510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K193103

B Applicant

PerkinElmer Inc.

C Proprietary and Established Names

NeoBase 2 Non-derivatized MSMS Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NQL	Class II	21 CFR 862.1055 - Newborn Screening Test System For Amino Acids, Free Carnitine, And Acylcarnitines Using Tandem Mass Spectrometry	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Addition of a mass spectrometry system to a previously cleared assay.

B Measurand:

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

C Type of Test:

Quantitative measurement by mass spectrometry

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B 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 NeoBase 2 Non-derivatized MSMS kit

ANALYTE NAME	ABBREVIATION
Amino acids	
Alanine	Ala
Arginine	Arg
Argininosuccinic acid	Asa
Citrulline	Cit
Glutamine\Lysine [1]	Gln\Lys
Glutamic acid	Glu
Glycine	Gly
Leucine\Isoleucine\Hydroxyproline [1]	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 [1]	C3DC\C4OH
Butyrylcarnitine	C4
Methylmalonyl\3-Hydroxy-isovalerylcarnitine [1]	C4DC\C5OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutarylcarnitine\3-Hydroxy-hexanoylcarnitine [1]	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 1	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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use only.

The NeoBase 2 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 lysophospholipid profiles. Users should follow local guidelines for follow-up and confirmation testing.

The NeoBase 2 Non-derivatized MSMS assay does not screen for rare or newly recognized metabolic disorders whose indicative biochemical markers are not listed in Table 1. above. Patients with CPT-II deficiency typically show elevated blood levels of long-chain acylcarnitine species, especially palmitoyl-carnitine (C16) and/or oleoyl-carnitine (C18:1). With the myopathic form of CPT II primary markers C16 and C18:1 may not be elevated.

Based on limited amount of publications and newborn screening program findings, most infants with SCAD identified through newborn screening programs have remained well and asymptomatic, whereas in children identified clinically demonstrate severe symptoms. Clinically identified children often have only SCAD variants (625G>A, 511C>T), which may have C4 concentrations within normal range.

ABCD1 gene variants that are of unknown significance (VOUS) may not be screen positive with primary marker C26-LPC for x-ALD.

In the measurement of C0 as a marker for CUD, it is possible that a cut-off lower than 10^{th} percentile may yield a false negative result.

Please note that the 25-26% recovery of Asa is lower compared to other analytes measured with NeoBase 2 Nonderivatized MSMS assay. 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.
Known causes for anomalous analytical assay results are:

- sample not uniformly saturated with blood

- sample disk punched too close to the edge of the blood spot
- poorly collected specimens, e.g. excessive milking or squeezing the puncture may cause hemolysis of the specimen or a mixture of tissue fluids with the specimen. Layering successive drops of blood in the specimen may affect the measured results.
- improperly dried specimens e.g. heating or stacking the specimen collection devices during the drying process
- humidity and moisture or exposure to direct sunlight are detrimental to the dried blood spot sample. Samples and controls can be pre-punched to microplate 2 hours before addition of EWS.
- non-eluting blood spot due to deterioration of sample
- contamination of blood spot filter paper e.g. with fecal material, urine and liquid infant formula.
- disinfectants such as alcohol swabs with a pain killer benzocaine, chlorhexidine digluconate, or lidocaine used to wipe off the heel of newborn in specimen collection can interfere with the screening analytes. In addition, avoid using other disinfectants (e.g. hexachlorophene or povidone-iodine, or equivalent commercially available products).

Variables such as hematocrit, prematurity, preterm birth, maternal diseases, medications and total parenteral nutrition may affect the interpretation of the values produced. Age-related variations in the amino acid, acylcarnitine and free carnitine concentrations are known. Certain acylcarnitines may tend to be significantly lower in older infants than in newborns and free carnitine can be significantly higher in older children than in newborns.

D Special Instrument Requirements:

Perkin Elmer QSight 210 MD Screening System that consists of: QSight 210 MD Mass Spectrometer, Simplicity 3Q MD Software, QSight HC Autosampler MD, QSight Binary Pump MD, Perkin Elmer MSMS Workstation Software

IV Device/System Characteristics:

A Device Description:

Each NeoBase 2 Non-derivatized MSMS kit contains reagents for 960 assays. This kit is designed to be used with 3045-0010 NeoBase 2 Non-derivatized Assay Solutions (consisting of Neo MSMS Flow Solvent and NeoBase 2 Extraction Solution) and 3046-0010 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

B Principle of Operation:

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.

SA is a reactive diketone. As such, this analyte tends to react with amine groups of amino acid residues of peptides and proteins present in blood. Therefore, SA is generally bound to proteins in the sample. In the NeoBase 2 assay, SA is derivatized with hydrazine (a stronger base than the amino acid residues). This reaction displaces the interaction of SA with proteins and replaces it with a more stable interaction with the diamine, hydrazine. The reaction creates a very stable pyrazole-like product which is then extracted with the other analytes measured in this assay. In consequence, SA is measured in the MSMS assay as the derivative 3-(5-methyl-1H-pyrazol-3-yl) propanoic acid designated here with the acronym MPP. Finally, quantitation of SA is accomplished by including an isotope-labeled analog of MPP as internal standard in the EWS.

In addition to SA, the extraction and measurement of ASA requires the use of NeoBase 2 Succinylacetone Assay Solution.

The measurement of amino acids, succinylacetone, free carnitine, acylcarnitines, nucleosides and lysophospholipids with the NeoBase 2 assay involves extraction of analytes from dried blood spots with a solution containing labeled internal standards and analysis using a tandem mass spectrometry (MSMS) system in Multiple Reaction Monitoring (MRM) mode. The response of each analyte relative to its corresponding internal standard is proportional to the analyte concentration.

The analytes present in the sample extract are introduced to the mass spectrometer via the sample delivery system. In the ESI ion source, the analytes acquire a positive or negative charge and are transferred from solution into the gaseous phase. The ions are further transferred into the mass spectrometer, which consists of two sets of quadrupoles (MS1 and MS2) and a collision cell between the quadrupoles. The mass spectrometer sorts and separates the ions according to their mass to charge ratio (m/z value).

In the MRM acquisition mode, MS1 is set to select a particular precursor ion. After MS1 selection, the precursor ion is sent to the collision cell where collision induced dissociation (CID) takes place and the precursor ion is fragmented to several product ions. Thereafter, only a selected specific product ion is allowed to pass through MS2 to reach the detector and to record an analyte specific precursor-product ion MRM-transition. All non-specified product ions are filtered out.

