cit/Arg</p>

Table 30: Continued

Organic Acid Disorders	
Glutaric acidemia Type I	HMG
C5DC ^{1,2,3,4,6,7}	¹ C5-3M-DCOH (hydroxymethylglutaryl carnitine)
C5DC, C5DC/C8, C5DC/C16 ⁵	^{2,5} C5OH
Propionic acidemia	⁴ C5:1, C5OH, C6DC
C3, C2 ¹	⁶ C5OH, C6DC
C3 ^{2,7}	⁷ C5-3M-DC (methylglutaryl carnitine), C5OH
C3, C3/C2 ^{3,6}	3-MMC (3-MCC)
C3/C2 ⁴	^{1,2,5,6,7} C5OH
C3, C3/C0, C3/C2 ⁵	Isovaleric acidemia
Methylmalonic acidemia	^{1,2,3,7} C5
C3, C2 ¹	^{4,5} C5, C5/C2
C3 ²	⁶ C5, C5/C3, C5/C2
C3, C3/C2 ³	Multiple CoA carboxylase deficiency
C3/C2 ⁴	⁴ C3/C2, C5OH
C3, C3/C0, C3/C2 ⁵	⁵ C5OH
C3, C4DC, C3/C2 ⁶	⁷ C3, C5OH
C3, C3-2M-DC ⁷	2-Methylbutyryl Co-A Dehydrogenase deficiency
Malonic aciduria	⁷ C5
C3DC ²	Isobutyryl CoA dehydrogenase deficiency
Beta-Ketothiolase deficiency	² C5
C5:1, C5OH ^{2,4,6,7}	⁷ C4

Appendix References

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510(k) Summary

This summary of 510(k) safety and effectiveness information is being supplied in accordance with the requirements of the SMDA of 1990 and 21 CFR 807.92

The assigned 510(k) number is K031878.

Date: June 30, 2004

Submitted by: PerkinElmer Life and Analytical Sciences
3985 Eastern Road
Norton, Ohio 44203

Telephone: 330-825-4525 ext 170 or 617-350-9305
Fax: 330-825-8520

Contact person: Cindy Lloyd, Manager, Regulatory Affairs/Quality Assurance

Trade Name: NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit (MS-8970)
Common Name: Amino Acid and Acylcarnitine Screening Test Kit, or NeoGram AAAC kit
Classification Name: Not defined

Legally marketed predicate device(s): NeoGram PKU by Tandem Mass Spectrometry
Wallac Neonatal Leucine Kit
Astoria Pacific Phenylalanine Test Kit
Astoria Pacific Tyrosine Test Kit
Isolab Neonatal Phenylalanine Test Kit

Device description: The assay involves the extraction of dried blood spots with a solution containing stable, isotopically labeled internal standards. The response of each amino acid, free carnitine and acylcarnitine relative to the isotopically labeled standards in the kit is proportional to their actual concentration. The analysis of the material is performed on a tandem mass spectrometer. Control material contained in the kit allows for verification of performance for the test run.

Intended use: This kit is intended for the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper. Table 1 details the analytes measured by the kit. Quantitative analysis of amino acids, free carnitine and acylcarnitines and their relationship with each other is intended to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism. This kit is to be used for **In Vitro diagnostic** use only, by trained, qualified laboratory personnel.

Table 1. Analytes Measured by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit.

ANALYTE NAME	ABBREVIATION
Amino Acids	
Alanine	Ala
Arginine	Arg
Citrulline	Cit
Glycine	Gly
Leucine	Leu
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Tyrosine	Tyr
Valine	Val
Carnitines	
Free carnitine	C0
Acetylcarnitine	C2
Propionylcarnitine	C3
Malonylcarnitine	C3DC
Butyrylcarnitine	C4
3-Hydroxy-butyrylcarnitine	C4OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutarylcarnitine	C5DC
3-Hydroxy-isovalerylcarnitine	C5OH
Hexanoylecarnitine	C6
Adipylcarnitine	C6DC
Octanoylecarnitine	C8
Octenoylcarnitine	C8:1
Decanoylecarnitine	C10
Decenoylcarnitine	C10:1
Decadienoylcarnitine	C10:2
Dodecanoylecarnitine	C12
Dodecenoylcarnitine	C12:1
Tetradecanoylecarnitine (Myristoylcarnitine)	C14
Tetradecenoylcarnitine	C14:1
Tetradecadienoylcarnitine	C14:2
3-Hydroxy-tetradecanoylecarnitine	C14OH
Hexadecanoylecarnitine (palmitoylcarnitine)	C16
Hexadecenoylcarnitine	C16:1
3-Hydroxy-hexadecanoylecarnitine	C16OH
3-Hydroxy-hexadecenoylcarnitine	C16:1OH
Octadecanoylecarnitine (Stearoylcarnitine)	C18
Octadecenoylcarnitine (Oleylcarnitine)	C18:1

