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

A. 510(k) Number:

k173568

B. Purpose for Submission:

New Device

C. Measurand:

Amino acids, free carnitine, acylcarnitines, succinylacetone, nucleotides and lysophospholipids

D. Type of Test:

Quantitative measurement by mass spectrometry

E. Applicant:

Wallac Oy, a subsidiary of PerkinElmer Inc.

F. Proprietary and Established Names:

NeoBase 2 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:

   Class 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 2 Non-derivatized MSMS kit is intended for the measurement and evaluation of amino acid, succinylacetone, free carnitine, acylcarnitine, nucleoside and lysophospholipid concentrations (Table 1) with a tandem mass spectrometer from newborn heel prick blood specimens dried on filter paper. Quantitative analysis of these analytes and their relationship with each other is intended to provide analyte concentration profiles that may aid in screening newborns for metabolic disorders.

   Table 1. Analytes measured by the NeoBase2 Non-derivatized MSMS kit:

Analyte Name	Abbreviation
Amino acids	
Alanine	Ala
Arginine	Arg
Argininosuccinic acid	Asa
Citrulline	Cit
Glutamine\Lysine (*)	Gln\Lys
Glutamic acid	Glu
Glycine	Gly
Leucine\Isoleucine\Hydroxyproline (*)	Leu\Ile\Pro-OH
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Proline	Pro
Tyrosine	Tyr
Valine	Val
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

Analyte Name	Abbreviation
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\ Heptadecanoylcarnitine (*)	C16:1OH\C17
Octadecanoylcarnitine (Stearoylcarnitine)	C18
Octadecenoylcarnitine (Oleylcarnitine)	C18:1
Octadecadienoylcarnitine (Linoleylcarnitine)	C18:2
3-Hydroxy-octadecanoylcarnitine	C18OH
3-Hydroxy-octadecenoylcarnitine	C18:1OH
3-Hydroxy-octadecadienoylcarnitine	C18:2OH
Ketones	
Succinylacetone	SA
Nucleosides	
Adenosine	ADO
2'-deoxyadenosine	D-ADO
Lysophospholipids	
C24:0 lysophosphatidylcholine	C24:0-LPC
C26:0 lysophosphatidylcholine	C26:0-LPC

(*) Analytes in these rows are either isomers or isobars and cannot be distinguished in the tandem mass spectrometry experiment.

3. Special conditions for use statement(s):

For prescription use.

The NeoBase2 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, acylcarnitine, nucleoside and lysophopholipid profiles. Users should follow local guidelines for follow-up and confirmatory testing.

4. Special instrument requirements:

   Perkin Elmer TQD MS/MS Newborn Screening System.

I. Device Description:

Each NeoBase 2 Non-derivatized MSMS kit contains reagents for 960 assays. The kit is designed to be used with NeoBase 2 Non-derivatized Assay Solutions consisting of Neo MSMS Flow Solvent and NeoBase 2 Extraction Solution and NeoBase 2 Succinylacetone Assay Solution.

- NeoBase 2 Internal Standards
- NeoBase 2 Controls Low, High - 3 filter paper cassettes (Whatman, no. 903) containing 3 spots of each level per cassette
- Microplate, U-bottomed - 20 plates
- Adhesive microplate covers - 20 sheets
- Barcode labels for the plates - 30 pcs (10 different barcodes, 3 pcs of each)
- Lot-specific quality control certificate

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):

   NeoBase Non-derivatized MSMS kit

2. Predicate 510(k) number(s):

   k093916

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The NeoBase2 Non-derivatized MSMS kit is intended for the measurement and evaluation of amino acid, succinylacetone, free carnitine, acylcarnitine concentrations with a tandem mass spectrometer from newborn heel prick blood specimens dried on filter paper.	Same
Disorders screened	Amino-, organic-, and fatty acid metabolic disorders	Same
Test Principle	Analytes in sample are measured by tandem mass spectrometry through analyte-specific mass transitions appropriate for each type of analyte. The extracted analytes are measured for set time periods and compared to the signal intensities produced by the corresponding isotope-labeled internal standards. The concentrations are determined by comparing the signal intensities of the known standards to the measured analytes.	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
Assay format	Non-derivatized (analytes measured in their native	Same

Similarities		
Item	Device	Predicate
	forms)	

Differences		
Item	Device	Predicate
Analytes Measured	Amino acids, free carnitine, acylcarnitines, succinylacetone, nucleosides, and lysophospholipids	Amino acids, free carnitine, acylcarnitines, and succinylacetone
MSMS Instrument Flow Solvent	84% Acetonitrile 16% water 0.1% Formic Acid	78% Methanol, 22% water, 3mM oxalic acid

K. Standard/Guidance Document Referenced (if applicable):

Clinical and Laboratory Standards Institute (CLSI) EP5-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP7-A2 Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition

L. Test Principle:

The measurement of amino acids, succinylacetone, free carnitine, acylcarnitines, nucleosides, and lysophopholipids with the NeoBase 2 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 the analyte concentration.

In the NeoBase 2 Non-derivatized MSMS Kit, data is acquired in the Multiple Reaction Monitoring (MRM) mode. During this acquisition, a collisionally induced product 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 2 Non-derivatized MSMS assay is accomplished for the

amino acids, carnitines, nucleosides and lysophospholipids by adding extraction working solution (EWS) containing NeoBase 2 Extraction Solution and NeoBase 2 Internal Standards to the sample during the incubation step. However, for the extraction and measurement of SA, the compound needs to be derivatized. This takes place simultaneously with the extraction of other analytes by addition of an aliquot of the NeoBase 2 Succinylacetone Assay Solution to the EWS.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three precision studies were conducted using dried blood spot punches following the recommendations in the CLSI guideline EP05-A3. The repeatability and within-laboratory variation for NeoBase 2 Non-derivatized MSMS kit were based on 80 determinations: 40 plates measured over 20 working days, each plate with 2 replicates per sample. The samples were measured with the TQD MSMS system. Between-lot variation is based on 75 determinations: 15 plates measured over five working days using three kit lots, each plate having 5 replicates per sample. One TQD was used. Between-instrument variation was based on 50 determinations. 10 plates were measured with two TQDs over 5 working days, each plate having 5 replicates per sample. The results are summarized below:

Ala

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	141	5.7	4.3	8.9	6.7	4.1	2.8	7.6	5.4	12	8.8
2	347	17	5.0	20	5.9	6.2	1.8	11	3.1	24	6.9
3	407	16	3.9	18	4.4	4.2	1.0	18	4.4	26	6.4
4	519	24	4.5	27	5.1	15	3.1	23	4.2	38	7.3
5	2832	114	4.0	171	6.0	82	3.0	0.05	<0.01	190	6.7

Arg

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	7.5	0.49	6.1	0.85	11	0.47	6.8	<0.01	<0.01	0.97	13
2	23	1.4	6.0	1.6	6.9	0.11	0.49	0.61	2.6	1.7	7.6
3	67	2.8	4.2	3.5	5.2	1.7	2.6	0.57	0.85	3.9	5.8
4	157	6.6	4.1	8.8	5.4	5.2	3.5	2.8	1.8	11	6.8
5	1241	52	4.1	72	5.7	39	3.3	<0.01	<0.01	82	6.6

Asa

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.30	0.05	22	0.06	26	0.01	1.8	0.07	29	0.10	32
2	2.3	0.14	6.0	0.34	15	0.07	3.1	0.02	1.1	0.35	15
3	8.4	0.53	6.2	1.0	12	0.44	5.3	<0.01	<0.01	1.1	13
4	23	0.93	3.8	3.1	13	0.95	4.5	<0.01	0.01	3.2	14
5	50	1.8	3.4	7.0	13	2.1	4.6	0.19	0.38	7.3	15

Cit

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	10	0.79	8.3	1.1	11	0.33	3.0	0.70	6.6	1.3	13
2	69	3.9	5.8	4.9	7.2	2.1	3.1	1.7	2.4	5.6	8.2
3	205	10	4.9	11	5.2	5.4	2.7	4.7	2.3	13	6.3
4	480	27	5.5	33	6.8	11	2.3	12	2.5	37	7.6
5	926	39	4.2	55	5.9	27	3.0	0.03	<0.01	61	6.6

Gln\Lys

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	40	2.2	5.5	3.1	7.8	1.0	2.5	2.2	5.4	3.9	9.8
2	470	23	4.9	29	6.2	15	3.3	9.6	2.0	34	7.3
3	654	26	3.9	30	4.6	3.3	0.52	13	2.0	33	5.0
4	1043	47	4.4	56	5.3	37	3.7	27	2.6	72	6.9
5	2264	84	3.7	106	4.7	20	0.90	41	1.8	116	5.1

Gly

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	245	12	5.0	18	7.9	7.4	2.8	12	4.8	22	9.0
2	322	20	6.6	21	7.0	6.6	1.9	18	5.7	28	8.8
3	521	23	4.6	26	5.3	6.1	1.1	27	5.2	38	7.3
4	928	44	5.0	58	6.5	26	2.8	50	5.3	81	8.7
5	2752	128	4.8	171	6.4	77	2.7	95	3.4	210	7.6

Leu\Ile\Pro-OH

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	58	2.0	3.7	3.3	6.1	0.54	0.87	3.6	6.1	4.9	8.4
2	202	9.3	4.7	12	5.9	4.7	2.3	5.6	2.7	14	6.9
3	353	12	3.4	16	4.4	2.7	0.80	7.6	2.1	18	5.0
4	668	28	4.1	37	5.4	17	2.6	13	1.8	42	6.3
5	1137	48	4.1	77	6.7	13	1.2	22	1.8	81	7.1