V Substantial Equivalence Information:

A Predicate Device Name(s):

NeoBase 2 Non-derivatized MSMS Kit

B Predicate 510(k) Number(s):

K173568

C Comparison with Predicate(s):

Device & Predicate Device(s):	K193103	K173568
Device Trade Name	NeoBase 2 Non-derivatized MSMS Kit	Same
General Device Characteristic Similarities		
Intended Use/Indications 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 with a tandem mass spectrometer from newborn heel prick blood specimens dried on filter paper.	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-	Same

	labeled internal standards. The concentrations are determined by comparing the signal intensities of the known standards to the measured analytes.	
Sample Type	Punch from dried blood spot specimen	Same
Analytes Measured	See Table 1 above in Section III B.	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
General Device Characteristic Differences		
Instrument / Software Platform	PerkinElmer QSight 210MD Screening System: CTC PAL RSI Sample Manager, Spark Holland SPH1240 Binary Pump, Simplicity Software, and PerkinElmer MSMS Workstation Software	Waters TQD instrument with MassLynx v4.1 firmware, with Waters 1525 sample pump, with Waters 2777c autosampler, with Waters NeoLynx v4.1 software and with the PerkinElmer MSMS Workstation Software

VI Standards/Guidance Documents Referenced:

CLSI EP05-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 EP07: Interference testing in clinical chemistry; Approved guideline - Third edition

CLSI EP17-A2: Evaluation of detection capability for clinical laboratory measurement procedures; Approved guideline -- Second edition

FDA Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Newborn Screening Test Systems for Amino Acids, Free Carnitine, and Acylcarnitines Using Tandem Mass Spectrometry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Dried blood spot samples were prepared from pooled human whole blood from apparently healthy adults. To achieve concentrations of analytes near the lower end of the measuring range, samples were also prepared by diluting human red blood cell concentrate with either charcoal stripped human serum or 0.9% sodium chloride. Hematocrit was adjusted to between 50-55% before spiking with analyte stock solutions.

The repeatability and within-laboratory variation for NeoBase 2 Non-derivatized MSMS kit is based on 80 determinations on one instrument: 40 plates measured over 20 working days, each plate having 2 replicates per sample.

Between-lot variation is based on 75 determinations on one instrument: 15 plates measured over five working days using three kit lots, each plate having 5 replicates per sample.

The between instrument variation was determined from 50 total determinations (25 per instrument) on two instruments running 1 plate per day over 5 days. Each plate contained 5 replicates per sample.

Total imprecision was calculated by the sum of the variance of the repeatability, between lot, and between instrument results.

A summary of the results is presented in the tables below.

Ala

Sample	Totalmean µmol/L	Repeatability		Within-Lab		Between-lot		Between-instrument		Total Variation	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	161	11	6.5	17	9.8	4.1	2.8	19	12	26	16
2	361	21	5.5	27	7.1	6.2	1.8	17	4.6	32	9.0
3	414	24	5.4	30	6.8	4.2	1.0	3.4	0.84	30	7.3
4	518	28	5.3	34	6.3	15	3.1	0.04	0.01	37	7.2

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	0.45	6.0	0.62	8.3	0.47	6.8	<0.01	<0.01	0.78	10
2	23	1.3	5.7	1.7	7.3	0.11	0.49	<0.01	<0.01	1.7	7.6
3	69	3.4	4.9	3.7	5.4	1.7	2.6	<0.01	<0.01	4.1	5.9
4	157	5.2	3.3	8.0	5.0	5.2	3.5	1.1	0.67	9.6	6.1

Asa[1]

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	0.31	0.06	29	0.09	43	<0.01	0.74	0.03	14	0.10	32
2	2.2	0.14	6.7	0.24	11	0.07	3.1	0.10	4.3	0.27	12
3	8.1	0.39	5.0	0.76	9.9	0.44	5.3	0.66	7.8	1.1	14
4	21	0.74	3.6	1.7	8.1	0.95	4.5	1.7	7.8	2.6	12
5	57	2.3	3.8	5.6	9.2	2.1	4.6	5.7	8.7	8.2	14

[1] Asa is measured as a total concentration of Asa and its anhydrides.

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	1.1	12	1.2	12	0.33	3.0	<0.01	0.01	1.2	12
2	68	3.3	5.0	4.3	6.5	2.1	3.1	1.2	1.7	5.0	7.3
3	202	10	5.1	11	5.3	5.4	2.7	<0.01	<0.01	12	5.9
4	470	27	5.8	29	6.3	11	2.3	10	2.1	33	6.9
5	957	50	5.1	55	5.7	27	3.0	16	1.6	64	6.7

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	43	2.8	5.9	3.7	7.8	1.0	2.5	2.9	7.2	4.8	11
2	487	24	4.6	30	5.7	15	3.3	0.02	<0.01	34	6.9
3	675	35	4.9	43	6.0	3.3	0.52	<0.01	<0.01	43	6.4
4	1064	52	4.6	65	5.7	37	3.7	13	1.2	75	7.1
5	2274	94	3.9	136	5.7	20	0.90	0.13	0.01	138	6.1

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	247	16	6.6	24	9.8	7.9	3.0	3.9	1.6	25	10
2	324	19	6.3	23	7.6	6.6	1.9	4.7	1.5	25	7.6
3	524	32	6.3	41	8.1	6.1	1.1	0.01	<0.01	42	8.0
4	930	52	5.7	82	8.9	26	2.8	19	2.1	88	9.5

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	2.5	4.3	3.2	5.5	0.54	0.87	<0.01	<0.01	3.2	5.6
2	202	9.0	4.5	11	5.7	4.7	2.3	<0.01	<0.01	12	6.2
3	350	16	4.4	16	4.4	2.7	0.80	<0.01	<0.01	16	4.6
4	656	31	4.7	33	4.9	17	2.6	0.03	0.01	37	5.6
5	1121	45	3.9	54	4.7	27	2.5	<0.01	<0.01	60	5.4

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	2.2	0.28	18	0.28	18	0.26	8.3	0.21	11	0.43	20
2	51	2.6	5.1	3.1	6.2	1.9	3.7	0.58	1.1	3.7	7.3
3	155	8.2	5.4	9.1	5.9	2.3	1.5	<0.01	<0.01	9.4	6.1
4	369	19	5.0	23	6.3	9.8	2.7	4.2	1.1	26	6.9
5	696	26	3.7	37	5.2	14	2.0	0.01	<0.01	40	5.7