Octadecadienoylcarnitine (Linoleylcarnitine)	C18:2
3-Hydroxy-octadecanoylcarnitine	C18OH
3-Hydroxy-octadecenoylcarnitine	C18:1OH

Similarities/Differences with the predicate device(s):

- Similarities:
- Intended use is the same. All kits are for screening of newborns
 - All assays can measure specific analytes
 - All assays use dried blood spots on filter paper for sample collection
 - All assays use standards and controls dried onto filter paper
 - All assays are quantitative
 - Sample size (3 mm punched spot) is the same for all assays
 - All assays employ a 96 well microtiter plate
 - NeoGram PKU and NeoGram AAAC are both measured by tandem mass spectrometry

- Differences:
- Wallac Neonatal Leucine, Isolab Phenylalanine, Astoria-Pacific Phenylalanine and Tyrosine kits are fluorometric based; NeoGram AAAC is tandem mass spectrometry based
 - Wallac Neonatal Leucine, Isolab Phenylalanine, Astoria-Pacific Phenylalanine and Tyrosine kits measure one analyte. The NeoGram PKU kit measures two analytes. The NeoGram AAAC measures more than 40 analytes

The NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit was compared directly to the aforementioned predicate devices for the analytes that they measure: phenylalanine, tyrosine and leucine. However, because the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit measures more than forty analytes, a direct comparison for each analyte was performed with accepted home-brew methods that do screen for all the analytes screened for by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit. Additionally, overall screening results of a pilot study performed with the NeoGram AAAC kit are compared with the screening results of newborn screening programs that use the currently accepted practice for screening newborn for inborn errors of metabolism by tandem mass spectrometry.

Summary Data:

SUBSTANTIAL EQUIVALENCE TO THE CURRENTLY ACCEPTED PRACTICE FOR NEWBORN SCREENING BY TANDEM MASS SPECTROMETRY:

- *Direct comparison to an established tandem mass spectrometry method for newborn screening:* In this study, identical sample sets consisting of 50 samples each and representing reportable ranges were assayed in duplicate by the Neogram AAAC kit and the tandem mass spectrometry assays used at Baylor University Medical Center for the measurement of 18 analytes (7 amino acids and 11 carnitines). The slope of the regression lines (NeoGram AAAC values vs. Baylor values) for each analyte ranged from 0.64 to 1.12 and the correlation coefficients ranged from 0.94 to 0.99. Because of the good correlation, the NeoGram AAAC kit was deemed substantially equivalent to the established

(predicate) tandem mass spectrometry assay used at Baylor University. For more details, please refer to the document titled " Comparison of Methods, EP9A - NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit" in the Substantial Equivalence and Comparisons of Methods section of this submission.

- *Comparison of the CDC proficiency testing results:* In this study, the results of measuring CDC proficiency testing samples with the NeoGram AAAC kit are compared to the reported spiked levels by the CDC and the mean of the reported results from over 20 participating laboratories that use the currently accepted practice for newborn screening by tandem mass spectrometry. The CDC proficiency samples contain several amino acids and acylcarnitines enriched at different levels. The correlation of the NeoGram AAAC results to the sample enrichment levels reported by the CDC consistently in slopes that are close to 1 for comparison to both the participants' overall means and enrichment levels. Because of this good correlation it was concluded that the NeoGram AAAC kit is substantially equivalent to the current practice for newborn screening by tandem mass spectrometry. For more details please refer to the document titled " CDC Proficiency Testing for the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit" in the Substantial Equivalence and Comparisons of Methods section of this submission.
- *Comparison of screening results with the NeoGram AAAC kit to screening results from laboratories that use the currently accepted practice for newborn screening by tandem mass spectrometry:* In this study we compared the results of a pilot study in which more than 212,000 infants were screened using the NeoGram AAAC kit to the experience of three screening laboratories that perform newborn screening for amino, fatty, and organic acid metabolic defects using the currently accepted MS/MS methods (predicate devices). These groups have shown that their MS/MS newborn screening programs are safe and effective. These predicate programs include the New England, NeoGen Screening, and New South Wales MS/MS screening programs. The performance comparison is based mainly on overall screening results from large populations (137,000 to ~1,100,000 infants) screened by each program. This study reveals that NeoGram AAAC kit specificity (99.5%) is similar to that of the predicate programs (99.6 to 99.9%), the sensitivity of the NeoGram AAAC kit (100%) is similar to that of the predicate programs (99.6 to 100%), the false negative rate is also comparable with 0 false negatives for the NeoGram AAAC kit while 0 to 1 false negatives for the predicate programs, the positive predictive values (PPV) are also comparable with the NeoGram AAAC kit exhibiting a PPV of 5% while the PPV's of the predicate programs range from 5.5 to 25%. Overall the performance of the AAAC kit is comparable to that reported by established screening laboratories that use the currently accepted newborn screening MS/MS practice for screening amino, fatty, and organic acid metabolic defects and thus we conclude that the NeoGram AAAC kit is substantially equivalent to the currently MS/MS newborn screening practice and that the NeoGram AAAC kit is safe and effective for the screening of newborns for amino, fatty, and organic acid metabolic defects. For more details please refer to the document titled "Substantial Equivalence Comparison of the PerkinElmer Life and Analytical Sciences NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit to the Currently Accepted Practice for the Screening of Inborn Errors of Metabolism by Tandem Mass Spectrometry" in the Substantial Equivalence and Comparisons of Methods section of this submission.