Met

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	1.8	0.29	36	0.35	43	0.26	8.3	0.50	34	0.67	37
2	50	3.0	6.3	3.9	8.2	1.9	3.7	1.9	3.7	4.8	9.5
3	154	5.4	3.5	8.7	5.7	2.3	1.5	4.4	2.8	10	6.5
4	373	18	4.7	25	6.6	9.8	2.7	9.1	2.4	28	7.6
5	703	32	4.5	52	7.4	14	2.0	24	3.2	59	8.3

Orn

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	30	3.4	11	3.7	12	0.50	1.7	<0.01	<0.01	3.7	12
2	110	5.2	4.6	6.1	5.4	1.5	1.4	<0.01	<0.01	6.3	5.7
3	202	7.8	3.7	9.9	4.7	2.3	1.2	<0.01	<0.01	10	5.0
4	390	14	3.5	20	5.0	9.9	2.7	<0.01	<0.01	23	5.8
5	1305	43	3.2	86	6.3	18	1.5	0.12	0.01	88	6.7

Phe

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	22	1.1	5.3	1.5	7.2	0.43	1.7	1.1	4.8	1.9	8.3
2	128	6.0	4.8	7.4	5.9	3.5	2.7	2.1	1.6	8.5	6.6
3	343	11	3.3	15	4.3	4.7	1.4	0.04	0.01	16	4.5
4	790	37	4.6	43	5.4	29	3.8	2.8	0.35	52	6.6
5	1468	66	4.5	98	6.6	17	1.2	0.34	0.02	99	6.8

Pro

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	40	1.8	4.6	2.7	6.9	0.57	1.4	1.6	3.9	3.1	7.8
2	180	7.8	4.3	11	5.8	4.6	2.6	1.9	1.0	12	6.5
3	318	11	3.4	15	4.7	2.6	0.86	0.04	0.01	16	4.9
4	606	27	4.2	31	5.0	16	2.8	<0.01	<0.01	35	5.8
5	1467	50	3.3	87	5.8	36	2.6	0.04	<0.01	95	6.4

Tyr

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	20	1.2	6.3	1.8	8.9	0.52	2.6	0.65	3.1	1.9	9.6
2	110	4.8	4.4	6.2	5.7	3.6	3.3	1.1	0.99	7.3	6.6
3	268	10	3.8	12	4.5	4.1	1.6	2.4	0.89	13	4.9
4	596	28	4.7	33	5.5	15	2.6	0.01	<0.01	36	6.1
5	1585	67	4.1	90	5.6	40	2.6	0.02	<0.01	99	6.2

Val

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	57	2.2	4.2	3.3	6.3	0.81	1.3	3.0	5.3	4.5	8.0
2	209	9.8	4.6	13	6.0	5.6	2.8	2.4	1.1	14	6.7
3	321	9.7	2.9	14	4.4	4.2	1.4	2.2	0.67	15	4.7
4	554	24	4.2	31	5.4	15	3.0	<0.01	<0.01	35	6.3
5	1160	41	3.4	69	5.7	35	3.2	0.01	<0.01	78	6.7

C0

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	8.6	0.47	5.6	0.57	6.8	0.04	0.47	0.47	5.3	0.74	8.5
2	42	2.0	4.9	2.4	5.9	1.5	3.7	1.5	3.5	3.2	7.8
3	90	3.1	3.4	4.2	4.7	1.1	1.2	2.2	2.4	4.9	5.4
4	189	8.7	4.5	13	6.6	6.7	3.7	5.3	2.8	15	8.1
5	1236	59	4.7	79	6.3	31	2.6	0.02	<0.01	84	6.8

C2

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	3.6	0.15	4.4	0.25	7.2	0.09	2.5	0.09	2.6	0.28	7.9
2	12	0.63	5.2	0.76	6.3	0.47	3.9	0.29	2.3	0.94	7.7
3	19	0.62	3.4	0.81	4.4	0.09	0.49	0.51	2.7	0.97	5.2
4	31	1.4	4.5	1.8	5.9	0.66	2.2	1.1	3.6	2.2	7.3
5	328	13	4.1	20	6.1	11	3.3	<0.01	<0.01	23	6.9

C3

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.43	0.03	7.5	0.04	9.5	0.01	2.9	0.03	5.8	0.05	11
2	4.4	0.22	5.2	0.27	6.3	0.14	3.2	0.22	4.7	0.37	8.5
3	13	0.51	3.9	0.64	5.0	0.16	1.2	0.55	4.1	0.86	6.6
4	31	1.6	5.3	2.1	6.8	1.2	4.0	1.4	4.3	2.8	9.0
5	58	2.5	4.4	4.5	7.8	0.93	1.7	2.6	4.3	5.2	9.1

C4

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.06	0.01	11	0.01	12	<0.01	3.5	0.01	13	0.01	17
2	0.57	0.03	5.8	0.04	6.8	0.02	2.9	0.02	3.4	0.05	8.0
3	1.8	0.05	3.1	0.09	5.2	0.01	0.31	0.04	2.2	0.10	5.7
4	4.3	0.19	4.5	0.27	6.2	0.08	2.0	0.14	3.2	0.31	7.4
5	20	0.89	4.4	1.2	5.9	0.54	2.7	<0.01	<0.01	1.3	6.5

C5

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.04	0.01	23	0.01	24	<0.01	2.6	<0.01	8.6	<0.01	23
2	1.0	0.05	5.3	0.06	6.3	0.03	2.8	0.02	2.3	0.07	7.2
3	3.7	0.13	3.6	0.18	5.0	0.04	1.1	0.09	2.5	0.21	5.7
4	9.2	0.42	4.6	0.57	6.2	0.31	3.6	0.23	2.4	0.69	7.5
5	20	0.74	3.8	1.1	5.8	0.22	1.2	<0.01	<0.01	1.2	6.0

C5DC\C6OH

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV%
1	0.04	0.01	22	0.01	23	<0.01	9.2	<0.01	15	0.01	27
2	0.44	0.03	6.5	0.03	7.4	0.01	3.1	0.01	3.2	0.04	8.5
3	1.5	0.08	5.4	0.10	6.6	0.02	1.1	0.08	5.0	0.13	8.3
4	3.8	0.23	6.1	0.26	6.9	0.07	1.9	0.11	2.9	0.30	7.8
5	35	2.0	5.8	2.4	6.9	1.1	3.1	<0.01	<0.01	2.7	7.6

C6

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.02	<0.01	35	<0.01	35	<0.01	15	<0.01	22	<0.01	41
2	0.38	0.02	6.1	0.03	7.1	<0.01	2.4	0.02	5.7	0.04	9.3
3	1.4	0.04	3.2	0.07	5.2	0.03	1.8	0.05	3.6	0.09	6.5
4	3.5	0.16	4.5	0.20	5.7	0.10	3.1	0.13	3.7	0.26	7.5
5	22	0.97	4.3	1.3	5.6	0.73	3.3	<0.01	0.01	1.5	6.5

C8

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.03	0.01	26	0.01	30	<0.01	16	<0.01	8.3	0.01	36
2	2.1	0.11	5.2	0.13	6.6	0.08	3.8	0.06	3.0	0.17	8.1
3	7.6	0.30	3.9	0.35	4.6	0.12	1.6	0.12	1.6	0.39	5.1
4	19	1.0	5.2	1.2	6.1	0.64	3.6	0.31	1.5	1.4	7.3
5	36	1.8	5.0	2.6	7.1	0.44	1.3	0.71	1.9	2.7	7.5

C10

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.03	<0.01	18	0.01	23	<0.01	13	<0.01	10	<0.01	28
2	0.45	0.02	5.7	0.03	6.6	0.01	2.4	0.03	6.0	0.04	9.1
3	1.5	0.06	4.2	0.07	4.9	0.02	1.2	0.10	6.2	0.12	8.0
4	3.9	0.21	5.5	0.25	6.6	0.11	2.9	0.23	5.6	0.35	9.2
5	20	0.78	4.0	1.1	5.6	0.72	3.6	0.79	3.9	1.5	7.6

C12

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.02	<0.01	24	0.01	61	<0.01	5.2	<0.01	17	0.01	70
2	0.49	0.03	6.6	0.04	7.4	0.02	3.4	<0.01	0.73	0.04	8.0
3	1.8	0.07	4.0	0.08	4.8	0.01	0.76	0.05	2.7	0.10	5.5
4	4.4	0.24	5.4	0.27	6.2	0.16	3.7	0.09	1.9	0.33	7.4
5	20	0.67	3.3	1.1	5.3	0.49	2.4	<0.01	0.01	1.2	5.8

C14

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV%	SD	CV%
1	0.05	0.01	8.3	0.02	38	<0.01	3.5	<0.01	1.6	0.02	43
2	0.57	0.03	5.7	0.04	7.3	0.01	1.6	<0.01	0.03	0.04	7.5
3	1.8	0.07	3.8	0.08	4.4	0.03	1.6	<0.01	0.01	0.09	4.7
4	4.3	0.22	5.1	0.25	5.7	0.12	2.8	<0.01	0.01	0.28	6.4
5	19	0.66	3.5	1.0	5.4	0.43	2.3	<0.01	<0.01	1.1	5.9

C16

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	1.0	0.05	4.9	0.08	7.8	0.02	2.3	0.04	3.8	0.09	8.9
2	3.7	0.17	4.6	0.18	5.1	0.13	3.6	0.13	3.3	0.26	7.0
3	11	0.42	3.9	0.51	4.7	0.07	0.62	0.23	2.1	0.56	5.2
4	25	1.1	4.6	1.5	6.1	0.35	1.5	0.61	2.4	1.6	6.7
5	60	2.0	3.4	3.2	5.3	1.3	2.2	1.1	1.8	3.6	6.0

