510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k083130

B. Purpose for Submission:
New device

C. Measurand:
Amino acids, free carnitine, acylcarnitines, and succinylacetone

D. Type of Test:
Quantitative measurement by mass spectrometry

E. Applicant:
PerkinElmer, Inc.

F. Proprietary and Established Names:
NeoBase Non-derivatized MSMS kit

G. Regulatory Information:
1. Regulation section:
   21 CFR §862.1055 Newborn screening test system for amino acids, free carnitine, and acylcarnitines using tandem mass spectrometry

2. Classification:
   II

3. Product code:
   NQL

4. Panel:
   Chemistry (75)

H. Intended Use:
1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:
   The NeoBase Non-derivatized MSMS kit is intended for the measurement and evaluation of amino acids, succinylacetone, free carnitine, and acylcarnitine concentrations from newborn heel prick blood samples dried on filter paper.

   Quantitative analysis of these analytes (listed in the tables below) and their

relationship with each other is intended to provide analyte concentration profiles that may aid in screening newborns for metabolic disorders.

Analyte Name	Abbreviation
Amino Acids	
Alanine	Ala
Arginine	Arg
Citrulline	Cit
Glycine	Gly
Leucine/Isoleucine/Hydroxyproline	Leu/Ile/Pro-OH
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Proline	Pro
Tyrosine	Tyr
Valine	Val

Analyte Name	Abbreviation
Carnitines	
Free carnitine	C0
Acetylcarnitine	C2
Propionylcarnitine	C3
Malonylcarnitine / 3-Hydroxy-butyrylcarnitine	C3DC/C4OH
Butyrylcarnitine	C4
Methylmalonyl / 3-Hydroxy-isovalerylcarnitine	C4DC/C5OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutarylcarnitine / 3-Hydroxy-hexanoylcarnitine	C5DC/C6OH
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	C18 OH
3-Hydroxy-octadecenoylcarnitine	C18:1 OH

Analyte Name	Abbreviation
Ketones	
Succinylacetone	SA

3. Special conditions for use statement(s):
   The NeoBase Non-derivatized MSMS Kit is a screening assay, not intended for confirmatory or prenatal testing. As with any other *in vitro* screening test, the data obtained using this kit should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician. A diagnostic procedure should be used for confirmation of presumptive abnormal amino acid, succinylacetone, free carnitine and acylcarnitine profiles. Users should follow local guidelines for follow-up and confirmatory testing.

4. Special instrument requirements:
   PerkinElmer MS^2 Tandem Mass Spectrometer System (MS^2) and PerkinElmer MSMS Quattro Micro (QMicro) Newborn Screening System

I. Device Description:
The test kit consists of the following components:
- Amino acid internal standards vial
- Acylcarnitine internal standard vial
- Dried blood spot controls (3 filter paper cassettes containing 3 spots of each low and high level per cassette)
- V-bottom heat-resistant micro plates
- Truncated V-bottom clear micro plates
- Aluminum foil microplate covers
- Adhesive microplate covers
- NeoBase Non-derivatized Assay Solutions (Flow Solvent and Extraction Solution)
- NeoBase Succinylacetone Assay Solution

This kit contains components manufactured from human blood. The source materials have been tested by FDA-approved methods for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies and found to be negative.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit

2. Predicate K number(s):
   k031878

3. Comparison with predicate:

Similarities		
Item	Proposed Device	Predicate Device k031878
Disorders Screened	Amino-, organic-, and fatty acid metabolic disorders	Same
Methodology	Microplate based tandem mass spectrometric assay	Same
Quantitative Nature	Quantitative by internal standardization	Same
Sample type	Newborn dried blood spots	Same
Throughput	Ninety-six tests per microtiter plate. Multiple plates can be analyzed	Same
Analysis Time	2 to 2.5 hours per plate.	Same
Calibrators	Internal calibration using several isotopically labeled standards, included as dried material in vials. Internal standards must be reconstituted with extraction solution prior to their use.	Same

Differences		
Item	Proposed Device	Predicate Device k031878
Analytes Measured	Amino acids, free carnitine, acylcarnitines, and succinylacetone	Amino acids, free carnitine, and acylcarnitines
Assay format	Non-derivatized (analytes measured in their native forms)	Derivatized (analytes converted to butyl esters prior to being measured)

K. Standard/Guidance Document Referenced (if applicable):
- CLSI Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative*

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Measurement Methods
- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Guideline EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples*
- CLSI Protocol C28-A2, *How to Define and Determine Reference Intervals in the Clinical Laboratory*

L. Test Principle:

The measurement of amino acids, succinylacetone, free carnitine, and acylcarnitines with the NeoBase assay involves extraction of dried blood spots from newborns with a solution containing stable-isotope labeled internal standards and analysis using a tandem mass spectrometry (MSMS) system. The response of each analyte relative to their corresponding stable-isotope labeled internal standard is proportional to analyte concentration.

In the NeoBase Non-derivatized MSMS Kit, data is acquired in the Multiple Reaction Monitoring (MRM) mode. During this acquisition, a collisionally induced of each analyte is measured for a set time period. Data acquisition and processing is performed by the software package included with the system.

The 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. The extracted sample is delivered to the ion source of the mass spectrometer by the liquid chromatography (LC) system consisting of the autosampler, micro pump(s) and solvent vacuum degasser. What is reported in the MSMS MRM spectrum is the m/z value of the precursor ions that generated a desired product.

Analyte extraction with the NeoBase assay is accomplished for the amino acids and carnitines by simply contacting the sample with the extraction solution containing the corresponding internal standards during the incubation step. However, succinylacetone requires a specific derivatization reaction during the incubation step for its extraction and measurement. The derivatization and extraction of succinylacetone takes place simultaneously with the extraction of other analytes by addition of an aliquot of the Succinylacetone Assay Solution to the extraction mixture containing internal standards.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*

   A reproducibility study was performed across multiple instruments, operators, assay lots and multiple sites. The study plan consisted of the analysis of dried blood spots samples at three different analyte concentration levels. The study included three MS^2 instruments and three QMicro instruments tested at three different sites as well as six different operators and three different NeoBase assay lots. Each run consisted of the analysis of 6 replicates of each sample level (6 distinct dried blood punches and sample preparations) by one operator using one assay lot on one instrument. Each day, there were two runs (two different operators) per instrument and assay lot. The analysis was carried out

for five days for each instrument and assay lot. Overall, there were a total of 30 runs per instrument (5 runs per operator and assay lot on each instrument). Each operator performed a run on each of 5 distinct days (not necessarily consecutively) for an instrument and assay lot.

The total variability or Total %CV was determined as the square root of the sum of squares of the variation components mentioned above and according the equation 1, where Wr is the Within-Run variation, Br is the Between-Run component of variation, Bl is the Between-Lot variation and Bi/o is the Between-Instrument and Operator contribution to variability.

$$\text{Total imprecision} = [(Wr)^2 + (Br)^2 + (Bi/o)^2 + (Bl)^2]^{1/2}$$

Analyte	ALA						ARG					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	662.2	849.3	1893.2	713.6	880.1	1898.3	77.7	152.0	591.4	81.3	155.8	599.2
WR %CV Avg	5.1	5.4	5.4	5.1	4.6	4.7	4.5	4.3	4.6	4.7	4.2	4.3
Br % CV Avg	5.5	4.8	5.3	5.2	4.9	4.0	4.0	3.9	4.3	4.2	3.9	3.5
Bi/o % CV	3.3	3.1	3.4	7.3	7.5	6.2	3.2	1.9	2.6	4.8	6.8	7.3
BI %CV	3.5	2.2	2.4	3.3	2.0	2.3	3.2	1.5	2.0	2.7	2.3	2.9
Total %CV	9.0	8.2	8.6	10.9	10.3	9.1	7.5	6.3	7.1	8.4	9.2	9.6