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	29	1.9	6.7	2.9	10	0.50	1.7	0.89	2.9	3.1	11
2	109	4.1	3.8	7.0	6.4	1.5	1.4	2.0	1.8	7.4	6.8
3	204	10	4.9	12	5.7	2.3	1.2	<0.01	<0.01	12	5.8
4	382	14	3.8	17	4.5	9.9	2.7	11	2.7	23	5.9

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	5.1	1.2	5.8	0.43	1.7	<0.01	<0.01	1.3	5.8
2	127	4.3	3.4	5.6	4.5	3.5	2.7	1.2	0.94	6.7	5.3
3	340	15	4.5	16	4.8	4.7	1.4	<0.01	<0.01	17	5.0
4	778	35	4.5	43	5.5	29	3.8	7.3	0.91	52	6.7
5	1436	39	2.6	65	4.4	17	1.2	23	1.6	71	4.9

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	2.2	5.6	0.57	1.4	<0.01	<0.01	2.3	5.7
2	178	7.0	3.9	8.1	4.5	4.6	2.6	2.0	1.1	9.5	5.3
3	316	14	4.4	15	4.7	2.6	0.86	<0.01	<0.01	16	4.9
4	596	28	4.5	30	4.8	16	2.8	6.9	1.1	34	5.7

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	1.5	7.5	0.52	2.6	<0.01	<0.01	1.5	7.7
2	109	4.1	3.9	4.7	4.5	3.6	3.3	1.4	1.3	6.1	5.6
3	264	9.2	3.6	9.2	3.6	4.1	1.6	<0.01	<0.01	10	3.8
4	586	21	3.6	26	4.6	15	2.6	2.4	0.39	30	5.1

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	55	2.4	4.5	2.8	5.2	0.81	1.3	<0.01	<0.01	2.9	5.2
2	205	8.7	4.2	11	5.4	5.6	2.8	3.6	1.8	13	6.4
3	314	15	4.8	15	4.8	4.2	1.4	<0.01	<0.01	16	5.1
4	540	27	4.9	29	5.2	15	3.0	10	1.9	34	6.4

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	8.4	0.46	5.6	0.54	6.5	0.04	0.47	<0.01	0.01	0.55	6.5
2	42	1.8	4.4	2.1	5.0	1.5	3.7	1.1	2.6	2.8	6.7
3	89	4.9	5.4	5.4	5.9	1.1	1.2	0.01	0.02	5.5	6.1
4	186	8.3	4.3	11	5.5	6.7	3.7	3.0	1.6	13	6.9

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	0.17	4.8	0.21	5.7	0.09	2.5	<0.01	<0.01	0.22	6.3
2	12	0.58	4.7	0.72	5.9	0.47	3.9	0.06	0.47	0.87	7.1
3	18	0.87	4.7	0.91	4.9	0.09	0.49	<0.01	<0.01	0.91	5.0
4	30	1.3	4.3	1.5	4.9	0.66	2.2	<0.01	<0.01	1.7	5.5

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	0.44	0.03	6.7	0.04	8.1	0.01	2.9	<0.01	0.01	0.04	9.1
2	4.5	0.18	3.8	0.27	5.5	0.14	3.2	0.03	0.76	0.30	6.6
3	13	0.73	5.1	0.95	6.6	0.16	1.2	<0.01	<0.01	0.96	7.2
4	32	1.6	4.8	2.3	6.8	1.2	4.0	0.36	1.2	2.6	8.3

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	0.01	0.01	11
2	0.57	0.03	4.9	0.03	5.5	0.02	2.9	<0.01	0.78	0.04	6.2
3	1.8	0.09	4.8	0.09	4.8	0.01	0.31	<0.01	<0.01	0.09	4.8
4	4.2	0.18	4.1	0.20	4.6	0.08	2.0	0.04	0.93	0.22	5.2

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	19	0.01	19	<0.01	2.6	<0.01	0.01	0.01	17
2	1.0	0.04	4.2	0.06	6.3	0.03	2.8	<0.01	<0.01	0.07	6.7
3	3.6	0.17	4.9	0.18	5.2	0.04	1.1	<0.01	<0.01	0.19	5.2
4	8.9	0.37	4.2	0.43	4.9	0.31	3.6	<0.01	<0.01	0.53	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	0.03	0.01	17	0.01	23	<0.01	9.2	<0.01	5.5	0.01	25
2	0.44	0.03	7.5	0.03	7.9	0.01	3.1	0.01	1.5	0.04	8.6
3	1.5	0.09	5.9	0.10	6.2	0.02	1.1	<0.01	<0.01	0.10	6.3
4	3.8	0.22	5.7	0.24	6.2	0.07	1.9	0.07	1.7	0.26	6.7

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	0.38	0.02	4.4	0.02	6.0	0.01	2.4	<0.01	<0.01	0.02	6.4
2	1.4	0.07	5.0	0.07	5.0	0.03	1.8	0.01	0.59	0.07	5.3
3	3.4	0.16	4.7	0.17	4.9	0.10	3.1	<0.01	<0.01	0.20	5.8

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	2.0	0.10	4.8	0.12	6.0	0.08	3.8	<0.01	<0.01	0.14	7.1
2	7.5	0.35	4.7	0.37	4.9	0.12	1.6	<0.01	<0.01	0.39	5.2
3	18	0.86	4.6	0.99	5.3	0.64	3.6	0.10	0.55	1.2	6.4
4	35	1.3	3.5	1.6	4.4	0.44	1.3	0.56	1.6	1.7	5.0

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	0.46	0.02	5.5	0.03	6.8	0.01	2.4	<0.01	<0.01	0.03	7.2
2	1.6	0.09	5.8	0.11	7.1	0.02	1.2	<0.01	<0.01	0.11	7.1
3	3.9	0.24	6.2	0.29	7.7	0.11	2.9	0.04	1.1	0.32	8.2

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	0.49	0.02	5.1	0.03	6.4	0.02	3.4	<0.01	<0.01	0.04	7.2
2	1.8	0.10	5.8	0.10	5.8	0.01	0.76	<0.01	<0.01	0.10	5.8
3	4.3	0.25	5.8	0.26	6.0	0.16	3.7	<0.01	<0.01	0.30	7.0