C18

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV%	SD	CV %
1	0.51	0.02	4.3	0.05	10	0.010	2.0	<0.01	0.01	0.05	11
2	1.3	0.06	4.8	0.09	6.8	0.03	2.7	<0.01	<0.01	0.10	7.4
3	3.0	0.10	3.3	0.14	4.7	0.03	1.0	<0.01	<0.01	0.15	4.9
4	6.3	0.25	3.8	0.29	4.4	0.03	0.58	<0.01	<0.01	0.29	4.6
5	38	1.4	3.5	2.0	5.2	0.79	2.2	<0.01	<0.01	2.2	5.7

SA

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.39	0.04	20	0.04	23	0.02	3.2	0.16	47	0.17	43
2	3.6	0.32	9.0	0.62	18	0.09	2.4	<0.01	<0.01	0.63	17
3	13	0.79	5.8	2.3	17	0.22	1.6	<0.01	0.01	2.3	17
4	37	2.1	5.6	6.4	17	0.77	2.2	<0.01	<0.01	6.5	17
5	75	5.1	6.4	14	18	0.15	0.22	0.03	0.04	14	19

ADO

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.15	0.02	14	0.02	16	<0.01	2.1	0.02	17	0.03	22
2	1.4	0.11	8.3	0.13	9.3	0.02	1.3	0.04	2.8	0.13	9.9
3	5.5	0.23	4.1	0.28	5.0	0.07	1.2	0.08	1.4	0.29	5.3
4	15	0.70	4.7	0.83	5.5	0.23	1.6	0.15	1.0	0.87	6.0
5	29	1.0	3.4	1.8	6.2	0.43	1.5	<0.01	0.01	1.9	6.4

C26:0-LPC

Sample	Total mean µmol/L	Repeatability		Within-lab		Between-lot		Between-instrument		Total variation	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	0.22	0.03	13	0.03	13	0.01	4.5	<0.01	0.02	0.03	13
2	0.69	0.06	9.2	0.08	11	0.01	1.1	0.02	2.8	0.08	11
3	1.4	0.11	7.4	0.13	8.4	0.01	0.47	0.11	8.2	0.17	12
4	2.9	0.15	4.9	0.21	6.8	0.01	0.55	0.18	6.3	0.28	9.7
5	5.5	0.34	5.6	0.43	7.1	0.10	2.1	0.49	8.8	0.66	12

The reproducibility of the NeoBase2 Non-derivatized MSMS assay was determined in an additional study on the TQD MSMS Screening System across 2 external sites and one internal site. The reproducibility is based on 75 determinations: in each laboratory 5 plates were measured over 5 working days using one kit lot and each plate having 5 replicates per sample. The results are summarized below:

Ala

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	336	19	5.9	25	7.5	32	9.5
2	430	23	5.5	38	8.9	45	10
3	693	38	5.6	71	10	81	11

Arg

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	8.2	1.1	13	0.69	8.4	1.3	15
2	49	2.4	4.8	2.0	4.1	3.1	6.3
3	175	9.0	5.1	6.5	3.7	11	6.3

Asa

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.53	0.16	29	0.36	67	0.39	73
2	6.7	0.54	8.1	0.11	1.7	0.55	8.3
3	29	1.9	6.4	1.6	5.3	2.5	8.3

Cit

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	21	2.3	10	0.38	1.8	2.4	11
2	91	6.5	7.1	0.88	0.97	6.5	7.2
3	292	19	6.5	9.7	3.3	21	7.3

Gln\Lys

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	517	35	6.9	10	2.1	37	7.2
2	738	45	6.2	12	1.6	47	6.4
3	1409	110	7.8	52	3.7	121	8.6

Gly

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	320	23	7.5	12	3.8	26	8.4
2	592	37	6.3	25	4.2	44	7.6
3	1398	95	6.8	62	4.5	114	8.1

Leu\Ile\Pro-OH

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	179	9.2	5.2	5.2	2.9	10	5.9
2	273	12	4.5	8.0	2.9	14	5.3
3	549	30	5.5	22	4.0	37	6.8

Met

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	15	1.4	9.2	0.37	2.4	1.5	9.5
2	96	5.7	5.9	5.4	5.6	7.8	8.2
3	337	21	6.3	23	7.1	31	9.5

Orn

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	112	8.8	7.9	9.2	8.2	12	11
2	200	8.4	4.2	1.3	0.62	8.5	4.2
3	484	23	4.8	3.7	0.76	23	4.9

Phe

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	62	3.1	5.0	0.48	0.77	3.1	5.0
2	190	9.2	4.8	2.5	1.3	9.5	5.0
3	573	31	5.5	10	1.9	33	5.8

Pro

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	150	8.8	5.8	4.0	2.6	9.6	6.4
2	304	13	4.5	4.3	1.4	14	4.7
3	759	37	5.0	24	3.2	45	5.9

Tyr

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	57	3.9	6.8	1.1	1.9	4.0	7.0
2	227	11	5.0	8.3	3.6	13	6.2
3	736	41	5.6	26	3.6	49	6.7

Val

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	191	9.9	5.2	4.4	2.3	10	5.7
2	295	12	4.2	8.3	2.8	15	5.1
3	601	33	5.5	12	2.1	35	5.9

C0

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	22	1.3	6.0	1.1	4.9	1.7	7.8
2	75	3.4	4.5	3.4	4.4	4.8	6.3
3	233	13	5.8	6.8	2.9	15	6.5

C2

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	11	0.63	5.5	0.38	3.4	0.74	6.4
2	20	0.98	4.8	0.55	2.7	1.1	5.5
3	46	2.6	5.6	1.8	3.8	3.1	6.7

C3

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	1.2	0.07	5.9	0.04	3.5	0.08	6.8
2	8.5	0.39	4.6	0.41	4.8	0.57	6.7
3	29	1.9	6.3	1.9	6.3	2.7	8.9

C4

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.14	0.01	9.8	<0.01	1.8	0.01	10.
2	1.3	0.06	5.1	0.07	5.6	0.10	7.6
3	4.6	0.30	6.5	0.28	6.1	0.41	8.9

C5

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.10	0.02	18	<0.01	8.8	0.02	20
2	2.6	0.13	4.8	0.09	3.3	0.15	5.8
3	10	0.56	5.5	0.45	4.4	0.71	7.0

C5DC\C6OH

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.058	0.01	21	<0.01	8.6	0.01	22
2	1.0	0.07	6.8	0.06	5.8	0.09	9.0
3	3.8	0.30	7.7	0.27	7.1	0.40	10

C6

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.041	<0.01	19	<0.01	7.5	<0.01	20
2	0.96	0.05	4.9	0.04	4.3	0.06	6.5
3	3.7	0.22	6.0	0.21	5.6	0.30	8.2

C8

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.07	0.01	16	<0.01	13	0.02	20
2	1.9	0.11	5.7	0.07	4.0	0.13	6.9
3	7.2	0.43	6.0	0.35	4.8	0.55	7.6

C10

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.08	<0.01	8.3	<0.01	3.8	<0.01	9.1
2	0.87	0.04	4.9	0.05	5.9	0.07	7.7
3	3.2	0.21	6.6	0.22	6.8	0.30	9.5

C12

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.04	<0.01	15	<0.01	9.0	<0.01	17
2	0.77	0.05	6.2	0.02	2.8	0.05	6.8
3	2.9	0.19	6.4	0.10	3.4	0.21	7.2

C14

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.11	<0.01	8.8	<0.01	4.8	0.01	10
2	1.1	0.06	5.4	0.06	5.6	0.09	7.8
3	4.0	0.23	5.8	0.28	7.0	0.37	9.1

C16

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	1.1	0.07	6.0	0.06	5.0	0.09	7.8
2	8.1	0.37	4.6	0.31	3.9	0.49	6.0
3	27	1.4	5.2	1.3	4.6	1.9	6.9

C18

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.65	0.03	5.3	0.01	1.6	0.04	5.5
2	2.1	0.11	5.2	0.04	1.7	0.12	5.5
3	6.5	0.38	5.8	0.18	2.7	0.42	6.4

SA

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.28	0.08	26	0.05	17	0.09	32
2	7.0	0.72	10	0.76	10	1.0	14
3	28	2.4	8.4	3.1	10	3.9	13

ADO

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.37	0.04	10	0.02	5.4	0.04	12
2	2.0	0.14	6.8	0.11	5.2	0.17	8.6
3	7.9	0.44	5.5	0.28	3.6	0.52	6.5

C26:0-LPC

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.22	0.04	16	0.04	17	0.05	24
2	0.58	0.07	11	0.06	10	0.09	15
3	1.8	0.15	8.3	0.20	11	0.25	13

b. Linearity/assay reportable range:

Linearity on the NeoBase 2 was determined by testing dried blood spots with 12 analyte levels spanning the ranges defined below in three different linearity studies per analyte. A spike and recovery study was also conducted. Summary of these studies are presented in the tables below.