Analyte	CIT						GLY					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	86.7	147.6	486.6	86.3	145.6	487.0	551.5	730.7	1740.2	613.7	763.9	1781.6
WR %CV Avg	6.6	6.3	5.5	5.2	5.2	4.7	7.6	6.7	6.6	10.7	10.2	8.0
Br % CV Avg	4.1	4.1	4.3	4.0	3.8	3.4	5.0	4.1	4.4	5.4	4.8	4.2
Bi/o % CV	3.2	2.3	2.9	6.3	6.4	5.5	4.0	3.6	3.7	8.2	8.1	5.8
BI %CV	3.3	1.8	2.4	3.2	2.8	3.3	3.6	3.0	2.1	7.4	3.6	2.9
Total %CV	9.0	8.1	8.0	9.7	9.5	8.7	10.6	9.1	9.0	16.3	14.3	11.1

Analyte	LEU						MET					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	301.8	369.6	750.3	309.1	371.4	742.2	45.4	76.2	257.6	47.4	76.9	259.0
WR %CV Avg	4.5	4.6	4.6	4.5	4.4	4.4	6.0	5.7	4.9	6.0	4.7	4.6
Br % CV Avg	4.2	3.9	4.2	3.8	3.6	3.4	5.0	3.9	4.3	4.2	4.0	4.0
Bi/o % CV	3.3	1.9	2.6	6.8	7.2	6.7	3.5	3.0	3.4	12.2	11.4	10.2
BI %CV	3.3	2.3	2.4	3.8	3.2	3.5	3.5	2.5	2.3	9.0	6.0	6.5
Total %CV	7.8	6.7	7.2	9.8	9.7	9.4	9.2	7.9	7.7	16.8	14.3	13.5

Analyte	ORN						PHE					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	204.6	292.3	785.5	198.8	283.2	767.7	125.5	187.4	549.6	131.8	191.3	548.9
WR %CV Avg	4.9	4.6	4.9	4.8	4.4	4.5	4.7	4.6	4.7	4.7	4.3	4.3
Br % CV Avg	5.7	5.0	5.7	4.0	3.9	3.4	4.5	4.0	4.4	3.7	3.3	3.2
Bi/o % CV	10.1	10.0	10.6	7.3	7.6	6.8	3.3	2.0	2.5	6.9	6.8	5.9
BI %CV	3.4	2.1	2.3	3.3	3.5	3.8	2.8	1.6	1.8	3.7	2.7	3.0
Total %CV	13.0	12.3	13.2	10.2	10.2	9.6	7.8	6.6	7.1	9.8	9.1	8.5

Analyte	PRO						SA					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	279.6	383.5	959.6	286.4	375.4	914.9	2.72	4.89	17.9	2.85	4.95	17.9
WR %CV Avg	5.1	5.1	4.9	5.0	4.4	4.3	7.9	6.6	5.0	8.3	6.4	4.7
Br % CV Avg	4.5	4.4	4.9	3.9	3.8	3.5	5.3	5.8	4.9	6.8	6.5	7.5
Bi/o % CV	7.1	7.9	8.8	9.0	8.5	6.6	4.5	4.0	4.6	5.7	7.1	9.7
BI %CV	3.2	1.8	1.9	5.6	3.5	3.4	4.2	3.2	3.4	2.5	3.3	2.6
Total %CV	10.3	10.5	11.4	12.4	10.9	9.3	11.3	10.2	9.0	12.4	12.0	13.4

Analyte	TYR						VAL					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	164.2.	26.18	833.3	166.3	260.1	823.8	266.1	423.7	655.0	280.0	335.0	666.0
WR %CV Avg	5.0	4.7	4.6	4.8	4.5	4.5	4.6	4.7	4.6	4.4	4.4	4.4
Br % CV Avg	3.9	3.6	4.4	3.6	3.5	3.3	5.0	4.4	4.8	3.7	3.5	3.3
Bi/o % CV	2.9	2.2	2.8	5.3	5.5	4.4	3.8	3.4	3.7	5.3	5.8	5.3
BI %CV	2.5	2.0	2.4	3.1	2.8	3.4	3.2	2.0	2.5	4.4	3.8	4.1
Total %CV	7.4	6.7	7.4	8.6	8.4	7.9	8.4	7.6	8.0	8.9	8.9	8.7

Analyte	C0						C2					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	107.3	189.8	663.4	106.8	187.7	653.4	31.4	53.7	181.4	32.3	54.8	183.0
WR %CV Avg	5.2	5.0	5.2	5.2	4.8	4.7	5.4	5.3	5.4	5.5	5.1	5.1
Br % CV Avg	5.2	4.6	5.4	3.6	3.4	3.5	4.3	4.0	4.4	3.5	3.4	3.2
Bi/o % CV	2.8	2.9	3.0	5.4	5.2	5.1	2.7	2.7	2.3	4.5	4.6	4.2
BI %CV	4.6	3.7	3.5	3.3	3.0	3.1	6.7	6.1	5.4	5.3	5.4	5.4
Total %CV	9.2	8.3	8.8	9.0	8.4	8.4	9.9	9.4	9.1	9.6	9.4	9.1

Analyte	C3						C4					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	3.89	6.49	21.3	3.90	6.45	21.3	1.61	2.99	10.8	1.59	2.94	10.8
WR %CV Avg	6.0	5.4	5.5	5.3	5.1	5.1	6.0	5.7	5.6	5.6	5.2	5.2
Br % CV Avg	4.1	4.4	4.7	3.7	3.6	3.2	4.4	3.9	4.4	4.9	4.4	4.0
Bi/o % CV	3.4	2.3	2.7	3.9	4.1	3.5	3.9	2.4	2.7	3.8	3.8	3.6
BI %CV	5.3	4.0	5.0	5.1	4.3	4.6	5.7	5.1	5.0	4.5	4.7	4.3
Total %CV	9.6	8.8	9.2	9.1	8.6	8.4	10.1	8.9	9.1	9.5	9.1	8.7

Analyte	C5						C5DC					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	0.98	1.86	6.82	1.02	1.91	7.07	3.23	6.26	23.4	3.04	5.93	22.7
WR %CV Avg	6.1	5.7	5.8	5.7	5.1	5.2	6.1	5.8	5.7	7.9	6.9	7.3
Br % CV Avg	4.4	4.4	4.9	3.9	3.5	3.2	6.6	6.6	7.1	3.9	3.8	4.4
Bi/o % CV	3.5	3.4	3.4	5.1	5.5	4.6	7.0	5.0	3.8	4.9	4.5	3.9
BI %CV	6.9	6.2	5.8	5.2	5.2	5.0	5.3	3.8	3.9	3.7	4.8	4.0
Total %CV	10.8	10.2	10.1	10.0	9.8	9.1	12.6	10.8	10.6	10.7	10.2	10.1

Analyte	C6						C8					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	1.52	2.96	11.3	1.43	2.83	10.8	1.28	2.51	9.60	1.28	2.50	9.61
WR %CV Avg	5.9	5.7	5.6	6.1	5.5	5.6	6.0	5.7	5.3	5.4	5.0	5.0
Br % CV Avg	4.2	4.0	4.5	8.8	8.4	9.4	4.8	4.3	4.5	3.9	3.8	3.3
Bi/o % CV	4.2	2.1	2.2	10.0	9.4	10.4	3.8	2.4	3.1	4.5	5.3	4.8
BI %CV	6.8	5.1	5.2	4.5	5.1	4.1	6.3	5.3	5.5	4.8	4.7	5.2
Total %CV	10.8	8.9	9.1	15.4	14.6	15.6	10.6	9.3	9.4	9.4	9.4	9.2