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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	9.0	0.01	10	<0.01	3.5	<0.01	0.02	0.01	11
2	0.56	0.03	5.1	0.03	6.0	0.01	1.6	0.01	1.9	0.04	6.4
3	1.8	0.09	5.0	0.09	5.0	0.03	1.6	<0.01	<0.01	0.09	5.3
4	4.3	0.24	5.7	0.25	5.8	0.12	2.8	0.09	1.9	0.29	6.8

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	0.99	0.05	5.1	0.06	5.8	0.02	2.3	<0.01	<0.01	0.06	6.1
2	3.6	0.16	4.4	0.20	5.7	0.13	3.6	<0.01	<0.01	0.24	6.6
3	11	0.52	5.0	0.66	6.4	0.07	0.62	<0.01	<0.01	0.67	6.3
4	24	1.2	5.2	1.5	6.3	0.35	1.5	<0.01	<0.01	1.5	6.4

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	0.49	0.03	5.5	0.03	5.9	0.01	2.0	<0.01	0.01	0.03	6.2
2	1.2	0.06	4.5	0.08	6.1	0.03	2.7	0.01	1.1	0.09	6.9
3	2.9	0.15	4.9	0.15	5.1	0.03	1.0	<0.01	<0.01	0.16	5.4
4	6.2	0.28	4.4	0.34	5.3	0.03	0.58	<0.01	<0.01	0.34	5.5

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	17	0.05	19	0.01	2.1	<0.01	0.02	0.05	13
2	3.5	0.20	6.3	0.25	7.9	0.09	2.4	<0.01	0.01	0.27	7.7
3	13	0.62	5.4	1.0	8.6	0.22	1.6	<0.01	<0.01	1.0	8.1
4	35	1.3	4.0	2.6	7.8	0.77	2.2	0.01	0.04	2.7	7.8
5	85	5.4	5.6	8.4	8.7	1.7	3.1	<0.01	<0.01	8.6	10

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	0.13	0.02	19	0.02	20	<0.01	2.1	<0.01	0.02	0.02	17
2	1.4	0.07	5.1	0.10	6.8	0.02	1.3	<0.01	<0.01	0.10	7.1
3	5.7	0.20	3.5	0.22	3.8	0.07	1.2	<0.01	<0.01	0.23	4.0
4	15	0.50	3.2	0.58	3.7	0.23	1.6	0.04	0.28	0.62	4.1
5	30	0.81	2.6	1.2	3.8	0.43	1.5	0.35	1.2	1.3	4.3

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	0.26	0.04	16	0.06	23	0.01	4.5	<0.01	0.01	0.06	23
2	0.78	0.08	10	0.10	12	0.01	1.1	<0.01	<0.01	0.10	12
3	1.5	0.11	7.1	0.11	7.7	0.01	0.47	<0.01	<0.01	0.11	7.7
4	3.0	0.18	5.8	0.19	6.4	0.01	0.55	0.10	3.0	0.22	7.3
5	5.5	0.29	5.0	0.37	6.5	0.10	2.1	0.11	1.9	0.40	7.3

The reproducibility of the NeoBase 2 Non-derivatized MSMS assay was determined on the QSight across 2 external sites and one internal site. Dried blood spots were prepared from adult human whole blood adjusted to a hematocrit of between 50-55% and spiked with analyte stock solutions . The reproducibility is based on 75 determinations: in each laboratory 5 plates measured over 5 working days using one kit lot and each plate having 5 replicates per sample. The results of reproducibility, between- and within-laboratory precisions are presented in the table below.

Ala

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	313	17	5.4	11	3.5	20	6.5
2	426	25	5.9	4.4	1.0	25	6.0
3	755	38	5.0	10	1.4	39	5.2

Arg

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	8.6	0.52	6.0	0.10	1.2	0.53	6.2
2	45	2.3	5.0	1.7	3.8	2.8	6.3
3	154	6.5	4.2	4.7	3.0	8.0	5.2

Asa[1]

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.73	0.17	24	0.11	15	0.21	28
2	9.9	0.79	8.0	1.3	14	1.6	16
3	39	2.3	5.9	4.6	12	5.1	13

Cit

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	27	3.1	12	0.29	1.1	3.1	12
2	141	7.4	5.3	6.0	4.2	9.5	6.8
3	463	25	5.4	8.7	1.9	27	5.8

Gln/Lys

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	520	29	5.6	12	2.4	31	6.0
2	741	40	5.4	40	5.4	57	7.6
3	1402	82	5.9	54	3.8	98	7.0

Gly

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	316	20	6.4	6.0	1.9	21	6.7
2	515	35	6.7	10	2.0	36	7.0
3	1112	70	6.3	14	1.3	71	6.4

Leu/Ile/Pro-OH

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	189	9.0	4.8	2.2	1.2	9.2	4.9
2	325	16	5.1	13	4.1	21	6.5
3	725	38	5.2	12	1.7	40	5.5

Met

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	16	1.1	6.7	0.73	4.5	1.3	8.1
2	108	5.9	5.5	4.2	3.9	7.2	6.7
3	372	20	5.4	7.9	2.1	22	5.8

Orn

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	112	4.4	4.0	5.0	4.5	6.7	6.0
2	179	9.5	5.3	5.3	2.9	11	6.1
3	374	20	5.2	4.3	1.2	20	5.3

Phe

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	60	2.7	4.6	0.52	0.86	2.8	4.6
2	215	12	5.4	6.4	3.0	13	6.1
3	663	31	4.7	4.2	0.63	32	4.8

Pro

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	138	6.0	4.3	2.8	2.0	6.6	4.8
2	242	13	5.4	3.8	1.6	14	5.6
3	543	29	5.4	3.2	0.59	30	5.4

Tyr

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	60	2.4	4.1	0.04	0.06	2.4	4.1
2	197	9.4	4.8	6.2	3.2	11	5.7
3	601	29	4.8	7.3	1.2	30	4.9

Val

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	207	9.5	4.6	2.1	1.0	9.7	4.7
2	315	18	5.8	14	4.6	23	7.3
3	637	36	5.7	15	2.4	39	6.2

C0

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	25	1.3	5.0	0.35	1.4	1.3	5.1
2	73	4.1	5.7	1.7	2.3	4.5	6.1
3	210	11	5.3	1.4	0.66	11	5.3

C2

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	12	0.53	4.5	0.14	1.2	0.55	4.7
2	28	1.4	4.9	1.1	3.8	1.8	6.2
3	75	3.8	5.1	0.92	1.2	3.9	5.2