Analyte	Linear range lower limit (µmol/L)	Linear range upper limit (µmol/L)
Ala	145	1580
Arg	1.95	311
Asa	0.42	67.7
Cit	10.1	934
Gln\Lys	38.7	2190
Gly	264	1820
Leu\Ile\Pro-OH	56.6	1420
Met	2.37	826
Orn	30.1	799
Phe	21.9	1470
Pro	39.4	1250
Tyr	19.8	1240
Val	55.8	1110
C0	8.16	392
C2	3.52	139
C3	0.44	58.4
C4	0.06	10.4
C5	0.05	17.0
C5DC\C6OH	0.03	6.99
C6	0.02	7.48
C8	0.06	38.7
C10	0.03	6.93
C12	0.02	9.15
C14	0.05	8.61

C16	1.00	51.5
C18	0.48	11.8
SA	0.28	122
ADO	0.18	36.9
C26:0-LPC	0.22	7.46

Results from the three replicate linearity runs:

Analyte	R^2 Range	Slope Range
Ala	0.994 - 0.997	0.696 - 0.786
Arg	0.992 - 0.995	0.655 - 0.799
Asa-Total	0.992 - 0.993	0.193 – 0.219
Cit	0.995 - 0.997	0.681 - 0.786
Gln	0.994 - 0.996	0.719 - 0.830
Gly	0.995 - 0.998	0.697 - 0.819
Leu\Ile\Pro-OH	0.996 - 0.998	0.680 - 0.762
Met	0.998 - 0.998	0.665 - 0.741
Orn	0.998 - 0.998	0.612 - 0.673
Phe	0.996 - 0.998	0.678 - 0.796
Pro	0.996 - 0.999	0.648 - 0.708
Tyr	0.997 - 0.999	0.692 - 0.826
Val	0.995 - 0.998	0.701 - 0.789
C0	0.996 - 0.998	0.764 - 0.858
C2	0.996 - 0.998	0.725 - 0.835
C3	0.996 - 0.998	0.870 - 1.047
C4	0.997 - 0.997	0.699 - 0.802
C5	0.997 - 0.999	0.635 - 0.723
C5DC\C6OH	0.998 - 0.998	0.587 - 0.685
C6	0.998 - 0.998	0.702 - 0.812
C8	0.996 - 0.998	0.705 - 0.818
C10	0.997 - 0.998	0.664 - 0.803
C12	0.997 - 0.998	0.743 - 0.836
C14	0.997 - 0.999	0.766 - 0.888
C16	0.994 - 0.996	0.855 - 0.992
C18	0.997 - 0.998	0.823 - 0.924
C26	0.999 - 0.999	1.247 - 1.768
SA	0.998 - 0.999	0.499 - 0.519
ADO	0.999 - 0.999	0.958 - 1.098
C26:0-LPC	0.996 - 0.998	1.228 - 1.320

Recovery:
Analyte recovery for the NeoBase Non-derivatized MSMS kit was evaluated using the Perkin Elmer TQD tandem mass spectrometry system over a total of 21 runs, consisting of samples with 5 different levels analyzed in 6 replicates per run. The resuls are summarized below:

Analyte	Recovery with kit lot 1 (%)	Recovery with kit lot 2 (%)	Recovery with kit lot 3 (%)	Mean recovery over kit lots (%)
ADO	101.8	89.3	98.9	96.7
Ala	103.3	107.1	111.2	107.2
Arg	87.2	75.4	84.8	82.5
Asa	27.5	23.1	25.9	25.5
Cit	92.6	82.1	89.8	88.1
Gln	107.9	102.1	109.7	106.5
Gly	105.7	87.9	94.5	96.0
Leu	95.3	85.0	91.7	90.7
Met	89.1	78.8	85.2	84.4
Orn	84.5	78.0	84.2	82.2
Phe	94.7	81.3	88.0	88.0
Pro	95.3	81.0	88.1	88.1
SA	48.9	46.0	50.1	48.4
Tyr	92.0	82.2	88.7	87.6
Val	103.6	90.2	98.0	97.3
C0	123.9	97.9	107.2	109.7
C2	107.7	96.6	102.3	102.2
C3	125.4	109.1	119.3	118.0
C4	91.9	78.6	86.6	85.7
C5	87.0	74.6	82.2	81.3
C5DC	82.5	72.9	79.4	78.2
C6	99.6	84.6	95.4	93.2
C8	99.1	86.1	95.1	93.4
C10	103.0	89.9	99.0	97.3
C12	94.1	83.1	94.1	90.4
C14	97.6	86.5	95.0	93.0
C16	120.7	107.4	117.6	115.3
C18	105.5	96.7	103.8	102.0
C26	122.2	109.6	123.8	118.5
C26:0-LPC	109.2	110.0	118.3	112.5

The following limitation is included in the package insert for Asa:
"Please note that the 25.5% recovery of Asa (slope range =0.193-0.219) is lower compared to other analytes measured with NeoBase 2 Non-derivatized MSMS assay (Table 12). Incomplete recovery may cause difference in measured analyte level

compared to alternative methods. Therefore, it is important that each laboratory establishes its own reference range and cut-off values with the NeoBase 2 Non-derivatized MSMS assay."

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

NeoBase 2 Non-derivatized MSMS kit is traceable to NIST standards and has been anchored to the non-labeled reference compounds, which are the primary calibrators of the kit. The concentration of labelled secondary calibrators is assigned against the primary calibrators.

d. *Detection limit:*

15 serially diluted dried blood spots samples in a blood matrix spiked with internal standards were used for the analytical sensitivity study. Extracted samples were pipetted onto the plates in 6 replicates of each, from the lowest dilution level to the highest dilution level.

At the below listed concentrations the sponsor claims that they meet an imprecision critera of CV less than or equal to 20%.

Analyte	**Analytical sensitivity limit (µmol/L)**
Ala	1.75
Arg	0.37
Asa [1]	0.18
Cit	2.64
Gln\Lys	0.81
Gly	4.35
Leu\Ile\Pro-OH	0.40
Met	6.31
Orn	1.75
Phe	0.29
Pro	0.35
Tyr	1.75
Val	0.22
C0	0.17
C2	0.04
C3	0.02
C4	0.01
C5	0.02
C5DC\C6OH	0.02

Analyte	Analytical sensitivity limit (µmol/L)
C6	0.05
C8	0.01
C10	0.01
C12	0.01
C14	0.01
C16	0.02
C18	0.01
SA	0.24
ADO	0.08
C26:0-LPC	0.08

e. *Analytical specificity:*

Dried blood spot samples representing two different analyte concentrations were tested in an interference study. The substances tested for interference included endogenous substances and possible sample contaminants. The effect of 25 substances was assessed by the paired-difference method at two analyte concentration levels (both normal and abnormal) with the NeoBase 2 Non-derivatized MSMS kit. Additionally, 9 substances that are abundant in the DBS sample matrix and have potential for unspecific effects on the test results were studied. Results confirmed that 11 of the 25 substances caused a significant change in the test result. These 11 compounds were further evaluated by dose-response testing.

At the following concentrations, the bias between the test and control samples did not exceed 15% with overall 95% confidence. The sponsor claims that the following substances were found not to interfere with the assay at the concentration indicated.

Tested substance	Added concentration of tested substance in blood
Formiminoglutamic acid (Figlu)	37.1 µmol/L
O-Acetyl-L-serine	1000 µmol/L
6-Aminocaproic Acid	6.07 µmol/L
DL-Malic acid	3000 µmol/L
4-Aminoantipyrine	500 µmol/L
Propranolol	7.74 µmol/L
2,5-dihydroxybenzoic acid	127 µmol/L
Bilirubin conjugated	15 mg/dL
Bilirubin unconjugated	10 mg/dL
Calcifediol	250 nmol/L
Acetaminophen	5.5 mg/dL

For the stubstances that did interfere with the assay, the following was added to the package insert of the device:

Sarcosine: Amino acid derivative Sarcosine was found to interfere with the assay by increasing the measured Ala concentration. Sarcosine concentrations above 62.5 µmol/L may cause a false positive screening result. Sarcosine concentration in plasma ranges from 0–625 µmol/L in newborns aged 0–1 months. However, Sarcosine does not exist in healthy newborns; it is only found at detectable amount when a newborn is affected by hypersarcosinemia. Therefore, Sarcosine is unlikely to interfere with Ala in routine testing.

Creatine: Non-essential amino acid Creatine was found to interfere with the assay by increasing the measured Ala and Leu concentrations. Creatine concentrations above 1500 µmol/L with Ala or above 4500 µmol/L with Leu may cause a false positive screening result. The normal expected Creatine level in newborns aged 0–1 months is 107–640 µmol/L. Therefore, Creatine is unlikely to interfere with Ala and Leu in routine testing.

Verapamil metabolite D617: D617 is a metabolite of calcium channel blocker Verapamil. D617 was found to interfere with the assay by increasing the measured Asa concentration. D617 concentrations from 0.97 µmol/L may cause a false positive screening result on Asa. Therapeutic concentration range in plasma for Verapamil is 0.11–1.32 µmol/L. D617 has been found to present appr. 20% of the given oral dose excreted in to urine, i.e. the D617 level is very unlikely to exceed the concentration of 0.97 µmol/L in whole blood. Therefore, Verapamil metabolite D617 is unlikely to interfere with Asa in routine testing. Nevertheless, newborns given Verapamil or exposed to the compound during pregnancy or breastfeeding could screen positive for Asa.

L-Lysine: L-Lysine was found to interfere with the assay by increasing the measured Gln and Glu concentrations. L-Lysine is an essential amino acid and is isobaric to NeoBase 2 analyte Gln. NeoBase 2 assay cannot separate the compounds, and the result of Gln is a sum of Gln and L-Lysine (Gln\Lys). The reference plasma level of L-Lysine, Gln and Glu in newborns aged 0–1 months are 92–325 µmol/L, 376–709 µmol/L and 62–620 µmol/L, respectively. L-Lysine may cause a false positive screening result to Gln. The proposed cut-off area measured with NeoBase 2 assay for Gln is generally high (e.g. 99.0% percentile, 1120 µmol/L). An additional 730 µmol/L dose of L-Lysine would be needed to raise the Gln concentration from endogenous level (465 µmol/L) to the cut-off area (1100 µmol/L). For Glu, L-Lysine concentrations from 355 µmol/L may cause a false positive screening result. Since the whole blood used in the interference samples contains also endogenous L-Lysine, the sum of spiked and endogenous L-Lysine levels are in the upper part of newborn physiological L-Lysine range. In hyperlysinemia, the concentration of L-Lysine in blood plasma is relatively high. Plasma L-Lysine levels have been reported to exceed 600 µmol/L and can reach up to 2000 µmol/L. When blood specimen is taken from a

newborn with such a condition, L-Lysine may cause false positive screening results to Gln and/or Glu.