Analyte	C10						C12					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	1.29	2.54	9.76	1.26	2.46	9.45	1.44	2.85	11.0	1.44	2.84	10.9
WR %CV Avg	6.1	5.3	5.5	5.5	5.2	5.1	5.6	5.2	5.2	5.5	5.3	5.2
Br % CV Avg	4.5	4.0	4.9	3.9	3.8	3.5	4.3	3.9	4.8	3.9	3.6	3.2
Bi/o % CV	3.1	1.9	2.1	4.9	4.4	4.1	3.1	2.2	2.6	5.1	4.9	4.5
BI %CV	6.6	5.6	5.6	5.1	4.7	5.0	6.2	5.7	5.2	4.9	4.7	4.7
Total %CV	10.5	8.9	9.5	9.8	9.1	8.9	9.9	8.9	9.2	9.8	9.3	8.9

Analyte	C14						C16					
Platform	QMicro			MS^2			QMicro			MS^2		
DBS Levels	1	2	4	1	2	4	1	2	4	1	2	4
Grand mean	1.25	2.42	9.14	1.26	2.41	9.16	4.41	7.94	28.1	4.49	7.95	28.1
WR %CV Avg	5.3	5.0	5.2	5.5	5.1	5.3	5.2	5.0	5.0	5.4	5.2	5.1
Br %CV Avg	4.5	4.1	4.9	3.8	3.9	3.3	4.6	4.0	4.8	3.8	3.3	3.3
Bi/o % CV	3.1	2.4	2.8	5.6	5.6	5.2	2.8	1.8	2.4	5.8	5.6	5.1
BI %CV	6.4	5.7	5.7	4.8	4.8	5.1	6.9	6.1	6.3	5.2	5.3	5.7
Total %CV	10.0	9.0	9.5	9.9	9.7	9.6	10.2	9.0	9.6	10.3	9.8	9.7

Analyte	C18					
Platform	QMicro			MS^2		
DBS Levels	1	2	4	1	2	4
Grand mean	2.82	4.96	17.2	2.82	4.90	17.0
WR %CV Avg	5.4	5.2	5.2	5.5	5.1	5.3
Br %CV Avg	4.5	4.0	4.8	3.7	3.3	3.1
Bi/o % CV	2.7	1.6	2.0	4.7	4.7	4.2
BI %CV	7.2	6.3	6.2	5.2	5.2	5.4
Total %CV	10.4	9.2	9.6	9.7	9.3	9.2

An in-house precision study of the NeoBase Non-derivatized MSMS kit was performed using the QMicro Tandem Mass Spectrometry System. The data for the precision evaluation was acquired using multiple reaction monitoring (MRM) mode.

The testing for the precision was based on the CLSI guideline--Evaluation of Precision Performance of Clinical Chemistry (EP5-A) -- using three different levels of dried blood spots denoted in this study as Low, Mid, and High samples. These blood spots contained enriched levels of amino acids, succinylacetone (SA), free carnitine and acylcarnitines spiked into whole blood. The analyte concentrations of these spots cover significant clinical ranges (endogenous to above cutoffs). Additionally, one low and high blood spot controls were included for each run and used as plate qualifiers for this study.

Briefly, two runs per day, for twenty days over a period of one month were performed among four operators (five runs each) using the same lot of materials including DBS, extraction solution, flow solvent, and succinylacetone assay solution following the NeoBase assay procedure. Each of the runs within the same day was separated by at least two hours. All of the runs were acquired on one single instrument.

Analyte	ALA			ARG			CIT		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	603.2	1106.8	3044.5	97.2	374.8	1443.2	82.0	231.3	778.9
Total CV (%)	8.0	8.2	7.7	7.5	7.5	7.1	9.1	8.3	7.2
Analyte	GLY			LEU			MET		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	525.9	1009.6	2783.3	265.4	460.4	1190.9	46.1	140.1	500.6
Total CV (%)	9.1	9.0	7.5	7.7	7.8	6.4	7.9	7.9	6.2
Analyte	SA			ORN			PHE		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	2.9	10.4	40.8	226.7	491.1	1464.3	143.3	359.6	1186.6
Total CV (%)	11.9	9.1	9.2	7.6	8.0	7.9	8.2	7.8	6.7
Analyte	PRO			TYR			VAL		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	263.0	555.0	1648.5	172.3	463.7	1554.6	262.5	448.8	1154.4
Total CV (%)	7.1	8.7	6.7	8.1	7.4	6.8	8.4	8.7	6.9

Analyte	C0			C2			C3		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	92.6	298.2	1055.5	28.8	86.2	295.3	3.5	9.9	33.9
Total CV (%)	8.4	8.8	7.4	8.4	8.0	7.0	9.1	8.0	6.7
Analyte	C4			C5			C5DC		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	1.3	4.5	16.4	1.0	3.6	13.4	2.2	8.4	31.8
Total CV (%)	7.8	8.8	7.5	8.9	8.3	7.8	8.5	8.0	7.6

Analyte	C6			C8			C10		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	1.3	4.9	18.6	1.1	4.3	15.7	1.2	4.6	17.4
Total CV (%)	8.2	8.7	7.0	8.4	9.0	7.7	8.3	8.4	6.6
Analyte	C12			C14			C16		
Level	Low	Mid	High	Low	Mid	High	Low	Mid	High
Mean (µM)	1.2	4.6	17.7	1.2	4.4	16.8	4.4	14.1	50.1
Total CV (%)	8.4	8.3	7.2	7.4	8.3	7.2	8.3	8.3	6.9
Analyte	C18								
Level	Low	Mid	High						
Mean (µM)	2.8	9.2	33.2						
Total CV (%)	8.9	8.2	6.6						

b. *Linearity/assay reportable range:*

Linearity:
The linearity of the assay was determined by testing dried blood spot samples enriched with known and increasing analyte concentrations that covered the entire clinical range (endogenous to above cut-off levels). Twelve concentrations of each analyte were tested, and there were six replicates for each level. Finally, three low and high blood spot controls were included for each run and used as plate qualifiers for this study (all control results must be within 3 SDs of the assigned value). The study design consisted of one run per day over a total of four days. The linearity was determined by plotting the measured analyte concentrations and a function of the corresponding known enrichment levels and performing linear regression analysis of the data. The linear ranges ($R^2 \geq 0.99$ for all) for the assay on the QMicro Tandem Mass Spectrometry System are presented below.

	ALA	ARG	CIT	GLY
R^2 range	0.994-0.999	0.996-1.000	0.996-1.000	0.995-0.999
Slope range	0.76-0.79	0.76-0.80	0.86-0.91	0.80-0.90
	LEU	MET	ORN	PHE
R^2 range	0.995-0.999	0.995 – 1.000	0.995 – 1.000	0.995 – 1.000
Slope range	0.86-0.91	0.89-0.93	0.88-0.94	0.88-0.94
	PRO	SA	TYR	VAL
R^2 range	0.996-1.000	0.996-0.99	0.997-0.999	0.995-1.000
Slope range	0.86-0.92	0.72-0.74	0.84-0.88	0.87-0.92

	C0	C2	C3	C4
R^2 range	0.996-1.000	0.995-1.000	0.996-0.999	0.995-0.999
Slope range	0.81-0.85	0.83-0.88	0.80-0.86	0.75-0.78
	C5	C5DC	C6	C8
R^2 range	0.994-0.999	0.998-0.999	0.993-0.999	0.994 – 1.000
Slope range	0.82-0.85	0.90-0.93	0.85-0.89	0.72-0.75

	C10	C12	C14	C16
R^2 range	0.996-0.999	0.995-0.999	0.996-1.000	0.995-0.999
Slope range	0.93-0.99	0.81-0.86	0.89-0.94	1.13-1.16
	C18			
R^2 range	0.995-0.999			
Slope range	0.90-0.93			

The linear ranges ($R^2 \geq 0.99$ for all) for the assay on the MS^2 platform are presented below.