SUBSTANTIAL EQUIVALENCE TO PREDICATE DEVICES:

- *Substantial equivalence to NeoGram PKU by Tandem Mass Spectrometry Kit:* In this study, the phenylalanine, tyrosine, and phenylalanine-to-tyrosine ratio levels were measured from 5529 newborn samples in parallel by the NeoGram AAAC and the NeoGram PKU assays. The mean phenylalanine value observed with the NeoGram AAAC assay was 67.8 $\mu\text{mol/L}$ with a standard deviation of 15.0 $\mu\text{mol/L}$. For comparison, the NeoGram PKU assay mean phenylalanine result was 66.9 $\mu\text{mol/L}$ with a standard deviation of 14.8 $\mu\text{mol/L}$. The mean tyrosine value observed with the NeoGram AAAC assay was 100.8 $\mu\text{mol/L}$ with a standard deviation of 39.9 $\mu\text{mol/L}$ while a mean tyrosine value of 103.5 $\mu\text{mol/L}$ with a standard deviation of 41.8 $\mu\text{mol/L}$ was obtained with the NeoGram PKU assay. The mean Phe/Tyr ratios obtained were 0.74 (1SD=0.25) and 0.72 (1SD=0.27) for the NeoGram AAAC and the NeoGram PKU assays, respectively. The screening of these samples by both methods also resulted in 100% positive and negative agreement. Therefore, we conclude that the NeoGram AAAC kit is substantially equivalent to the predicate NeoGram PKU by Tandem Mass Spectrometry Kit. For more details, please refer to the documents titled "Substantial Equivalence Comparison of the PerkinElmer Life and Analytical Sciences NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit to the PerkinElmer Life and Analytical Sciences NeoGram PKU Tandem Mass Spectrometry kit" and "Comparison of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Assay to the NeoGram PKU Tandem Mass Spectrometry Assay" in the Substantial Equivalence and Comparisons of Methods section of this submission.
- *Substantial equivalence to Isolab Neonatal Phenylalanine Test Kit:* The results of screening 5529 newborn samples for their measurement of phenylalanine by the NeoGram AAAC kit and the Isolab Neonatal Phenylalanine Test Kit reveal these two kits have 100% positive and negative agreement. In addition, identical sample sets consisting of 50 samples each and representing reportable ranges were assayed for phenylalanine (Phe) in duplicate by the NeoGram AAAC kit and the Isolab Neonatal Phenylalanine Test Kit. The slope of this direct comparison (NeoGram AAAC phe vs Isolab Phe) results in a slope of 0.86 and a correlation coefficient of 0.964. Thus we conclude that the NeoGram AAAC kit is substantially equivalent to the Isolab Neonatal Phenylalanine Test Kit. For more details, please refer to the documents titled "Substantial Equivalence Comparison of the PerkinElmer Life and Analytical Sciences NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit to the PerkinElmer Life and Analytical Sciences NeoGram PKU Tandem Mass Spectrometry kit"; "Comparison of the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Assay to the NeoGram PKU Tandem Mass Spectrometry Assay", and "Comparison of Methods, EP9A - NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit" in the Substantial Equivalence and Comparisons of Methods section of this submission.
- *Substantial equivalence to the Astoria Pacific Phenylalanine and Astoria Pacific Tyrosine Test Kits:* In this study, 203,844 newborn samples were measured for phenylalanine, tyrosine and the phenylalanine-to-tyrosine (Phe/Tyr) ratio in parallel by the NeoGram AAAC and the Astoria Pacific Phenylalanine and Tyrosine tests kits. The mean phenylalanine, tyrosine, and Phe/Tyr for the normal population were mM/L 90.9, mM/L 111.3, and 0.94 for the NeoGram AAAC kit, respectively; and mM/L 111.3, mM/L 159.4, and 0.73 for the Astoria Pacific Phenylalanine and Tyrosine kits, respectively. The screening results of this large population resulted in 79% positive agreement and 99.98% negative agreement. These results indicate that the NeoGram AAAC kit performs comparably to the Astoria Pacific