L-Glutamic acid: Non-essential amino acid and NeoBase 2 analyte L-Glutamic acid (Glu) was found to interfere with the assay by increasing the measured Met concentration. Glu concentrations above 2250 µmol/L may cause a false positive screening result on Met. The reference plasma level of Glu in newborns aged 0–1 months is 62–620 µmol/L. Therefore, Glu is very unlikely to interfere with Met in routine testing.

L-Asparagine: Non-essential amino acid L-Asparagine was found to interfere with the assay by increasing the measured Orn concentration. L-Asparagine concentrations above 375 µmol/L may cause a false positive screening result on Orn. The reference plasma level of L-Asparagine in newborns aged 0–1 months is 29–132 µmol/L. Therefore L-Asparagine is unlikely to interfere with Orn in routine testing.

L-Ornithine (Orn): NeoBase 2 analyte L-Ornithine (Orn) was found to interfere with the assay by increasing the measured Pro concentration. The interference is caused by mass transition overlap between a fragment of Orn formed in the ion source and Pro. Orn concentrations above 188 µmol/L may cause a false positive screening result on Pro. The proposed cut-off area measured with NeoBase 2 assay for Pro is above 200 µmol/L (e.g. 99.0% percentile, 235 µmol/L). An additional 638 µmol/L dose of Orn would be needed to raise the Pro concentration from endogenous level (155 µmol/L) to the cut-off area (240 µmol/L). The proposed normal population for Orn measured with NeoBase 2 assay (e.g. 99% percentiles range between 152–194 µmol/L) is on much lower concentration level than the needed additional dose. It is unlike that Orn interferes with Pro in routine testing.

L-Methionine sulfone: Amino acid derivative L-Methionine sulfone was found to interfere with the assay by increasing the measured Tyr concentration. L-Methionine sulfone concentrations above 15.6 µmol/L may cause a false positive screening result on Tyr. No reference concentration in newborns was found for methionine sulfone. However, because methionine sulfone is an oxidation product of methionine sulfoxide, and methionine sulfoxide is a product of methionine (Met), the highest concentration of methionine sulfone should not exceed the normal level of Met in infants aged 0–1 year (10–60 µmol/L). L-Methionine sulfone dose of 62.5 µmol/L increased the endogenous Tyr concentration (53 µmol/L) to 76 µmol/L. Since the proposed cut-off area measured with NeoBase 2 assay for Tyr is higher (e.g. 99.0% percentile, 188 µmol/L), L-Methionine sulfone is unlikely to interfere routine testing.

Lidocaine: Lidocaine was found to interfere with the assay by decreasing the measured C4 concentration. Lidocaine concentrations above 25.6 µmol/L may cause a false negative screening result on C4. Lidocaine is a drug used to e.g. numb tissues. If disinfectant pads containing lidocaine are used to wipe off the heels of newborns in specimen collection, there is potential for lidocaine to be carried into the specimen. However, the residual amount of this compound present after skin preparation should

only be a fraction of the effective interfering level and the less likely to interfere. Therapeutic concentration range in plasma for Lidocaine is 5.1–25.6 µmol/L. Concentrations above 25.6 µmol/L are reported as toxic. Therefore, Lidocaine is unlikely to interfere routine testing.

Albumin: High albumin concentrations were found to interfere with the assay. When total albumin is above 3.16 g/dL, the interference caused an increase in the measured ADO concentrations. The reference range for albumin in infant plasma/serum aged 0–12 months is 2.8–4.7 g/dL corresponding to albumin concentration of 1.4–2.4 g/dL in whole blood.

Intralipid (Triglyceride): High intralipid concentrations were found to interfere with the assay. When total triglycerides are above 0.82 g/dL, the interference caused an increase in the measured Glu concentration. When more than 0.10 g/dL of intralipid was added to blood containing 0.07 g/dL of endogenous triglycerides (i.e. tested total triglycerides above 0.17 g/dL) the interference caused an increase in the measured C24:0-LPC concentration. The reference range for triglycerides in newborns aged 0–7 days has been reported to be from 0.02 to 0.18 g/dL in serum corresponding to triglyceride concentration in whole blood of 0.01 to 0.09 g/dL.

Chlorhexidine digluconate: Chlorhexidine digluconate was found to interfere with the assay by increasing the measured C24:0-LPC and C26:0-LPC concentrations. Chlorhexidine digluconate is a cationic broad-spectrum antimicrobial agent belonging to the bis(biguanide) family. Its mechanism of action involves destabilization of the outer bacterial membrane. Chlorhexidine digluconate amounts above 0.03% may cause false positive screening results on C24:0-LPC and C26:0-LPC. If disinfectant pads containing chlorhexidine digluconate are used to wipe off the heels of newborns in preparation for sample collection, there is potential for chlorhexidine digluconate to be carried into the sample. It can be estimated that in the worst case, 1 µL of the 3% skin disinfection solution might contaminate a 75 µL blood droplet, which corresponds to an amount of 0.04% Chlorhexidine digluconate in the sample. Therefore, it is unlikely that Chlorhexidine digluconate will interfere with C24:0-LPC and C26:0-LPC in routine testing.

Hemoglobin: High hemoglobin concentrations were found to interfere with the assay. Total hemoglobin above 21.7 g/dL caused a decrease in the measured C24:0-LPC by 19% and in the measured C26:0-LPC by 28%. Total hemoglobin above 20.4 g/dL caused an elevation in the measured ADO by 20%.

The effect of hematocrit on Arg was evaluated. Hematocrit values below 43% increased Arg results by 20%, and hematocrit values above 63% decreased Arg results by 30%. Although interference by hemoglobin and hematocrit was observed with concentrations within the newborn reference ranges (12.0–22.0 g/dL for hemoglobin, 35–65% for hematocrit), it is concluded based on the external study results that the interferences are not pronounced enough to impair the separation of the affected and un-affected cases. Interference by hemoglobin could cause false

negative screening results only if C24:0-LPC would be used as the sole marker for X-ALD. C24:0-LPC should always be used together with the primary marker C26:0-LPC.

Benzocaine: Disinfectants such as alcohol swabs with a topical anesthetic benzocaine should not be used to wipe off the heel of a newborn. Benzocaine and Phe are isomers having the same mass to charge ratio of 166.1. Therefore, benzocaine may cause falsely elevated Phe concentration and a false positive phenylketonuria (PKU) screening result.

C5 isomer pivalylcarnitine: Pivalic acid may cause false positive screening result for Isovaleric acidemia (IVA), whose marker is acylcarnitine C5. Pivalic acid can be liberated from a prodrug containing esterified pivalic acid (such as pivalic-ester containing antibiotics, corticosteroids or other pharmaceuticals) administered to mothers or newborns. Pivalic acid is further metabolized to pivalylcarnitine which is an isomer of C5, and therefore pivalic acid can cause falsely elevated C5 results. Falsely elevated C5 concentrations have been reported in newborns due to pivalylcarnitine interference. Administration of pivalic acid containing prodrugs can lead to carnitine depletion. In addition, falsely elevated C5 concentrations have been connected to cases where pivalylcarnitine originated from neopentanoate esters present in nipple-fissure unguent used by the breastfeeding mothers.

C8 isomer valproylcarnitine: Medication valproic acid administered to mothers or newborns may interfere with the screening of Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) or Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT), whose diagnostic marker is acylcarnitine C8. Valproic acid is metabolized to valproylcarnitine, which is isomer of C8, and can cause falsely elevated C8 concentration. False positive MCAD results have been measured in newborns due to valproylcarnitine interference.

C3DC\C4OH, C4DC\C5OH and C5DC\C6OH: Analytes in the pairs C3DC\C4OH; C4DC\C5OH; and C5DC\C6OH; are all natural acylcarnitines that can be present in dried blood spots and cannot be separated in the NeoBase 2 assay due to mass transition overlap. The mass to charge ratios of the precursor ions are 248 (for C3DC, C4OH), 262 (for C4DC, C5OH), and 276 (for C5DC, C6OH) and they all have the same identifying product ion (m/z 85). As a result, the NeoBase 2 Non-derivatized MSMS kit reports the results for these analytes in the pair together as a sum, which is very much the same as the case for Leu\Ile\Hydroxyproline, and Gln\Lys. Because of this overlap, the results for these analytes should be reported as the cumulative concentration of the two analytes in the pair.

C16:1OH\C17: C16:1OH is a marker among other hydroxylated long chain acylcarnitines for Long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) and Trifunctional protein deficiency (TFP). Heptadecanoylcarnitine (C17) has been identified as a marker specific for Propionic acidemia (PROP) and Methylmalonic acidemia (MUT). C17 and C16:1OH are isomers, having the same

mass to charge ratio of m/z 414, and are always measured in NeoBase 2 assay as a sum of both analytes. In screening of LCHAD, TFP, PROP and MUT, the cumulative sum concentration of C16:1OH and C17 increases. It is recommended to confirm positive screening result with 2nd tier analysis, which is capable of separating C16:1OH and C17 and identify specifically the disorder.

C26:0-LPC: Elevated C26:0-LPC concentrations have been measured in newborn blood spots and post-natal blood specimens taken from children diagnosed by Aicardi Goutières Syndrome (AGS) leading to false positive results in first tier X-ALD screening.