	ALA	ARG	CIT	GLY
R^2 range	0.998 - 0.999	0.999 - 1	0.999 - 1	0.998 - 0.999
Slope range	0.75 - 0.79	0.88 - 0.93	0.97 - 1.02	0.84 - 0.94
	LEU	MET	ORN	PHE
R^2 range	0.998 - 1	0.999 - 1	0.999 - 1	0.999 - 1
Slope range	0.89 - 0.93	0.88 - 0.92	0.82 - 0.85	0.89 - 0.94
	PRO	SA	TYR	VAL
R^2 range	0.999 - 1	0.999 - 1	0.999 - 1	0.998 - 1
Slope range	0.83 - 0.87	0.53 - 0.57	1.06 - 1.11	0.73 - 0.77

	C0	C2	C3	C4
R^2 range	0.999 – 1.00	0.999 – 1.00	0.999 – 1.00	0.999 – 1.00
Slope range	1.11 - 1.19	0.92 - 0.97	0.86 - 0.91	0.99 - 1.05
	C5	C5DC	C6	C8
R^2 range	0.999 – 1.00	0.998 – 1.00	0.999 – 1.00	0.999 – 1.00
Slope range	0.96 - 1.03	1.01 - 1.15	0.71 - 0.75	0.89 - 0.96
	C10	C12	C14	C16
R^2 range	0.999 – 1.00	0.999 – 1.00	0.999 – 1.00	0.999 – 1.00
Slope range	0.91 - 0.99	1.07 - 1.14	0.95 - 1.03	0.71 - 0.76
	C18			
R^2 range	0.998 – 1.00			
Slope range	0.89 - 0.95			

Recovery:

Analyte recovery for the NeoBase Non-derivatized MSMS kit was evaluated using the QMicro Tandem Mass Spectrometry System over five runs. Each run consisted of one plate containing 8 replicates each of endogenous samples and samples enriched at five different levels (levels L1 through L5) of the analytes of interest. Analytical runs were validated by including three low and three high blood spot controls (lot # Pilot 1) for each run and used as plate qualifiers for this study.

% Recovery= (Mean Measured concentration - Mean Endogenous concentration)
            ―――――――――――――――――――――――――――――――――――
                        Mean Spiked concentration

In total, the analysis consists of 146 daily recovery determinations for each analyte. The grand means of the 146 daily recovery determinations obtained in this study together with the corresponding standard deviations and 95% confidence interval for the data sets for each analyte are presented in the tables below:

		ALA	ARG	CIT	GLY	LEU	MET	ORN	PHE	PRO	SA	TYR	VAL
QMicro	Mean (%)	92	87	96	93	93	88	91	95	93	64	96	88
	SD (%)	12	8	7	12	10	6	8	7	8	6	9	9
	95% CI (%)	69-116	72-102	83-109	69-117	72-113	75-101	75-108	81-109	78-108	52-77	79-114	69-106
MS²	Mean (%)	83	87	95	86	88	86	91	89	84	62	102	78
	SD (%)	10	7	11	17	8	6	6	6	8	7	10	10
	95% CI (%)	63-104	73-100	73-116	51-120	72-103	73-98	78-103	76-101	68-100	48-76	81-122	58-97

		C0	C2	C3	C4	C5	C5DC	C6	C8	C10	C12	C14	C16	C18
QMicro	Mean (%)	91	93	94	91	91	99	91	90	97	93	92	93	91
	SD (%)	11	7	8	9	7	8	5	11	5	9	5	13	7
	95% CI (%)	70-112	79-108	78-110	72-109	78-105	83-115	82-101	68-113	86-108	75-112	82-102	68-118	77-105
MS²	Mean (%)	107	97	95	92	94	104	83	96	95	103	94	84	94
	SD (%)	14	8	10	14	10	8	10	13	9	14	6	15	13
	95% CI (%)	80-134	80-113	76-115	64-121	74-114	87-121	63-103	70-121	78-112	75-130	81-107	55-114	69-119

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Internal standards as well as dried blood spots controls are included with the assay. The following tables provide a list of the relevant internal standards and DBS controls for each analyte in the NeoBase Non-derivatized MSMS Kit:

Amino acids:

Analyte	Internal Standard	Control
Ala	$^2H_4$-Ala	Ala
Arg	$^2H_4$, ^{13}C-Arg	Cit
Cit	$^2H_2$-Cit	Cit
Gly	$^{15}N,2$-^{13}C-Gly	Gly
Leu/Ile/Pro-OH	$^2H_3$-Leu	Leu
Met	$^2H_3$-Met	Met
Orn	$^2H_6$-Orn	Cit
Phe	$^{13}C_6$-Phe	Phe
Pro	$^{13}C_5$-Pro	Pro
Tyr	$^{13}C_6$-Tyr	Tyr
Val	$^2H_4$Val	Val

Carnitines:

Analyte	Internal Standard	Control	Analyte	Internal Standard	Control	Analyte	Internal Standard	Control
C0	$^2H_9$-C0	C0	C8	$^2H_3$-C8	C8	C14OH	$^2H_3$-C14	C14
C2	$^2H_3$-C2	C2	C8:1	$^2H_3$-C8	C8	C16	$^2H_3$-C16	C16
C3	$^2H_3$-C3	C3	C10	$^2H_3$-C10	C10	C16:1	$^2H_3$-C16	C16
C3DC/C4OH	$^2H_3$-C4	C4	C10:1	$^2H_3$-C10	C10	C16OH	$^2H_3$-C16	C16
C4	$^2H_3$-C4	C4	C10:2	$^2H_3$-C10	C10	C16:1OH	$^2H_3$-C16	C16
C4DC/C5OH	$^2H_9$-C5	C5	C12	$^2H_3$-C12	C12	C18	$^2H_3$-C18	C18
C5	$^2H_9$-C5	C5	C12:1	$^2H_3$-C12	C12	C18:1	$^2H_3$-C18	C18
C5:1	$^2H_9$-C5	C5	C14	$^2H_3$-C14	C14	C18:2	$^2H_3$-C18	C18
C5DC/C6OH	2H6-C5DC	C5DC	C14:1	$^2H_3$-C14	C14	C18OH	$^2H_3$-C18	C18
C6	$^2H_3$-C6	C6	C14:2	$^2H_3$-C14	C14	C18:1OH	$^2H_3$-C18	C18
C6DC	$^2H_6$-C5DC	C5DC						

Ketones:

Analyte	Internal Standard	Control
SA	$^{13}C_5$-MPP	SA

Value Assignment:

Internal Standards: The value assignment of the internal standards consists of the production of reference stock solutions of the corresponding non-labeled analytes. These solutions are of known concentrations and thus are used to determine the concentration of the individual internal standards in each vial type. The procedure consists of preparing three separate stock solutions of amino acids and of acylcarnitines. Each solution is prepared individually and the material is individually weighed for each solution.

The result of this process is three individually prepared solutions for each of the acylcarnitines and amino acids (the stock solutions are mixtures of each analyte type). Separately, internal standard vials are reconstituted (either amino acids or acylcarnitines). The reconstituted internal standards are then diluted and mixed with dilutions of the corresponding non-labeled reference solutions. Three random vials of each lot (either amino acid or acylcarnitine internal standards) are used for each of the stock solutions for a total of 9 vials analyzed. The resulting mixtures of internal standards and reference solutions are processed using the derivatized assay procedure. Using the known concentrations of the reference stock solutions, the concentration of each internal standard is determined per vial and per reference stock solutions. The results are analyzed for consistency within plate, within vial, within stock solution and between plate, between vial and between reference stock solution. If the data is consistent across all reference solutions and vials, the average concentration of each internal standard obtained from these experiments is then assigned as the lot assigned value.