C3

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	1.1	0.07	6.2	0.03	2.3	0.08	6.6
2	9.7	0.52	5.3	0.30	3.1	0.60	6.2
3	34	1.8	5.2	0.35	1.0	1.8	5.3

C4

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.15	0.01	7.1	<0.01	2.1	0.01	7.4
2	1.4	0.08	5.5	0.06	4.6	0.10	7.2
3	4.9	0.27	5.5	0.09	1.9	0.29	5.9

C5

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.084	0.01	8.7	<0.01	2.4	0.01	9.0
2	2.2	0.13	6.1	0.07	3.2	0.15	6.9
3	8.2	0.42	5.2	0.11	1.3	0.44	5.3

C5DC/C6OH

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.057	0.01	14	0.01	16	0.01	21
2	0.78	0.05	6.5	0.06	7.2	0.08	9.7
3	2.9	0.17	6.1	0.13	4.4	0.22	7.5

C6

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.037	<0.01	12	<0.01	6.2	0.01	13
2	1.1	0.06	5.5	0.04	3.5	0.07	6.5
3	4.0	0.22	5.6	0.07	1.7	0.23	5.8

C8

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.078	0.01	12	<0.01	2.2	0.01	12
2	4.9	0.26	5.3	0.17	3.5	0.31	6.4
3	19	1.0	5.4	0.17	0.93	1.0	5.5

C10

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.11	0.01	7.9	<0.01	2.0	0.01	8.2
2	0.97	0.06	6.3	0.05	5.1	0.08	8.1
3	3.5	0.23	6.4	0.08	2.4	0.24	6.9

C12

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.045	0.01	11	<0.01	2.3	0.01	11
2	1.1	0.07	6.2	0.04	3.6	0.08	7.2
3	4.3	0.26	6.1	0.06	1.3	0.27	6.3

C14

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.10	0.01	6.5	<0.01	1.9	0.01	6.7
2	1.1	0.07	5.7	0.04	3.4	0.08	6.7
3	4.1	0.22	5.4	0.04	0.88	0.22	5.5

C16

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	1.0	0.05	4.8	0.03	2.9	0.06	5.6
2	7.6	0.43	5.6	0.30	3.9	0.52	6.8
3	26	1.4	5.3	0.38	1.5	1.4	5.5

C18

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.70	0.04	5.1	0.02	2.3	0.04	5.6
2	2.1	0.10	5.0	0.08	3.7	0.13	6.3
3	6.0	0.27	4.5	0.08	1.4	0.28	4.7

SA

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.23	0.04	19	0.03	12	0.05	22
2	15	1.3	8.5	0.43	2.9	1.3	9.0
3	59	4.5	7.7	1.5	2.5	4.8	8.1

ADO

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.11	0.01	12	0.01	9.5	0.02	16
2	3.8	0.23	5.9	0.21	5.5	0.31	8.1
3	17	0.90	5.4	0.70	4.2	1.1	6.8

C26:0-LPC

Sample	Total mean µmol/L	Within-lab		Between-lab		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%
1	0.27	0.07	24	0.13	48	0.15	54
2	0.69	0.08	11	0.16	23	0.18	25
3	2.0	0.16	7.9	0.21	11	0.26	14

2. Linearity:

Dried blood spot samples were prepared from pooled human whole blood from apparently healthy adults. To achieve concentrations of analytes near the lower end of the measuring range, samples were also prepared by diluting human red blood cell concentrate with either

stripped human serum or 0.9% sodium chloride. Hematocrit was adjusted to between 50-55% before spiking.

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 summary of these studies is presented in the tables below.

Upper and lower limits of the linear range.

	QSight	
	Linear range lower limit (µmol/L)	**Linear range upper limit (µmol/L)**
Ala	163	1450
Arg	1.84	359
Asa [1]	0.22	67.2
Cit	9.18	1040
Gln\Lys	42.2	2450
Gly	268	2070
Leu\Ile\Pro-OH	57.8	1430
Met	1.66	802
Orn	25.6	807
Phe	20.9	1500
Pro	37.3	1240
Tyr	19.7	1270
Val	51.0	1130
C0	7.80	407
C2	3.53	147
C3	0.41	64.2
C4	0.05	11.3
C5	0.04	17.8
C5DC\C6OH	0.04	7.30
C6	0.03	7.99
C8	0.10	41.2
C10	0.04	7.24
C12	0.10	10.3
C14	0.05	9.45
C16	0.84	46.7
C18	0.48	12.8
SA	0.24	88.2
ADO	0.17	41.3
C26:0-LPC	0.22	7.08

1 Asa is measured as a total concentration of Asa and its anhydrides.

R^2 and Slope values.

Analyte	R^2 value	Slope value
Ala	0.987	0.664
Arg	0.994	0.794
Asa	0.994	0.202
Cit	0.995	0.779
Gln	0.991	0.835
Gly	0.993	0.826
Leu\Ile\Pro-OH	0.991	0.739
Met	0.996	0.698
Orn	0.994	0.657
Phe	0.994	0.763
Pro	0.993	0.683
Tyr	0.995	0.751
Val	0.991	0.759
C0	0.994	0.856
C2	0.993	0.837
C3	0.994	1.017
C4	0.996	0.814
C5	0.995	0.714
C5DC\C6OH	0.994	0.688
C6	0.995	0.802
C8	0.993	0.822
C10	0.991	0.724
C12	0.994	0.848
C14	0.994	0.858
C16	0.992	0.823
C18	0.996	0.918
C26	0.999	1.387
SA	0.996	0.375
ADO	0.999	1.122
C26:0-LPC	0.997	1.139

3. Analytical Specificity/Interference:

Dried blood spot samples representing two different analyte concentrations were tested in an interference study. Samples were prepared from human whole blood adjusted to a hematocrit of 50-55%. Two sample pools with different levels of analytes were tested: unspiked whole blood was used for the endogenous analyte level pools and high analyte level pools were prepared from whole blood spiked with analyte stock solutions. Potential interferants were added to sample pools to prepare test samples. Control samples were prepared by adding an equal volume of solvent that was used to dissolve the potential interferants. Sample pools were spotted on filter paper and allowed to dry. The substances tested for interference included endogenous substances and possible sample contaminants. The effect of 24 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. Results

confirmed that 12 of the 24 substances caused a significant change in the test result. These 12 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
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
Lidocaine	51.2 µmol/L

For the substances 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 31.3 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, Glu and Leu concentrations. Creatine concentrations above 450 µmol/L with Ala, above 1500 µmol/L with Leu or above 900 µmol/L with Glu may cause a false positive screening result. The normal expected Creatine level in newborns aged 0–1 months (107–640 µmol/L) in whole blood.