Interference from M+2 Isotopic Peaks: The analytes and internal standards measured in NeoBase 2 assay not only produce an M peak (the monoisotopic peak that is used in the measurement), but also M+1, M+2, and M+3 peaks. These additional M+n peaks are due to the naturally occurring heavier stable isotopes such as 13C, 15N or 18O. In tandem mass spectra of complex samples where many analytes are analyzed simultaneously (as is the case of the NeoBase 2 assay) the M+n peaks of one compound have the potential to overlap with the peaks generated by other compounds of neighbouring m/z and cause falsely elevated peaks. Potential M+2 peak interferences are as follows: M+2 peak of C5 overlaps with C3DC\C4OH; M+2 peak of C6 overlaps with C4DC\C5OH; and M+2 peak of C8 overlaps with C6DC. However, the effect is only significant when C5, C6, and C8 are present in high concentrations. At the endogenous concentrations the risk for false positive result with C3DC\C4OH, C4DC\C5OH and C6DC is negligible. When elevated level of C5, which is a marker for Isovaleric acidemia (IVA) and 2-Methylbutyrylglycinuria (2MBG), is observed, concentration of C3DC\C4OH must be evaluated. When elevated level of C6, which is a marker for Medium-chain acyl-CoA dehydrogenase deficiency (MCAD), is observed, concentration of C4DC\C5OH must be evaluated. When elevated level of C8, which is a marker for MCAD and Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT), is observed, concentration of C6DC must be evaluated. Conversely, when elevations are detected on C3DC\C4OH; C4DC\C5OH; or C6DC, it is recommended to evaluate the concentrations of C5, C6, and C8 to ensure these observations are not due to the M+2 effect described here.

Plasticizers: Plasticizers or other additives may leach from the plastic material used in the sample preparation, storage packages and medical equipment and interfere with the newborn screening results. For example, slip agent Oleamide (m/z 282) is known to interfere with C5DC IS having the same mass to charge ratio. Elevated C5DC IS intensity falsely decreases acylcarnitine C5DC analyte concentration and may cause false negative screening result on C5DC, which is a marker for Glutaric acidemia type I (GAI). Similarly, a common anti-static agent Lauric acid diethanolamide (LDEA, m/z 288) has been found to interfere with acylcarnitine C8 having the same mass to charge ratio. LDEA can lead to false positive C8 result, which is a marker for disorders Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and Medium-chain ketoacyl-CoA thiolase deficiency (MCKAT) disorder. In addition, falsely elevated C8 levels in two neonates treated with extracorporeal membrane

oxygenation (ECMO) has been identified. The C8 interference was traced to PVC tubing used in ECMO and a plasticizer Di-ethylhexyl phthalate (DEHP) used commonly in the manufacturing of PVC. Interference originated from a DEHP metabolite, 2-Ethylhexanoic acid, which was further metabolized in the exposed neonates to a C8 isomer, 2-ethylhexacosanoylcarnitine, can lead to false positive C8 test result. In the other neonate sample, also falsely elevated acylcarnitine C6DC concentration was detected. Interference was likely because of another plasticizer, di-(2-ethylhexyl) adipate (DEHA) metabolite adipic acid, which was metabolized to C6DC. If DEHA is metabolized to C6DC, which is a marker for 3-Hydroxy-3-methylglutaric acidemia (HMG), the false positive C6DC test result may occur. The NeoBase 2 assay is validated with the specific microplates and plate covers provided with the kit and any other items should not be used to avoid plasticizer or other additive contamination.

Carryover:
The carry-over evaluation for the NeoBase2 Non-derivatized MSMS kit was conducted by analyzing two plates using two TQD MSMS Screening Systems. Three low sample replicates were measured immediately after having measured a high sample replicate and the sample sequence was repeated twelve times during one run. No functionally significant carry-over effect was observed with any of the analytes.

Drift:
The drift study was performed using several sample types at different concentration levels to indicate possible drift. Neo Multilevel DBS samples, L1, L3 and L6 were selected to demonstrate the drift at endogenous concentrations, near cut-off concentrations and concentrations representing the upper part of the measuring range, respectively. In addition, the drift of the available control materials, NeoBase2 Controls Low (LC) and High (HC), and publicly available QA controls were included as samples in the study.

The drift study for the kit was completed via the analysis of 10 plates using several sample types at different concentration levels (from endogenous area to the upper part of the measuring range) to indicate possible drift. The results demonstrate that there is no drift for 35 hours continuous run time with the TQD, which is the maximum continuous run time when the autosampler is fully loaded. The fully prepared and sealed assay plate can be stored in the MSMS system autosampler at ambient temperature for 31 hours before the assay run starts.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

See clinical performance data below.

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):

A comparison of results from neonatal dried blood samples from two state laboratories within the United States and known positive neonatal dried blood samples from sample banks using the NeoBase 2 assay on the TQD system and the predicate test system was performed. Testing was performed with a total of 4456 presumptive negative neonatal dried blood samples. Included in the 4456 samples were 76 dried blood samples from affected babies, randomly distributed throughout the study.

The overall percent agreement is presented in the tables below at the different cut-offs.

Agreement between NeoBase2 and NeoBase Amino acids and Ketones with 99.5% cutoff

Analyte	N	Overall Agreement
Ala	4456	99.5 %
Arg	4456	99.8 %
Cit	4456	99.3 %
Gly	4456	99.6 %
Met	4456	99.7 %
Orn	4456	99.1 %
Phe	4456	99.4 %
Pro	4456	99.7 %
Tyr	4456	99.7 %
Val	4456	99.6 %
SA	4456	99.0 %

Agreement between NeoBase2 and NeoBase Carnitines with 99.5% cutoff

Analyte	N	Overall Agreement	Analyte	N	Overall Agreement
C0	4456	99.8 %	**C14**	4456	99.8 %
C3	4456	99.9 %	**C14:1**	4456	99.6 %
C4	4456	99.8 %	**C14:2**	4456	99.7 %
C5	4456	99.6 %	**C14OH**	4456	99.4 %
C5:1	4456	97.9 %	**C16**	4456	99.8 %
C6	4456	99.9 %	**C16:1**	4456	99.8 %
C6DC	4456	99.8 %	**C16OH**	4456	99.7 %
C8	4456	99.7 %	**C16:1OH**	4456	99.8 %
C8:1	4456	99.8 %	**C18**	4456	99.5 %
C10	4456	99.8 %	**C18:1**	4456	99.5 %
C10:1	4456	99.8 %	**C18:2**	4456	99.7 %
C10:2	4456	99.7 %	**C18OH**	4456	99.3 %
C12	4456	99.7 %	**C18:1OH**	4456	98.6 %
C12:1	4456	99.8 %			

Agreement between NeoBase2 and NeoBase Amino acids and Ketones with 99% cutoff

Analyte	N	Overall Agreement
Ala	4456	98.5 %
Arg	4456	99.8 %
Cit	4456	98.9 %
Gly	4456	99.1 %
Met	4456	99.7 %
Orn	4456	98.6 %
Phe	4456	99.0 %
Pro	4456	99.0 %
Tyr	4456	99.3 %
Val	4456	99.0 %
SA	4456	98.1 %

Agreement between NeoBase2 and NeoBase Carnitines with 99% cutoff

Analyte	N	Overall Agreement	Analyte	N	Overall Agreement
C0	4456	99.1 %	**C14**	4456	99.4 %
C3	4456	99.7 %	**C14:1**	4456	99.4 %
C4	4456	99.6 %	**C14:2**	4456	99.6 %
C5	4456	99.3 %	**C14OH**	4456	99.3 %
C5:1	4456	97.9 %	**C16**	4456	99.6 %
C6	4456	99.5 %	**C16:1**	4456	99.6 %
C6DC	4456	99.6 %	**C16OH**	4456	99.4 %

Analyte	N	Overall Agreement	Analyte	N	Overall Agreement
C8	4456	99.5 %	**C16:1OH**	4456	99.6 %
C8:1	4456	99.6 %	**C18**	4456	99.0 %
C10	4456	99.7 %	**C18:1**	4456	98.7 %
C10:1	4456	99.5 %	**C18:2**	4456	99.7 %
C10:2	4456	99.6 %	**C18OH**	4456	99.3 %
C12	4456	99.3 %	**C18:1OH**	4456	98.0 %
C12:1	4456	99.3 %			

Agreement between NeoBase2 and NeoBase analytes with 1% and 10% cutoff

Analyte	N	Overall Agreement
Cit	4456	98.2 %
C0 (10% cutoff)	4456	94.1 %
C2	4456	99.2 %
C3	4456	99.0 %
C16	4456	99.3 %
C18	4456	99.3 %
C18:1	4456	99.5 %

Summary of screening performance for different disorders

Amino acid disorders (AA): N=37				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase 2 Study Cutoffs**	**Detected with NeoBase Study Cutoffs**
Arginemia	ARG	2	2	2
Argininosuccinic acidemia	ASA	3	3	3
Benign hyperphenylalaninemia	H-PHE	4	4	4
Citrullinemia type I	CIT I	7	7	7
Classic phenylketonuria	PKU	4	4	4
Disorders of biopterin defect in cofactor biosynthesis	BIOPT-BS	1	1	1
Homocystinuria	HCY	1	1	1
Hypermethioninemia	MET	2	2	2
Maple syrup urine disease	MSUD	6	6	6
Ornithine transcarbamylase deficiency	OTCD	3	3	3
Tyrosinemia, type I	TYR I	2	2	2
Tyrosinemia, type II	TYR II	1	1	1
Tyrosinemia, type III	TYR III	1	1	1
Fatty acid oxidation disorders (FAO): N=26				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase2 Study Cutoffs**	**Detected with NeoBase Study Cutoffs**
Carnitine palmitoyltransferase	CPT II	1	1	1
Carnitine uptake defect	CUD	4	4	4
Long-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency	LCHAD	4	4	4
Medium-chain acyl-CoA dehydrogenase deficiency	MCAD	5	5	5
Short-chain acyl-CoA dehydrogenase deficiency	SCAD	4	4	4
Trifunctional protein deficiency	TFP	2	2	2
Very long-chain acyl-CoA dehydrogenase deficiency	VLCAD	6	6	6