Amino Acid Stable-isotope standards	Approx. amount per vial (µmol)
$^{15}N,2$-^{13}C-Glycine	2.5
$^{2}H_4$-Alanine	0.5
$^{2}H_8$-Valine	0.5
$^{2}H_3$-Leucine	0.5
$^{2}H_3$-Methionine	0.5
$^{13}C_6$-Phenylalanine	0.5
$^{13}C_6$-Tyrosine	0.5
$^{2}H_6$-Ornithine.HCl	0.5
$^{2}H_2$-Citrulline	0.5
$^{2}H_4, ^{13}C$-Arginine.HCl	0.5
$^{13}C_5$-Proline	0.5
$^{13}C_5$-3-(5-methyl-1H-pyrazol-3-yl)propanoic acid ($^{13}C_5$-MPP)	0.4
Acylcarnitine Stable-isotope standards	**Approx. amount per vial (µmol)**
$^{2}H_9$-Free carnitine ($^{2}H_9$-C0)	0.152
$^{2}H_3$-Acetylcarnitine ($^{2}H_3$-C2)	0.019
$^{2}H_3$-Propionylcarnitine ($^{2}H_3$-C3)	0.0114
$^{2}H_3$-Butyrylcarnitine ($^{2}H_3$-C4)	0.0076
$^{2}H_9$-Isovalerylcarnitine ($^{2}H_9$-C5)	0.0076
$^{2}H_6$-Glutarylcarnitine ($^{2}H_6$-C5DC)	0.0076
$^{2}H_3$-Hexanoylcarnitine ($^{2}H_3$-C6)	0.0076
$^{2}H_3$-Octanoylcarnitine ($^{2}H_3$-C8)	0.0076
$^{2}H_3$-Decanoylcarnitine ($^{2}H_3$-C10)	0.0076
$^{2}H_3$-Lauroylcarnitine ($^{2}H_3$-C12)	0.0152
$^{2}H_3$-Myristoylcarnitine ($^{2}H_3$-C14)	0.0152

$^2H_3$-Palmitoylcarnitine ($^2H_3$-C16)	0.0152
$^2H_3$-Octadecanoylcarnitine ($^2H_3$-C18)	0.0152

Dried Blood Spot Control*:* The assignment of the analyte concentrations in the NeoBase dried blood spot controls is performed using amino acid and acylcarnitine internal standards whose values have been assigned as described above. The value assignment consists in the determination of the analyte concentration in the low and high assay controls by analyzing the control material with the NeoBase assay procedure.

The dried blood spot are processed as indicated for the NeoBase assay. In this procedure, three different internal standard working solutions are prepared (all from the same internal standard assay lot) to avoid biases due to the preparation of the internal standard solutions. The blood spots are then processed using each internal standard solution and analyzed in 12 replicates and in three runs. The results of these experiments are analyzed for precision and consistency and if the data is consistent, the average concentration for each analyte is reported as the expected mean concentration expected for the lot.

Analytes included in the controls and approximate mean concentrations (µmol/L)		
Analyte	**Low control**	**High control**
Alanine (Ala)	930	1878
Citrulline (Cit)	100	300
Glycine (Gly)	920	2700
Leucine (Leu)	350	1100
Methionine (Met)	110	344
Phenylalanine (Phe)	240	674
Proline (Pro)	650	1690
Succinylacetone (SA)	5	15
Tyrosine (Tyr)	372	1156
Valine (Val)	383	867
Free carnitine (C0)	120	260
Acetylcarnitine (C2)	70	153
Propionylcarnitine (C3)	11	28
Butyrylcarnitine (C4)	2.7	6.5
Isovalerylcarnitine (C5)	1.3	3.3
Glutarylcarnitine (C5DC)	0.7	1.8
Hexanoylcarnitine (C6)	0.6	1.8
Octanoylcarnitine (C8)	0.6	1.8
Decanoylcarnitine (C10)	1.0	2.8
Lauroylcarnitine (C12)	1.6	5.2
Myristoylcarnitine (C14)	1.8	4.8
Palmitoylcarnitine (C16)	12.7	31.2
Octadecanoylcarnitine (C18)	2.3	5.0

Stability: Stability testing protocols and acceptance criteria were reviewed for blood spot and internal controls and kit components and found to be acceptable.

d. *Detection limit:*

The functional sensitivity of the NeoBase assay was evaluated by excising discs from each of two sets of blood spots (these blood spots contained a high and low enrichment levels of the analytes of interest). The sample discs were placed in microtiter plate wells and extracted with the NeoBase extraction solution. Upon completion of the extraction, the extracts from each level were pooled by transferring a small amount from each extraction well and into a common vial and mixed (this is referred to as "DBS extract diluents" below). Next, an aliquot of each of the pooled extracts was taken and spiked with amino acid and acylcarnitine internal standards resulting in a high internal standard concentration sample (Level 1). Sample level 1 was mixed with the DBS extract diluent in a 1:1 v/v ratio to obtain a Level 2 sample whose internal standard concentration is expected to be one half of that present in the Level 1 sample. This procedure was followed serially until a total of 17 dilutions were accomplished from which 18 sample levels with decreasing concentration of internal standards (Level 1 to 18) were obtained.
These solutions for each of the DBS levels were dispensed into microtiter plates. Additionally, 12 low plate controls and 12 high plate controls were extracted using NeoBase assay procedure and two rows of the neat NeoBase extraction solution was dispensed to ensure that Level18 which contains the lowest internal standard concentrations is not cross contaminated by preceding wells.

To determine the functional sensitivities of the analytes in the NeoBase assay, three different approaches were employed for data analysis: 1) a stepwise linear regression analysis, 2) a relative concentration response of adjacent dilutions and 3) the precision of the replicate samples. Thresholds were determined for each of the above parameters and the functional sensitivities were established as the lowest concentration that met all three criteria.

Analyte	Detection limit (µM)		Endogenous Conc. Range (µM)	Analyte	Detection limit (µM)		Endogenous Conc. Range (µM)
	QMicro	MS^2			QMicro	MS^2	
Ala	4.5	1.1	108-252	C0	0.2	0.19	1.8-4.2
ARG	0.6	0.6	6-14	C2	0.2	0.05	1.2-2.8
CIT	4.8	2.4	10-24	C3	0.03	0.06	1.5-3.5
GLY	50.4	100.9	120-280	C4	0.07	0.07	\0.12-0.28
LEU	1.3	1.3	72-168	C5	0.04	0.08	0.06-0.14
MET	2.5	2.5	9-21	C5DC	0.08	0.08	0.12-0.28
ORN	0.6	1.1	30-70	C6	0.08	0.08	0.06-0.14
PHE	0.3	0.6	36-84	C8	0.02	0.08	0.06-0.14
PRO	4.7	0.6	60-140	C10	0.04	0.02	0.06-0.14
SA	0.24	0.5	0.1-0.7	C12	0.04	0.01	0.06-0.14
TYR	1.2	1.1	36-84	C14	0.02	0.01	0.06-0.14
VAL	0.6	2.4	72-168	C16	0.1	0.05	0.6-1.4
				C18	0.04	0.01	0.3-0.7

e. *Analytical specificity:*
The analysis to identify potential interferents proceeded in the following manner:

1. A broad compilation of substances that could potentially interfere with the assay. The substances listed would be found in the clinical sample and a molecular mass overlap with any analytes or internal standards detected with the NeoBase assay. The initial list of potential interfering substances were compiled from the following sources:
   (a) 336 substances suggested by CLSI guideline EP7-A2 to be common interfering substances in clinical assays;
   (b) 122 substances from the NIST mass spectral database that had potential mass overlaps with analytes detected with the assay; and
   (c) 8 substances identified to be potential interfering substances in NeoGram AAAC Derivatized Tandem Mass Spectrometry kit. In total, the initial list considered 466 substances.
2. The list was refined by evaluating whether or not there was likelihood for any of these substances to be present in the neonatal sample.
3. The chemical structures of the remaining substances was examined and if the chemical structure of a substance indicated the potential to have the same mass-to-charge ratio (*m/z*) as an analyte or internal standard of interest, its structure was further examined to predict whether or not this substance would fragment in the tandem mass spectrometry experiment in such a way to produce the same mass transition as any of the analytes or internal standards in the assay. If the likelihood of a substance to fragment in such a way to interfere with the assay was predicted to be negligible, such substance was not considered a potential interference for the assay. If a substance, however, was predicted to potentially ionize and fragment in such a way that would produce the same mass transition

used for the detection of an analyte or internal standard in the assay, that substance was evaluated further.