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 0.72 µmol/L with Qsight 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 approximately 20% of the given oral dose excreted in to urine, i.e. the D617 level is very unlikely to exceed the concentration of 0.72 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 decreasing the measured Arg concentrations and increasing the measured Gln and Glu concentrations. L-Lysine concentrations above 1000 µmol/L caused a decrease in the measured Arg by 19%. Samples with Arg near the cutoff and suspected hyperlysinemia should be tested using a method that shows no interference by L-Lysine. 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 for Gln. The proposed cut-off area measured with NeoBase 2 assay for Gln is generally high (e.g. 99th percentile, 1200 µmol/L). For Glu, L-Lysine concentrations from 1500 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 is 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 750 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 93.8 µmol/L may cause a false positive screening result on Pro.

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 31.3 µmol/L with 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 months (reference plasma level is 10–60 µmol/L). L-Methionine sulfone dose of 62.5 µmol/L increased the endogenous Tyr concentration (56 µmol/L) to 69 µmol/L. Since the proposed cut-off area measured with

NeoBase 2 assay for Tyr is higher (e.g. 99th percentile, 192 μmol/L), L-Methionine sulfone 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. Therefore it is unlikely that albumin interferes with ADO in routine testing.

Intralipid (Triglyceride): High intralipid concentrations were found to interfere with the assay. When more than 0.25 g/dL of intralipid with was added to blood containing 0.07 g/dL of endogenous triglycerides (i.e. tested total triglycerides above 0.32 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. Therefore it is unlikely that triglycerides interfere with C24:0-LPC in routine testing.

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 with Qsight. 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 SA by 20% with Qsight. The decrease in the measured SA by high hemoglobin occurred as SA concentrations of 8.7 μmol/L. No interference by high hemoglobin (up to 22.9 g/dL) was observed at an SA concentration of 0.32 umol/L. Total hemoglobin above 21.7 g/dL caused an elevation in the measured Val by 16%. Total hemoglobin above 20.4 g/dL caused a decrease in the measured C24:0-LPC by 18–20%. Total hemoglobin above 19.2 g/dL caused an elevation in the measured ADO by 21–36%.

The effect of hematocrit on Arg was evaluated. Hematocrit values below 43% increased Arg results by 36%, and hematocrit values above 63% decreased Arg results by 28% and 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 XALD. C24:0-LPC should always be used together with the primary marker C26:0-LPC.

In addition to above findings, following potential interferences have been reported:

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 13908222-2 (en) 59 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 and contaminants from other consumables: 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. Diethylethanolamine (DEAE), which is used e.g. in cleaning agents, is known to interfere with amino acid Val and can cause falsely elevated results as observed during the external study. Similarly, dimethylethanolamine and ethylaminoethanol are known to interfere with amino acid Ala and can cause falsely elevated results as observed during the external study.

Carry Over:
The carry-over was measured using two QSights. Whole blood sample replicates close to endogenous levels were measured after enriched high concentration whole blood samples, and potential carry-over was calculated thereafter. No functionally significant carry-over effect was observed with any of the analytes.

Drift:
The possible drift was determined by analyzing 12 identical plates with one QSight using enriched whole blood samples at different concentration levels from endogenous to the upper part of the linear range. No significant drift was detected within the maximum continuous run time of 39 hours with the QSight when the autosampler is fully loaded.

4. Assay Reportable Range:

See Section 2. Linearity above.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The traceability of the NeoBase 2 Non-derivatized MSMS kit was reviewed in K173568 and found to be acceptable.

6. Detection Limit:

To prepare samples, internal standards were serially diluted to 15 levels, which were spiked into undiluted whole blood. Whole blood containing the serially diluted internal standards were pipetted onto filter paper, dried, and dried blood spot extraction was performed according to the NeoBase 2 kit insert. Extracted samples were pipetted onto plates in 6 replicates of each, from the lowest dilution level to the highest dilution level.

Analytes met the imprecision criterion of CV less than or equal to 20% at the concentrations listed below. A separate acceptance criterion was set for Asa. At the concentration listed in the table below, Asa met the criterion of imprecision within 1.02 SD at Asa concentrations <3 µmol/L.

Analyte	Limit of Quantitation (µmol/L)
Ala	3.66
Arg	0.64
Asa*	0.16
Cit	2.63
Gln\Lys	6.31
Gly	8.61
Leu\Ile\Pro-OH	0.40

Analyte	Limit of Quantitation (µmol/L)
Met	1.56
Orn	1.83
Phe	0.29
Pro	0.34
Tyr	1.84
Val	0.84
C0	0.18
C2	0.04
C3	0.02
C4	0.01
C5	0.01
C5DC\C6OH	0.04
C6	0.03
C8	0.10
C10	0.04
C12	0.10
C14	0.01
C16	0.02
C18	0.01
SA	0.24
ADO	0.07
C26:0-LPC	0.14

*Asa is measured as a total concentration of Asa and its anhydrides.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   See clinical performance data in Section VII C 3 below.

2. Matrix Comparison:

   Not applicable. This device is for use with newborn dried blood spots only.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

A screening performance study was conducted at one routine newborn screening laboratory in the United States. The study samples were punches taken from leftover DBS specimens submitted to the site for routine 1st tier newborn screening and archived, confirmed positive specimens obtained from the California Biobank Program. Study samples were tested in parallel on the QSight 210 MD MSMS platform and the TQD MSMS platform using the same NeoBase2 Non-derivatized MSMS kit lot and cut-off values established in a separate cutoff study. 2580 presumptive negative samples and 52 confirmed positive samples were tested on both the QSight and TQD MSMS platforms.

Disorders, conditions and sample numbers represented in confirmed positive specimen reporting along with the number of confirmed positive samples detected with the QSight and TQD Systems are shown in the table below. The screening performance of the NeoBase 2 Non-derivatized MSMS kit on QSight and TQD was compared in one routine screening laboratory. Using data from routine newborn screening, the cut-offs for both screening systems were determined by calculating the 99.5th and 99th percentile for all analytes except C2. For C2, C3, C16, C18, C18:1 and Cit the low cut-off applied is based on 1st percentile. For C0, the low cut-off applied is based on 10th percentile.