Organic acid conditions (OA): N=28				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase2 Study Cutoffs**	**Detected with NeoBase Study Cutoffs**
2-Methylbutyrylglycinuria	2MBG	2	2	2
3-Methylcrotonyl-CoA carboxylase deficiency	3MCC	4	4	4
3-Methylglutaconic academia type I	3MGA	1	1	1
Glutaric acidemia type I	GA I	6	6	6
Holocarboxylase synthetase deficiency	MCD	1	1	1
Isobutyrylglycinuria	IBG	1	1	1
Isovaleric acidemia	IVA	4	4	4
Methylmalonic acidemia (methylmalonyl-CoA mutase)	MUT	3	3	3
Methylmalonic acidemia (cbl disorders)	cblA, cblB	1	1	1
Propionic acidemia	PROP	5	5	5
Other disorders: N=8				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase2 Study Cutoffs**	**Detected with NeoBase Study Cutoffs**
Adenosine Deaminase Severe Combined Immunodeficiency	ADA-SCID	4	4	NA≠
X-linked Adrenoleukodystrophy	X-ALD	4	4	NA≠

≠ - not tested in Neobase instrument

2x2 tables using 99.5th percentile cutoff from site 1

Ala		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	0	9
	Negative	8	1762	1770
	Total	17	1762	1779

Arg		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	0	9
	Negative	4	1766	1770
	Total	13	1766	1779

C0		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	3	9
	Negative	2	1768	1770
	Total	8	1771	1779

C10		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	0	6
	Negative	5	1768	1773
	Total	11	1768	1779

C10:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	3	11
	Negative	1	1767	1768
	Total	9	1770	1779

C10:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	1	4	5
	Negative	0	1774	1774
	Total	1	1778	1779

C12		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	2	11
	Negative	3	1765	1768
	Total	12	1767	1779

C12:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	1	10
	Negative	2	1767	1769
	Total	11	1768	1779

C14		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	1	11
	Negative	2	1766	1768
	Total	12	1767	1779

C14:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	3	11
	Negative	2	1766	1768
	Total	10	1769	1779

C14:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	1	9
	Negative	4	1766	1770
	Total	12	1767	1779

C14OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	0	8
	Negative	6	1765	1771
	Total	14	1765	1779

C16		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	2	0	2
	Negative	1	1776	1777
	Total	3	1776	1779

C16:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	1	7
	Negative	2	1770	1772
	Total	8	1771	1779

C16:1OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	2	10
	Negative	2	1767	1769
	Total	10	1769	1779

C16OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	0	6
	Negative	3	1770	1773
	Total	9	1770	1779

C18		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	3	3	6
	Negative	1	1772	1773
	Total	4	1775	1779

C18:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	2	3	5
	Negative	7	1767	1774
	Total	9	1770	1779

C18:1OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	1	8
	Negative	2	1769	1771
	Total	9	1770	1779

C18:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	14	0	14
	Negative	4	1761	1765
	Total	18	1761	1779

C18OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	4	10
	Negative	0	1769	1769
	Total	6	1773	1779

C3		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	0	6
	Negative	2	1771	1773
	Total	8	1771	1779

C3DC\C4OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	11	20
	Negative	4	1755	1759
	Total	13	1766	1779

C4		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	2	12
	Negative	0	1767	1767
	Total	10	1769	1779

C4DC\C5OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	0	7
	Negative	0	1772	1772
	Total	7	1772	1779

C5		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	3	13
	Negative	1	1765	1766
	Total	11	1768	1779

C5:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	3	3	6
	Negative	3	1770	1773
	Total	6	1773	1779

C5DC\C6OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	9	18
	Negative	3	1758	1761
	Total	12	1767	1779

C6		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	0	9
	Negative	1	1769	1770
	Total	10	1769	1779

C6DC		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	3	3	6
	Negative	1	1772	1773
	Total	4	1775	1779

C8		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	3	9
	Negative	4	1766	1770
	Total	10	1769	1779

C8:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	3	13
	Negative	3	1763	1766
	Total	13	1766	1779

Cit		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	2	9
	Negative	12	1758	1770
	Total	19	1760	1779

Gly		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	4	3	7
	Negative	3	1769	1772
	Total	7	1772	1779

Leu\Ile\Pro-OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	12	2	14
	Negative	8	1757	1765
	Total	20	1759	1779

Met		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	3	13
	Negative	6	1760	1766
	Total	16	1763	1779

Orn		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	4	15	19
	Negative	6	1754	1760
	Total	10	1769	1779

Phe		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	14	3	17
	Negative	7	1755	1762
	Total	21	1758	1779

Pro		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	1	7
	Negative	0	1772	1772
	Total	6	1773	1779

SA		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	0	13	13
	Negative	2	1764	1766
	Total	2	1777	1779

Tyr		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	0	6
	Negative	2	1771	1773
	Total	8	1771	1779

Val		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	4	12
	Negative	5	1762	1767
	Total	13	1766	1779

2x2 tables with 99th percentile cutoff from site 1

Ala		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	21	6	27
	Negative	6	1746	1752
	Total	27	1752	1779

Arg		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	16	4	20
	Negative	1	1758	1759
	Total	17	1762	1779

C0		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	16	23
	Negative	5	1751	1756
	Total	12	1767	1779

C10		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	1	9
	Negative	5	1765	1770
	Total	13	1766	1779

C10:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	3	11
	Negative	1	1767	1768
	Total	9	1770	1779

C10:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	2	3	5
	Negative	5	1769	1774
	Total	7	1772	1779

C12		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	13	5	18
	Negative	5	1756	1761
	Total	18	1761	1779

C12:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	15	4	19
	Negative	6	1754	1760
	Total	21	1758	1779

C14		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	13	1	14
	Negative	6	1759	1765
	Total	19	1760	1779

C14:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	18	3	21
	Negative	9	1749	1758
	Total	27	1752	1779

C14:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	2	10
	Negative	4	1765	1769
	Total	12	1767	1779

C14OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	10	21
	Negative	3	1755	1758
	Total	14	1765	1779

C16		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	3	1	4
	Negative	5	1770	1775
	Total	8	1771	1779

C16:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	0	7
	Negative	6	1766	1772
	Total	13	1766	1779

C16:1OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	1	12
	Negative	2	1765	1767
	Total	13	1766	1779

C16OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	7	18
	Negative	4	1757	1761
	Total	15	1764	1779

C18		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	5	14
	Negative	9	1756	1765
	Total	18	1761	1779

C18:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	2	8
	Negative	12	1759	1771
	Total	18	1761	1779

C18:1OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	8	19
	Negative	20	1740	1760
	Total	31	1748	1779

C18:2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	19	0	19
	Negative	3	1757	1760
	Total	22	1757	1779

C18OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	7	3	10
	Negative	1	1768	1769
	Total	8	1771	1779

C3		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	12	1	13
	Negative	2	1764	1766
	Total	14	1765	1779

C3DC\C4OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	15	25
	Negative	5	1749	1754
	Total	15	1764	1779

C4		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	13	5	18
	Negative	0	1761	1761
	Total	13	1766	1779

C4DC\C5OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	2	11
	Negative	1	1767	1768
	Total	10	1769	1779

C5		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	16	6	22
	Negative	5	1752	1757
	Total	21	1758	1779

C5:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	3	3	6
	Negative	3	1770	1773
	Total	6	1773	1779

C5DC\C6OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	16	12	28
	Negative	6	1745	1751
	Total	22	1757	1779

C6		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	9	3	12
	Negative	7	1760	1767
	Total	16	1763	1779

C6DC		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	4	3	7
	Negative	2	1770	1772
	Total	6	1773	1779

C8		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	2	13
	Negative	9	1757	1766
	Total	20	1759	1779

C8:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	17	5	22
	Negative	1	1756	1757
	Total	18	1761	1779

Cit		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	10	8	18
	Negative	16	1745	1761
	Total	26	1753	1779

Gly		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	6	6	12
	Negative	13	1754	1767
	Total	19	1760	1779

Leu\Ile\Pro-OH		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	27	5	32
	Negative	6	1741	1747
	Total	33	1746	1779

Met		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	17	4	21
	Negative	3	1755	1758
	Total	20	1759	1779

Orn		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	22	30
	Negative	11	1738	1749
	Total	19	1760	1779

Phe		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	20	5	25
	Negative	7	1747	1754
	Total	27	1752	1779

Pro		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	11	2	13
	Negative	8	1758	1766
	Total	19	1760	1779

SA		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	0	18	18
	Negative	2	1759	1761
	Total	2	1777	1779

Tyr		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	8	2	10
	Negative	8	1761	1769
	Total	16	1763	1779

Val		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	14	5	19
	Negative	11	1749	1760
	Total	25	1754	1779

2x2 tables with 1st percentile cutoff at site 1

Cit		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	16	20	36
	Negative	16	1727	1743
	Total	32	1747	1779

C2		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	25	13	38
	Negative	13	1728	1741
	Total	38	1741	1779

C3		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	26	11	37
	Negative	21	1721	1742
	Total	47	1732	1779

C16		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	27	23	50
	Negative	1	1728	1729
	Total	28	1751	1779

C18		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	21	3	24
	Negative	14	1741	1755
	Total	35	1744	1779

C18:1		NEOBASE		
		Positive	Negative	Total
NEOBASE2	Positive	12	7	19
	Negative	4	1756	1760
	Total	16	1763	1779