4. Over all, 16 compounds were identified as potential interfering candidates. These 16 substances and the respective target analyte/internal standard with which they would interfere (listed in the parenthesis) are Aminocaproic acid (Leu), Asparagine (Orn), Creatine (Ala), Creatine (Leu), Dihydroxybenzoic acid (SA), Folic acid (C18:1OH), Formiminoglutamic acid or FIGLU (Arg), Hydroxylproline (Leu), Lidocaine (C4 IS), Malic acid (Leu IS), Methionine sulfone (Tyr), Ornithine (Pro), Salicylic acid (Orn), Sarcosine (Ala), Penicillamine (Met), Propranolol (C6), and Pseudoephedrine (Phe).

5. The 16 compounds identified above as potential candidates for interference with the assay were evaluated by subjecting them to daughter ion scan tandem mass spectrometry experiments. Fragmentation of the 16 interfering candidates confirmed that 14 of them generated fragments identical or close (within +/- 1 amu) to the daughter ion of the target analyte or internal standard. Therefore, they are considered as potential interfering substances in the assay and required further evaluation.

   These 14 interfering substances were further evaluated by the actual NeoBase assays in which pooled dried blood spot (DBS) extracts, both at endogenous level (referred to as Endo hereafter in this study) and analyte enriched level (referred to as Enriched hereafter), were spiked individually with each of the 14 potential interfering substances at the concentration suggested by CLSI EP7-A2 guidelines, results of which were compared with those obtained from the same pooled DBS extracts but measured without any spiking of the potential interfering substances. Results from these experiments indicated that 5 of the 14 substances evaluated caused a statistically significant change to the analyte measurement, and thus were confirmed to be potential interfering substances for the NeoBase assay. These substances are Asparagine, Creatine, Methionine sulfone, Sarcosine, and Hydroxyproline. These 5 compounds were further evaluated by performing dose response experiments in which the interfering substance was spiked in DBS extract at increasing concentrations to determine the concentration at which the interference becomes significant. None of the interferents should impact screening with the NeoBase assay (e.g. false positives).

6. The second study consisted in the evaluation of substances not expected to have a mass transition overlap but that could affect the assay by ion suppression or other indirect processes. In these experiments, eight substances (see table below) were spiked individually into blood. This spiked blood was then used to make DBS at endogenous and 3 enriched analyte levels. Reference control DBS (free of interfering substances) were also made. Results from these experiments indicated that none of these 8 substances interferes with the NeoBase assay.

Potential Interfering Substances		Concentration tested
1	Glucose	120 mg/L
2	EDTA	0.994 µg/mL
3	Heparin	21 µg/mL
4	Albumin	5000 µg/mL
5	PEG	100 µM
6	Heptanodione	5000 µM
7	2-butanone	5000 µM
8	Citrate-Phosphate-Dextrose (CPD)	14.7 mg/mL of Citrate tri-sodium
		1.8 mg/ml of citric acid
		14.1 mg/ml of dextrose
		1.2 mg/mL of monobasic sodium phosphate

7. Finally, the potential interference of Chlorhexidine gluconate (the active ingredient in Chlorascrub SwabRM) with NeoBase assay was also examined. This compound was first examined by adding the solution squeezed out of Chlorascrub Swab to NeoBase DBS assay (at both endogenous and enriched analyte levels) before incubation. Results showed that this compound caused significant changes in the measured concentrations for a number of analytes compared to reference assay (no Chlorascrub Swab solution added) and the total ion chromatogram (TIC) intensity was suppressed substantially. Tandem mass spectrometry fragmentation analysis was performed to investigate the observation in the screening test, and dose response study of chlorhexidine gluconate was also performed. Chlorhexidine gluconate interferes with quantitation of the C10 and C5 internal standards at concentrations ranging from 450 – 2790 µmol/L, respectively, however the residual amount of the compound found on the skin after Chlorascrub Swab use should only be a small percentage of the amount known to interfere.

These compounds and the details of their potential to interfere are all described in the package insert for the device.

Carryover:
The study samples were DBS prepared using enriched concentrations of amino acids, succinylacetone, free carnitine, and acylcarnitines spiked into whole blood. DBS samples were extracted according to the NeoBase assay procedure and pooled. Sample pooling was performed in order to minimize sample variation. Pooled samples were dispensed to microtiter plates 4 and data acquired using multiple reaction monitoring (MRM) mode. Quality control samples (two replicates each Low and High controls) were analyzed at the beginning, middle, and end of each run.

The study was designed to characterize carryover for three cutoff situations: 1) "more than" cutoff positive carryover; 2) "less than" cutoff positive carryover; and 3) negative carryover.

When using a "more than" cutoff, a patient is flagged if an analyte is above a certain concentration cutoff. In this case, positive carryover from one sample into another could result in a False Positive determination by artificially increasing the analyte concentration above its cutoff. This type of carryover was characterized by acquiring a range of high concentration samples (LL1 → LL4) each followed by three borderline samples (BL1 → BL3). The borderline sample was chosen such that analyte concentrations are in the region of the estimated cutoff ranges. Five runs were acquired on the QMicro and MS^2 platforms.

The results for the study demonstrate that for SA carryover may occur in cases where a Tyrosinemia Type I patient with drastically elevated SA is followed by a borderline patient. This is noted in the package insert. The clinical impact of this result is a potential increased repeat testing rate due to increased false positive outcome. Since the magnitude of the carryover is not great for SA, only the repeat testing rate is affected and not the safety or efficacy of the assay. Additionally, this disorder is not very prevalent (incidence rate 1:100,000-1:125,000; ref. 1, 2), thus instances in which carry over may be present will be very infrequent and thus the increased repeat rate will not be substantial.

Carryover was not detected for other analytes.

Drift:
The potential of the NeoBase Non-derivatized MSMS kit to be subject to drift was evaluated. The data for the drift study was acquired using multiple reaction monitoring (MRM) mode. The experiment was set to run continuously for a total elapsed assay time of approximately 24 hours. This timeframe covers the area of concern for practical use of the assay; that is, within day drifting.

For this study, dried blood spots were prepared using three enriched concentrations (denoted in this study as Low, Mid, and High) of amino acids, succinylacetone, free carnitine and acylcarnitines spiked into whole blood.

Three low and three high blood spot controls were included for each run and used as run qualifiers for this study. Each plate was considered to contain the equivalent of two runs. The time elapsed during the acquisition of each run was 1.5 hours. Therefore, the study analyzed a total of 16 runs (time points) collected throughout one 24 hour-period. The mean from the first run was used as time zero reference. Analysis of the data indicates that the assay variability over the 24-hour test period is well within the expected precision of the assay as demonstrated by Between Run % CV, therefore no drift was detected.