Amino acid disorders (AA): N=19				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase 2 Study Cutoffs (Qsight)**	**Detected with NeoBase 2 Study Cutoffs (TQD)**
Arginemia	ARG	2	2	2
Argininosuccinic acidemia	ASA	2	2	2
Benign hyperphenylalaninemia	H-PHE	2	2	2
Citrullinemia type I	CIT I	2	2	2
Classic phenylketonuria	PKU	2	2	2
Hypermethioninemia	MET	2	2	2
Maple syrup urine disease	MSUD	2	2	2
Ornithine transcarbamylase deficiency	OTCD	2	2	2
Tyrosinemia, type I	TYR I	3	3	3
Fatty acid oxidation disorders (FAO): N=12				
Carnitine palmitoyltransferase	CPT II	2	1*	1*
Carnitine uptake defect	CUD	2	2	2

Amino acid disorders (AA): N=19				
Disorder	**Abbreviation**	**Number of positive specimens**	**Detected with NeoBase 2 Study Cutoffs (Qsight)**	**Detected with NeoBase 2 Study Cutoffs (TQD)**
Long-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency	LCHAD	2	2	2
Medium-chain acyl-CoA dehydrogenase deficiency	MCAD	2	2	2
Short-chain acyl-CoA dehydrogenase deficiency	SCAD	2	1*	1*
Very long-chain acyl-CoA dehydrogenase deficiency	VLCAD	2	2	2
Organic acid conditions (OA): N=16				
Glutaric acidemia type I	GA I	2	2	2
Holocarboxylase synthetase deficiency	MCD	2	2	2
Isobutyrylglycinuria	IBG	2	2	2
Isovaleric acidemia	IVA	3	3	3
Methylmalonic acidemia	MMA	2	2	2
Malonic acidemia	MAL	2	2	2
Propionic acidemia	PROP	3	3	3
Other disorders: N=5				
Adenosine Deaminase Severe Combined Immunodeficiency	ADA-SCID	2	2	2
X-linked Adrenoleukodystrophy	X-ALD	3	2**	3

*Confirmed positive samples for CPT II and SCAD included in this study failed to screen positive for their respective conditions on either the TQD or QSight platforms using the primary markers. These samples were also not screened positive during the original screening by the state laboratory newborn screening program.

**One confirmed positive sample for X-ALD screened positive by the 99th percentile of the primary marker on the TQD but failed to screen positive on the QSight at either the 99th percentile or the 99.5th percentile cutoffs. The patient was followed-up and showed no symptoms of X-ALD at 3.5 years of age. The patient's ABCD1 gene had a variant of unknown significance and there is also no family history of X-ALD.

The overall percent agreement at the respective cut-offs between the NeoBase2 Kits run on the QSight and TQD Systems are presented below.

Overall agreement for NeoBase2 results between QSight and TQD

Analyte	Overall Agreement 99.5th Percentile	Overall Agreement 99th Percentile	Analyte	Overall Agreement 1st Percentile	Analyte	Overall Agreement 10th Percentile
Ala	99.5 %	99.2 %	Cit	98.3 %	C0	92.4 %
Arg	99.8 %	99.8 %	C2	99.0 %		
Asa	99.8 %	99.8 %	C3	99.7 %		
Cit	99.7 %	99.4 %	C16	99.7 %		
Gln\Lys	99.7 %	99.1 %	C18	99.2 %		
Glu	99.7 %	99.1 %	C18:1	99.7 %		
Gly	99.9 %	99.4 %				
Leu\Ile\Pro-OH	99.9 %	99.6 %				
Met	99.9 %	99.8 %				
Orn	99.7 %	99.3 %				
Phe	99.8 %	99.5 %				
Pro	99.4 %	98.8 %				
Tyr	99.7 %	99.6 %				
Val	99.9 %	99.6 %				
SA	99.2 %	99.1 %				
C0	99.5 %	99.3 %				
C3	99.6 %	99.7 %				
C3DC\C4OH	99.4 %	99.5 %				
C4	99.7 %	99.7 %				
C4DC\C5OH	99.8 %	99.8 %				
C5	99.9 %	99.8 %				
C5:1	100 %	100 %				
C5DC\C6OH	99.5 %	99.3 %				
C6	99.8 %	99.4 %				
C6DC	99.4 %	99.4 %				
C8	99.7 %	99.8 %				
C8:1	99.8 %	99.4 %				
C10	99.9 %	99.6 %				
C10:1	99.8 %	99.2 %				
C10:2	99.9 %	99.6 %				

Analyte	Overall Agreement 99.5th Percentile	Overall Agreement 99th Percentile	Analyte	Overall Agreement 1st Percentile	Analyte	Overall Agreement 10th Percentile
C12	99.7 %	99.4 %				
C12:1	99.7 %	99.7 %				
C14	99.5 %	99.5 %				
C14:1	99.9 %	99.7 %				
C14:2	99.7 %	99.4 %				
C14OH	99.7 %	99.7 %				
C16	99.4 %	99.3 %				
C16:1	99.0 %	98.4 %				
C16OH	99.3 %	99.3 %				
C16:1OH\C17	99.8 %	99.4 %				
C18	99.6 %	99.4 %				
C18:1	99.6 %	99.2 %				
C18:2	99.7 %	99.2 %				
C18OH	98.9 %	98.9 %				
C18:1OH	99.7 %	99.7 %				
C18:2OH	99.9 %	99.8 %				
C24:0-LPC	97.0 %	95.5 %				
C26:0-LPC	99.8 %	99.5 %				

D 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.

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.

E Expected Values/Reference Range:

Typical expected NeoBase 2 Non-derivatized MSMS kit results for neonatal populations are presented below for two separate studies performed on the QSight 210 MD Screening System: the cutoff determination study, and the screening performance study. These studies were conducted at the same state screening laboratory. These results were obtained from testing of

leftover routine newborn screening samples at one routine screening laboratory in the United States on the QSight Platform.

Below are results calculated from 2530 presumptive unaffected dried newborn blood sample tested during the cutoff determination study. The population means, medians, 1st percentile and 99th percentile are shown in micromolar (µmol/L) quantities.