2x2 tables with 10th percentile cutoff at site 1

C0		NeoBase	
		Positive	Negative
NeoBase2	Positive	123	19
	Negative	150	1487

2x2 tables with 99.5th percentile cutoff from Site 2

Ala		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	4
	Negative	10	2649

Arg		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	6
	Negative	1	2654

Cit		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	5
	Negative	11	2647

Gly		NeoBase	
		Positive	Negative
NeoBase2	Positive	1	0
	Negative	12	2664

Leu\Ile\Pro-OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	3
	Negative	10	2648

Met		NeoBase	
		Positive	Negative
NeoBase2	Positive	12	1
	Negative	2	2662

Orn		NeoBase	
		Positive	Negative
NeoBase2	Positive	17	7
	Negative	10	2643

Phe		NeoBase	
		Positive	Negative
NeoBase2	Positive	22	4
	Negative	11	2640

Pro		NeoBase	
		Positive	Negative
NeoBase2	Positive	9	4
	Negative	8	2656

Tyr		NeoBase	
		Positive	Negative
NeoBase2	Positive	23	5
	Negative	8	2641

Val		NeoBase	
		Positive	Negative
NeoBase2	Positive	17	2
	Negative	8	2650

C0		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	5
	Negative	0	2662

C3		NeoBase	
		Positive	Negative
NeoBase2	Positive	6	1
	Negative	1	2669

C3DC\C4OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	12	1
	Negative	15	2649

C4		NeoBase	
		Positive	Negative
NeoBase2	Positive	11	6
	Negative	3	2657

C4DC\C5OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	2
	Negative	4	2661

C5		NeoBase	
		Positive	Negative
NeoBase2	Positive	9	13
	Negative	0	2655

C5:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	5	88
	Negative	0	2584

C5DC\C6OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	9	8
	Negative	4	2656

C6		NeoBase	
		Positive	Negative
NeoBase2	Positive	11	5
	Negative	0	2661

C6DC		NeoBase	
		Positive	Negative
NeoBase2	Positive	4	1
	Negative	5	2667

C8		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	4
	Negative	2	2655

C8:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	6	3
	Negative	2	2666

C10		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	5
	Negative	0	2658

C10:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	2
	Negative	2	2663

C10:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	3	6
	Negative	4	2664

C12		NeoBase	
		Positive	Negative
NeoBase2	Positive	21	7
	Negative	3	2646

C12:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	17	4
	Negative	3	2653

C14		NeoBase	
		Positive	Negative
NeoBase2	Positive	17	5
	Negative	3	2652

C14:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	21	10
	Negative	2	2644

C14:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	11	5
	Negative	5	2656

C14OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	15
	Negative	4	2648

C16		NeoBase	
		Positive	Negative
NeoBase2	Positive	4	2
	Negative	4	2667

C16:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	6	1
	Negative	3	2667

C16OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	6	6
	Negative	5	2660

C16:1OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	1
	Negative	3	2663

C18		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	13
	Negative	6	2644

C18:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	12	14
	Negative	0	2651

C18:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	9	10
	Negative	0	2658

C18OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	2
	Negative	25	2636

C18:1OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	2
	Negative	59	2603

SA		NeoBase	
		Positive	Negative
NeoBase2	Positive	2	22
	Negative	7	2646

2x2 tables using 99th percentile cutoff at site 2

Ala		NeoBase	
		Positive	Negative
NeoBase2	Positive	19	4
	Negative	49	2605

Arg		NeoBase	
		Positive	Negative
NeoBase2	Positive	35	5
	Negative	1	2636

Cit		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	9
	Negative	16	2632

Gly		NeoBase	
		Positive	Negative
NeoBase2	Positive	6	2
	Negative	17	2652

Leu\Ile\Pro-OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	31	20
	Negative	7	2619

Met		NeoBase	
		Positive	Negative
NeoBase2	Positive	24	1
	Negative	6	2646

Orn		NeoBase	
		Positive	Negative
NeoBase2	Positive	30	18
	Negative	13	2616

Phe		NeoBase	
		Positive	Negative
NeoBase2	Positive	33	10
	Negative	23	2611

Pro		NeoBase	
		Positive	Negative
NeoBase2	Positive	26	12
	Negative	24	2615

Tyr		NeoBase	
		Positive	Negative
NeoBase2	Positive	34	8
	Negative	11	2624

Val		NeoBase	
		Positive	Negative
NeoBase2	Positive	43	16
	Negative	11	2607

C0		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	21
	Negative	0	2636

C3		NeoBase	
		Positive	Negative
NeoBase2	Positive	11	4
	Negative	8	2654

C3DC\C4OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	1
	Negative	19	2644

C4		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	12
	Negative	0	2645

C4DC\C5OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	9
	Negative	10	2638

C5		NeoBase	
		Positive	Negative
NeoBase2	Positive	29	20
	Negative	0	2628

C5:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	5	88
	Negative	0	2584

C5DC\C6OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	12	15
	Negative	4	2646

C6		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	7
	Negative	7	2643

C6DC		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	3
	Negative	10	2654

C8		NeoBase	
		Positive	Negative
NeoBase2	Positive	27	8
	Negative	4	2638

C8:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	19	6
	Negative	4	2648

C10		NeoBase	
		Positive	Negative
NeoBase2	Positive	19	1
	Negative	5	2652

C10:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	4
	Negative	14	2643

C10:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	3	6
	Negative	4	2664

C12		NeoBase	
		Positive	Negative
NeoBase2	Positive	28	16
	Negative	6	2627

C12:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	19	16
	Negative	3	2639

C14		NeoBase	
		Positive	Negative
NeoBase2	Positive	20	12
	Negative	8	2637

C14:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	28	11
	Negative	5	2633

C14:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	7
	Negative	7	2647

C14OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	15
	Negative	4	2648

C16		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	4
	Negative	10	2650

C16:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	10	10
	Negative	1	2656

C16OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	7	5
	Negative	10	2655

C16:1OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	7
	Negative	10	2646

C18		NeoBase	
		Positive	Negative
NeoBase2	Positive	26	16
	Negative	13	2622

C18:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	40
	Negative	2	2621

C18:2		NeoBase	
		Positive	Negative
NeoBase2	Positive	23	10
	Negative	1	2643

C18OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	2
	Negative	25	2636

C18:1OH		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	2
	Negative	59	2603

SA		NeoBase	
		Positive	Negative
NeoBase2	Positive	2	49
	Negative	14	2612

2x2 tables using 1st percentile cutoff at site 2

Cit		NeoBase	
		Positive	Negative
NeoBase2	Positive	14	40
	Negative	6	2617

C2		NeoBase	
		Positive	Negative
NeoBase2	Positive	15	5
	Negative	4	2653

C3		NeoBase	
		Positive	Negative
NeoBase2	Positive	16	6
	Negative	5	2650

C16		NeoBase	
		Positive	Negative
NeoBase2	Positive	19	9
	Negative	0	2649

C18		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	13
	Negative	3	2648

C18:1		NeoBase	
		Positive	Negative
NeoBase2	Positive	13	5
	Negative	7	2652

2x2 tables using 10th percentile cutoff at site 2

C0		NeoBase	
		Positive	Negative
NeoBase2	Positive	126	28
	Negative	64	2459

4. Clinical cut-off**:**

   The labeling for this device contains the following statement about cut-offs:

   The determination of presumptive positives for metabolic disorders in newborn is based on the use of a cut-off value, which distinguishes between presumptive negative and presumptive positive values.

   Please note that the values mentioned in this section should only be used as a guideline, and each laboratory shall establish its own reference range and cut-off-values.

NOTE: Do not use a cut-off value that is based on data collected at another site or using any other product than the 3044-001U NeoBase 2 Non-derivatized MSMS kit.

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 results from 2171 unaffected dried newborn blood sample. The population means and population standard deviation (SD) are shown in micromolar (μM) quantities.

Analyte	Mean	SD
Ala	299	106
Arg	12.8	8.82
Asa	0.29	0.10
Cit	15.1	5.39
Gln\Lys	771	171
Glu	288	74.5
Gly	547	156
Leu\Ile\Pro-OH	120	47.8
Met	22.7	6.50
Orn	94.4	48.1
Phe	54.5	12.2
Pro	156	42.8
Tyr	95.8	35.8
Val	114	40.5
C0	20.1	7.92
C2	22.2	9.31
C3	2.26	1.14
C3DC\C4OH	0.18	0.08
C4	0.23	0.12
C4DC\C5OH	0.26	0.08
C5	0.11	0.05
C5:1	0.01	0.00
C5DC\C6OH	0.11	0.04
C6	0.05	0.02
C6DC	0.06	0.03
C8	0.06	0.02
C8:1	0.10	0.05
C10	0.10	0.04
C10:1	0.04	0.01

Analyte	Mean	SD
C10:2	0.01	0.00
C12	0.11	0.06
C12:1	0.09	0.06
C14	0.23	0.09
C14:1	0.13	0.07
C14:2	0.02	0.01
C14OH	0.02	0.01
C16	3.74	1.55
C16:1	0.26	0.12
C16OH	0.03	0.01
C16:1OH\C17	0.05	0.02
C18	0.89	0.32
C18:1	1.23	0.40
C18:2	0.18	0.09
C18OH	0.01	0.01
C18:1OH	0.02	0.01
C18:2OH	0.01	0.00
SA	0.22	0.09
ADO	0.54	0.22
D-ADO	0.02	0.01
C24:0-LPCC20:0-LPC	0.490.17	0.120.10
C26:0-LPCC22:0-LPC	0.300.19	0.090.07
C24:0-LPC	0.49	0.12
C26:0-LPC	0.30	0.09

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10, and the special controls for this device type under 21 CFR 862.1055.

O. Conclusion:

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