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      The method comparison studies took place at two different US newborn screening laboratories that evaluated the NeoBase assay in parallel to the predicate device (identical specimens were analyzed as paired samples by both methods). The sample set consisted of 9416 random neonatal samples, 104 samples from with true positive diagnoses, and 320 artificially enriched dried blood spots. Correlation was established by assessing whether or not the methods were concordant in determining the paired samples to have analyte concentration values above or below their corresponding cutoffs. Examination on the number of concordant pairs for each analyte (cases in which both methods agreed) provided the percent agreements shown here:

Analyte	Total # Observations	% Agreement	Analyte	Total # Observations	% Agreement
ALA	2559	99.7%	C14	9813	99.9%
ARG	2564	100.0%	C16	9803	99.9%
CIT	9805	99.8%	C18	9781	100.0%
GLY	2474	99.8%	C4-OH/C3DC	2564	99.5%
LEU	9771	99.6%	C5:1	9840	100.0%
MET	9808	99.7%	C5-OH/C4DC	7276	98.4%
ORN	2554	99.7%	C6DC	9840	99.1%
PHE	9749	99.8%	C10:1	9840	100.0%
TYR	9803	99.9%	C12:1	2564	100.0%
VAL	9745	99.5%	C14:1	9840	99.9%
C0	9461	99.9%	C14:2	2564	99.9%
C2	9808	100.0%	C14-OH	2564	99.9%
C3	9781	99.9%	C16:1	2564	100.0%
C4	2559	99.9%	C16:1-OH	9840	100.0%
C5	9809	99.6%	C16-OH	9840	100.0%
C5DC	9840	97.2%	C18:1	9840	99.0%
C6	9840	100.0%	C18:1-OH	9840	100.0%
C8	9840	100.0%	C18:2	2564	99.9%
C10	9840	99.9%	C18-OH	9840	100.0%
C12	2559	99.9%			

The screening results for known positive samples:
A total of 104 stored true positive samples representing 19 different disorders were acquired and tested at Site 1 and 2 during the method comparison studies:

Disorder Abbrev.	Disorder Full name	Number Screen-ed	Detected by NeoBase Study Cutoffs	Detected by Predicate Study Cutoffs
3MCC	3-Methylcrotonyl-CoA Carboxylase Deficiency	9	9	9
CUD	Carnitine Uptake Defect	10	10	10
CTD	Carnitine Transporter Defect	1	1	1
CPT-1	Carnitine Palmitoyltransferase I Deficiency	1	1	1
GA-1	Glutaric acidemia, type 1	9	9	9
HCY	Homocystinuria	7	7	7
IVA	Isovaleric acidemia	9	9	9
2MBDD	2-Methylbutyryl-CoA Dehydrogenase Deficiency	1	1	1
MCAD	Medium-Chain Acyl-CoA Dehydrogenase Deficiency	16	16	16
MCD	Multiple CoA Carboxylase Deficiency	3	3	3
MMA	Methylmalonic Aciduria	2	2	2
PPA	Propionic Acidemia	3	3	3
MSUD	Maple Syrup Urine Disease	2	2	2
SCAD	Short-Chain Acyl-CoA Dehydrogenase Deficiency	1	1	1
PKU	Phenylketonuria	12	12	12
CPT-2	Carnitine Palmitoyltransferase II Deficiency	**1**	**0**	**0**
LCHAD	Long-Chain 3-hydroxyacyl-CoA Dehydrogenase Deficiency	5	5	5
VLCAD	Very Long-Chain Acyl-CoA Dehydrogenase Deficiency	**11**	**10**	**10**
VLCHAD	Very Long-Chain 3-hydroxyacyl-CoA Dehydrogenase Deficiency	1	1	1
TYR 1	Tyrosinemia Type 1	**4**	**4**	**0**

The two samples that neither of the two assays was able to detect based on the study cutoffs were a CPT-2 and a VLCAD case. The CPT-2 sample had been in storage for over three years and the VLCAD sample had been stored at room temperature for over one year. The CPT-2 sample was found originally during routine newborn screening at a state laboratory using the predicate device. It is possible that the acylcarnitine analytes in these samples had

experienced a significant degree of decay over time and thus causing the corresponding analytes to be below the study cutoffs. For both of these disorders, elevated levels of long chain acylcarnitines are expected, but neither device indicated that it measured analytes above the cut-offs. The package insert indicates that degradation of acylcarnitines may occur in patient samples, however, this should not impact newborn screening where samples should be tested within 10 days of sample collection.

US Site 1:

The correlation study at US Site 1 was executed using the MS^2 Tandem Mass Spectrometer System (MS^2) instruments to acquire all of the NeoBase and the NeoGram data. One lot of the NeoBase assay was used. All of the NeoBase data were acquired using full MRM method and all of the NeoGram data were acquired using the full scan method. A total of 31 full or partial plates containing retrospective presumptive negative (left over) neonatal samples were acquired totaling 2,500 neonatal samples. Additionally, 14 stored confirmed True Positive (TP) samples, 50 artificially enriched Presumptive Positive (PP) and Presumptive Borderline (PB) samples were acquired using both assays. (The PP and PB samples are artificially prepared DBS. The PP and PB samples were derived from analyte enriched DBS prepared by the sponsor.)
The following 2x2 tables indicate the results of each analyte after testing of paired samples on the predicate and proposed device. None of the discrepant results were from stored confirmed True Positive samples.

ALA		NeoGram		ARG		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	26	1	NeoBase	Above Cut-off	36	0
	Below Cut-off	6	2526		Below Cut-off	1	2527

CIT		NeoGram		GLY		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	45	5	NeoBase	Above Cut-off	17	5
	Below Cut-off	1	2505		Below Cut-off	1	2451

LEU		NeoGram		MET		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	34	6	NeoBase	Above Cut-off	47	4
	Below Cut-off	8	2501		Below Cut-off	6	2502

ORN		NeoGram		PHE		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	11	5	NeoBase	Above Cut-off	44	0
	Below Cut-off	2	2536		Below Cut-off	1	2509

TYR		NeoGram		VAL		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	28	3	NeoBase	Above Cut-off	38	5
	Below Cut-off	1	2522		Below Cut-off	7	2499

C0		NeoGram		C10		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	43	1	NeoBase	Above Cut-off	50	3
	Below Cut-off	1	2167		Below Cut-off	1	2510

C2		NeoGram		C3		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	21	0	NeoBase	Above Cut-off	27	0
	Below Cut-off	0	2538		Below Cut-off	2	2530

C4		NeoGram		C4 OH		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	40	2	NeoBase	Above Cut-off	0	13
	Below Cut-off	1	2516		Below Cut-off	1	2550

C5		NeoGram		C5DC		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	63	6	NeoBase	Above Cut-off	57	205
	Below Cut-off	14	2476		Below Cut-off	5	2297

C6DC		NeoGram		C8		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	6	17	NeoBase	Above Cut-off	50	1
	Below Cut-off	0	2541		Below Cut-off	0	2513

C5:1		NeoGram		C6		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	0	0	NeoBase	Above Cut-off	51	1
	Below Cut-off	1	2563		Below Cut-off	1	2511

C10:1		NeoGram		C12		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	1	1	NeoBase	Above Cut-off	44	1
	Below Cut-off	2	2560		Below Cut-off	1	2513

C12:1		NeoGram		C14		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	0	1	NeoBase	Above Cut-off	50	1
	Below Cut-off	0	2563		Below Cut-off	3	2510

C14:1		NeoGram		C14:2		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	1	1	NeoBase	Above Cut-off	1	1
	Below Cut-off	3	2559		Below Cut-off	1	2561

C14 OH		NeoGram		C16		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	0	1	NeoBase	Above Cut-off	26	3
	Below Cut-off	2	2561		Below Cut-off	4	2521

C16:1		NeoGram		C16:1 OH		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	1	0	NeoBase	Above Cut-off	0	0
	Below Cut-off	1	2562		Below Cut-off	1	2563

C16 OH		NeoGram		C18		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	0	0	NeoBase	Above Cut-off	42	1
	Below Cut-off	1	2563		Below Cut-off	0	2516

C18:1		NeoGram		C18:1 OH		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	1	1	NeoBase	Above Cut-off	0	0
	Below Cut-off	2	2560		Below Cut-off	0	2564

C18:2		NeoGram		C18 OH		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	3	1	NeoBase	Above Cut-off	0	0
	Below Cut-off	2	2558		Below Cut-off	1	2563

US SITE 2:

This evaluation study was executed using QMicro Tandem Mass Spectrometry System to acquire all of the NeoBase and NeoGram data. One lot of the proposed device was used. Both NeoBase and NeoGram data were acquired using MRM methods. A total of 78 full or partial plates containing retrospective (left over) presumptive negative neonatal samples were acquired totaling 6916 analyzed samples.