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)			
			1st	10th	99th	99.5th
Ala	374	362	226	281	627	672
Arg	8.17	<LOQ	<LOQ	<LOQ	25.8	32.7
Asa	0.39	0.37	0.19	0.26	0.75	0.82
Cit	14.7	14.1	7.85	10.3	26.9	29.7
Gln\Lys	739	724	439	556	1200	1300
Glu	254	245	147	183	443	471
Gly	534	519	314	394	924	997
Leu\Ile\Pro-OH	98	94.9	57.6	71.8	172	207
Met	21	20.2	11.6	15.4	38	44.1
Orn	74.8	71.2	36.4	49.4	152	166
Phe	54.3	53	35.2	42.1	91.1	97.3
Pro	141	137	82.2	103	243	267
Tyr	89.4	83.7	40.5	57	192	206
Val	95.7	92.3	55.7	69.3	174	187
C0	20.5	19.3	8.87	12.4	43.1	48.2
C2	21.8	20.8	9.73	13.6	44.4	48.4
C3	2.2	2.05	0.86	1.27	4.89	5.29
C3DC\C4OH	0.19	0.18	0.07	0.11	0.39	0.41
C4	0.23	0.21	0.1	0.14	0.65	0.73
C4DC\C5OH	0.25	0.24	0.12	0.16	0.51	0.56
C5	0.1	0.09	0.05	0.06	0.24	0.27
C5:1	0.01	0.01	<LOQ	0.01	0.02	0.02
C5DC\C6OH	0.11	0.1	0.05	0.07	0.23	0.26
C6	0.05	0.05	0.03	0.03	0.12	0.13
C6DC	0.07	0.06	<LOQ	0.04	0.13	0.14
C8	<LOQ	<LOQ	<LOQ	<LOQ	0.14	0.17
C8:1	0.1	<LOQ	<LOQ	<LOQ	0.23	0.25
C10	0.1	0.1	0.04	0.06	0.22	0.25

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)			
			1st	10th	99th	99.5th
C10:1	0.04	0.04	<LOQ	<LOQ	0.07	0.08
C10:2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C12	0.12	0.11	<LOQ	<LOQ	0.28	0.3
C12:1	<LOQ	<LOQ	<LOQ	<LOQ	0.25	0.28
C14	0.25	0.24	0.12	0.17	0.46	0.5
C14:1	0.14	0.13	0.05	0.08	0.33	0.37
C14:2	0.03	0.03	0.01	0.02	0.06	0.06
C14OH	0.03	0.03	0.01	0.02	0.06	0.06
C16	3.72	3.62	1.75	2.47	6.69	6.94
C16:1	0.24	0.24	0.1	0.15	0.42	0.46
C16OH	0.04	0.04	0.02	0.03	0.09	0.09
C16:1OH\C17	0.06	0.06	0.03	0.04	0.11	0.12
C18	0.92	0.88	0.44	0.6	1.75	1.91
C18:1	1.22	1.19	0.62	0.84	2.15	2.32
C18:2	0.17	0.16	0.08	0.11	0.38	0.43
C18OH	0.02	0.02	0.01	0.01	0.04	0.04
C18:1OH	0.05	0.05	0.02	0.03	0.13	0.14
C18:2OH	0.02	0.01	0.01	0.01	0.03	0.04
SA	0.25	0.24	<LOQ	<LOQ	0.4	0.44
ADO	0.77	0.74	0.38	0.53	1.4	1.47
D-ADO	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C24:0-LPC	0.5	0.47	0.29	0.36	0.95	1.02
C26:0-LPC	0.35	0.33	0.18	0.24	0.71	0.78

Below are results calculated from 2580 presumptive unaffected dried newborn blood sample tested during the screening performance study. The population means, medians, 1st percentile and 99th percentile are shown in micromolar (µmol/L) quantities.

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)	
			1st	99th
Ala	318	308	184	547
Arg	6.5	5.38	1.54	21.8
Asa	0.34	0.33	0.18	0.6
Cit	13.6	13.1	7.36	24.8
Gln\Lys	698	681	409	1110
Glu	252	244	144	431
Gly	499	486	315	806

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)	
			1st	99th
Leu\Ile\Pro-OH	88.7	86.5	54.8	147
Met	19.7	19.1	11.5	34
Orn	64.8	61.5	33.1	126
Phe	48.4	47.3	32.9	73.4
Pro	126	123	76.7	213
Tyr	83.4	78.7	38.3	178
Val	86	83.3	52.5	145
C0	18.2	17.1	8.04	37.3
C2	20.3	19.1	8.9	41.2
C3	2.07	1.93	0.78	4.7
C3DC\C4OH	0.18	0.17	0.07	0.39
C4	0.22	0.2	0.09	0.6
C4DC\C5OH	0.23	0.22	0.11	0.46
C5	0.09	0.08	0.04	0.19
C5:1	0.01	0.01	0	0.02
C5DC\C6OH	0.11	0.1	0.05	0.22
C6	0.05	0.05	0.02	0.11
C6DC	0.07	0.07	0.03	0.13
C8	<LOQ	<LOQ	<LOQ	0.13
C8:1	<LOQ	<LOQ	<LOQ	0.21
C10	0.09	0.09	0.04	0.21
C10:1	0.04	0.04	<LOQ	0.08
C10:2	<LOQ	<LOQ	<LOQ	<LOQ
C12	0.12	0.11	<LOQ	0.26
C12:1	<LOQ	<LOQ	<LOQ	0.25
C14	0.23	0.22	0.11	0.44
C14:1	0.14	0.13	0.05	0.34
C14:2	0.03	0.03	0.01	0.05
C14OH	0.03	0.02	0.01	0.05
C16	3.53	3.41	1.55	6.42
C16:1	0.25	0.24	0.11	0.47
C16OH	0.04	0.04	0.02	0.08
C16:1OH\C17	0.06	0.05	0.03	0.1
C18	0.85	0.82	0.39	1.59
C18:1	1.26	1.22	0.64	2.2
C18:2	0.18	0.17	0.08	0.39
C18OH	0.02	0.02	0.01	0.04
C18:1OH	0.05	0.04	0.02	0.1
C18:2OH	0.02	0.01	0.01	0.03
SA	0.22	0.22	0.15	0.35

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)	
			1st	99th
ADO	0.74	0.72	0.37	1.26
D-ADO	<LOQ	<LOQ	<LOQ	<LOQ
C24:0-LPC	0.59	0.56	0.36	1.18
C26:0-LPC	0.34	0.33	0.22	0.6

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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