Phenylalanine and Tyrosine kits and thus these three assays are substantially equivalent. For more details, please refer to the documents titled " Substantial Equivalence Comparison of the PerkinElmer Life and Analytical Sciences NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit to the Astoria-Pacific Phenylalanine and Tyrosine test kits" and " Comparison of NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit Results to three Predicate Devices: The Astoria-Pacific Fluorescent Phenylalanine and Tyrosine Assays and the NeoGram PKU Tandem Mass Spectrometry Kit" in the Substantial Equivalence and Comparisons of Methods section of this submission.

- *Substantial equivalence to the Wallac Neonatal Leucine Kit:* In this study, identical sample sets consisting of 50 samples each and representing reportable ranges were assayed for leucine concentrations in duplicate by the Neogram AAAC kit and the Wallac Neonatal Leucine Kit. The slope of the regression lines (NeoGram AAAC values vs. Wallac Leucine kit) was 0.79 and the correlation coefficient was 0.933. The slope and correlation coefficient obtained indicate that the NeoGram AAAC kit performs comparably to the Wallac Leucine kit for the measurement of Leucine concentrations and thus the NeoGram AAAC kit was deemed substantially equivalent to the Wallac Leucine kit. For more details, please refer to the document titled " Comparison of Methods, EP9A - NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit" in the Substantial Equivalence and Comparisons of Methods section of this submission.



INDICATIONS FOR USE STATEMENT

510(K) Number: **K031878**

Device Name: NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry kit.

Intended use: This kit is intended for the measurement and evaluation of amino acid, free carnitine and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper. Table 1 details the analytes measured by the kit. Quantitative analysis of amino acids, free carnitine and acylcarnitines and their relationship with each other is intended to provide analyte concentration profiles that may aid in the screening of newborns for one or more inborn errors of metabolism. This kit is to be used for **In Vitro diagnostic** use only, by trained, qualified laboratory personnel.

Table 1. Analytes Measured by the NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit.

ANALYTE NAME	ABBREVIATION
Amino Acids	
Alanine	Ala
Arginine	Arg
Citruline	Cit
Glycine	Gly
Leucine	Leu
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Tyrosine	Tyr
Valine	Val

ANALYTE NAME	ABBREVIATION
Carnitines	
Free carnitine	C0
Acetylcarnitine	C2
Propionylcarnitine	C3
Malonylcarnitine	C3DC
Butyrylcarnitine	C4
3-Hydroxy-butyrylcarnitine	C4OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutaryl carnitine	C5DC
3-Hydroxy-isovalerylcarnitine	C5OH
Hexanoylcarnitine	C6
Adipylcarnitine	C6DC
Octanoylcarnitine	C8
Octenoylcarnitine	C8:1
Decanoylcarnitine	C10
Decenoylcarnitine	C10:1
Decadienoylcarnitine	C10:2
Dodecanoylcarnitine	C12
Dodecenoylcarnitine	C12:1
Tetradecanoylcarnitine (Myristoylcarnitine)	C14
Tetradecenoylcarnitine	C14:1
Tetradecadienoylcarnitine	C14:2
3-Hydroxy-tetradecanoylcarnitine	C14OH
Hexadecanoylcarnitine (palmitoylcarnitine)	C16
Hexadecenoylcarnitine	C16:1
3-Hydroxy-hexadecanoylcarnitine	C16OH
3-Hydroxy-hexadecenoylcarnitine	C16:1OH
Octadecanoylcarnitine (Stearoylcarnitine)	C18
Octadecenoylcarnitine (Oleylcarnitine)	C18:1
Octadecadienoylcarnitine (Linoleylcarnitine)	C18:2
3-Hydroxy-octadecanoylcarnitine	C18OH
3-Hydroxy-octadecenoylcarnitine	C18:1OH

Prescription Use X
(21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use
(21 CFR 807 Subpart C)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Steven Atman

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K031878



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Self Revised

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Ornithine	Orn
Phenylalanine	Phe
Tyrosine	Tyr
Valine	Val