The neonatal samples were the left over routine newborn samples used for screening. Additionally, 90 stored confirmed True Positive (TP) samples, 270 Presumptive Positive (PP) and Presumptive Borderline (PB) samples were acquired using both assays. (The PP and PB samples were artificially prepared DBS. The PP and PB samples were derived from analyte enriched DBS prepared by the sponsor.)

(Alanine, Arginine, Glycine, Ornithine results not collected on predicate device because not state mandated for newborn screening)

The following 2x2 tables indicate the results of each analyte after testing of paired samples on the predicate and proposed device. None of the discrepant results were from stored confirmed True Positive samples.

CIT		NeoGram		LEU		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	245	5	NeoBase	Above Cut-off	212	17
	Below Cut-off	5	6994		Below Cut-off	7	6986

MET		NeoGram		PHE		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	199	6	NeoBase	Above Cut-off	215	16
	Below Cut-off	13	7031		Below Cut-off	2	6962

TYR		NeoGram		VAL		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	171	1	NeoBase	Above Cut-off	211	21
	Below Cut-off	0	7077		Below Cut-off	17	6947

C0		NeoGram		C2		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	220	5	NeoBase	Above Cut-off	108	1
	Below Cut-off	6	7018		Below Cut-off	1	7139

C3		NeoGram		C5		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	149	5	NeoBase	Above Cut-off	275	3
	Below Cut-off	0	7068		Below Cut-off	16	6956

C5DC		NeoGram		C6		NeoGram	
		Above Cut-off	Below Cut-off			Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	280	64	NeoBase	Above Cut-off	285	1
	Below Cut-off	5	6927		Below Cut-off	0	6990

C8		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	285	3
NeoBase	Below Cut-off	0	6988

C10		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	283	3
NeoBase	Below Cut-off	0	6990

C14		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	251	2
NeoBase	Below Cut-off	1	6995

C16		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	163	5
NeoBase	Below Cut-off	1	7080

C18		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	189	3
NeoBase	Below Cut-off	0	7030

C5:1		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	0	1
NeoBase	Below Cut-off	0	7275

C5OH/C4DC		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	78	107
NeoBase	Below Cut-off	12	7079

C6DC		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	12	68
NeoBase	Below Cut-off	0	7196

C10:1		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	10	0
NeoBase	Below Cut-off	1	7265

C16:1 OH		NeoGram Above Cut-off	NeoGram Below Cut-off
NeoBase	Above Cut-off	2	0
NeoBase	Below Cut-off	0	7274

C14:1		NeoGram			C16 OH		NeoGram	
		Above Cut-off	Below Cut-off				Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	9	1		NeoBase	Above Cut-off	5	0
	Below Cut-off	1	7265			Below Cut-off	0	7271

C18:1 OH		NeoGram			C18:1		NeoGram	
		Above Cut-off	Below Cut-off				Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	5	0		NeoBase	Above Cut-off	17	98
	Below Cut-off	0	7271			Below Cut-off	2	7159

C18 OH		NeoGram	
		Above Cut-off	Below Cut-off
NeoBase	Above Cut-off	5	0
	Below Cut-off	0	7271

b. *Matrix comparison:*
Not applicable. This device is for use with newborn dried blood spots only.

3. Clinical studies:
a. *Clinical Sensitivity:*
Not applicable.

b. *Clinical specificity:*
Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off:
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 cut-off values should be determined. Cut-off values for

reporting abnormal result levels for each analyte should be established by using statistical measurements (e.g. percentiles, means, and standard 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 amino acid, succinylacetone, free carnitine and acylcarnitine concentration profiles should be based on predetermined cut-offs obtained from pilot studies performed with the NeoBase Non-derivatized MSMS kit. If available, samples from patients with known disorders (true positives) should be run to provide additional guidance in setting conservative abnormal and borderline cut-off 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 cut-offs 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.

Presumptive positives:
Results that are above (or below if it is a low cut-off) the abnormal cut-offs should be considered presumptive positive. Retesting of the original specimen with the original method is recommended for all specimens designated as presumptive positive for one or more disorders or analytes. Follow local regulations and guidelines for the handling and reporting of presumptive positive results.

Borderline specimens:
Results that fall between the abnormal cut-off and the borderline cut-off should be considered borderline results. Retesting of the original specimen with the origina method is recommended for specimens whose initial results are borderline. Follow local regulations and guidelines for the handling and reporting of borderline results.

Repeat testing:
Regardless of whether retesting was initiated because of initial borderline or presumptive positive status, if the repeat testing result is above the abnormal cut-off (or below if it is a low cut-off) then the result should be considered presumptive positive. Follow local regulations and guidelines for the handling and reporting of presumptive positive results.

Regardless of whether retesting was initiated because of initial borderline or abnormal status, if the repeat testing result falls between the abnormal and borderline cut-offs, then all available data (initial and retesting) should be taken into consideration to report the values according to local regulations and guidelines.
Regardless of whether retesting was initiated because of initial borderline or abnormal status, follow local regulations and guidelines in cases where retesting results are below (or above if a low cut-off) the borderline cut-offs.

Presumptive negatives:
If all the initial results of any specimen, are below (or above if a low cut-off) all the borderline and abnormal cut-offs, the result can be treated as presumptive negative (or low risk) and reported appropriately.

5. Expected values/Reference range:
    Typical expected NeoBase Non-derivatized MSMS kit results for neonatal populations are presented below. These results were obtained at two US newborn screening facilities.

    The data represents 9,363 neonatal results. The population means and population standard deviation (SD) are shown in micromolar (μM) quantities.

Analyte	Population mean	Population SD	Analyte	Population Mean	Population SD
ALA	330	90	C6DC	0.17	0.12
ARG	13	10	C8	0.06	0.03
CIT	16	6	C8:1	0.15	0.06
GLY	436	149	C10	0.09	0.04
LEU	131	42	C10:1	0.07	0.02
MET	25	9	C10:2	0.01	0.01
ORN	121	44	C12	0.11	0.06
PHE	64	19	C12:1	0.10	0.07
PRO	169	45	C14	0.22	0.08
SA	0.72	0.23	C14:1	0.12	0.14
TYR	106	44	C14:2	0.03	0.02
VAL	115	32	C14-OH	0.02	0.01
C0	25	10	C16	2.99	1.08
C2	22	9	C16:1	0.21	0.09
C3	1.80	0.77	C16:1-OH	0.04	0.02
C3DC/C4-OH	0.13	0.07	C16-OH	0.03	0.01
C4	0.28	0.13	C18	0.86	0.29
C4DC/C5-OH	0.23	0.15	C18:1	1.43	0.45
C5	0.13	0.08	C18:1-OH	0.02	0.01
C5:1	0.02	0.01	C18:2	0.24	0.16
C5DC	0.18	0.08	C18-OH	0.01	0.01
C6	0.05	0.02			

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.