February 7, 2020

PerkinElmer Inc.
Eva Nalian
Sr. Manager Regulatory Affairs
940 Winter Street
Waltham, MA 02451

Re: K193103
Trade/Device Name: NeoBase 2 Non-derivatized MSMS Kit
Regulation Number: 21 CFR 862.1055
Regulation Name: Newborn Screening Test System for Amino Acids, Free Carnitine, and Acylcarnitines Using Tandem Mass Spectrometry
Regulatory Class: Class II
Product Code: NQL
Dated: November 7, 2019
Received: November 8, 2019

Dear Eva Nalian:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kellie B. Kelm, Ph.D.
Acting Director
Division Director of Chemistry and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*

K193103

Device Name

NeoBase™ 2 Non-derivatized MSMS kit

Indications for Use *(Describe)*

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

[1] Analytes in these rows are either isomers or isobars and cannot be distinguished in the tandem mass spectrometry experiment.

Table 1 (continued): Analytes measured by the NeoBase 2 Non-derivatized MSMS kit.

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

[1] Analytes in these rows are either isomers or isobars and cannot be distinguished in the tandem mass spectrometry experiment.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

This summary of safety and effectiveness information is supplied in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned number is K193103

Submitted by:	PerkinElmer, Inc. 940 Winter Street Waltham MA 02451
Contact Person:	Eva Nalian Tel: 647-633-8435
Trade Name:	NeoBase 2 Non-derivatized MSMS kit
Common Name:	Newborn screening test system for amino acids, free carnitine, and acylcarnitines using tandem mass spectrometry
Regulation:	21 CFR 862.1055
Classification:	II
Panel:	75 Chemistry
Product Code:	NQL
Predicate device:	NeoBase 2 Non-derivatized MSMS kit (K173568)

1. 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 - 1 vial
- 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.

Instruments used:

- QSight® 210 MD Screening System is comprised of:
    - QSight® 210 MD Mass Spectrometer
    - QSight® HC Autosampler MD
    - QSight® Binary Pump MD
    - Simplicity™ 3Q MD Software
- PerkinElmer MSMS Workstation software

2. Intended 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

[1] Analytes in these rows are either isomers or isobars and cannot be distinguished in the tandem mass spectrometry experiment.

Table 1 (continued): Analytes measured by the NeoBase 2 Non-derivatized MSMS kit.

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

[1] Analytes in these rows are either isomers or isobars and cannot be distinguished in the tandem mass spectrometry experiment.

3. Substantial Equivalency:

Characteristics	Proposed Device	Predicate (K173568)
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. 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.	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
Disorders Screened	Amino-, organic-, and fatty acid metabolic disorders	Same
Analytes Measured	Amino acids, free carnitine, acylcarnitines, succinylacetone, nucleosides, and lysophospholipids	Same
Instrument / Software Platform	PerkinElmer QSight 210MD Screening System: QSight® HC Autosampler MD,QSight® Binary Pump MD, Simplicity™ 3Q MD Software, 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
Sample Type	Punch from dried blood spot specimen	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
Throughput	Ninety-six tests per microtiter plate. Multiple plates can be analyzed	Same

The proposed device and predicate device utilize similar design shown to produce equivalent screening performance in a clinical setting. Both devices are intended for the measurement and evaluation of multiple metabolite concentrations from newborn heel prick blood samples 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.

4. Summary of the Studies:

Precision:

The precision was determined in accordance with CLSI document EP05-A3. The repeatability and within-laboratory variation for NeoBase 2 Non-derivatized MSMS kit is based on 80 determinations: 40 plates measured over 20 working days, each plate having 2 replicates per sample. One QSight was used. 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 is based on altogether 50 determinations: 10 plates were measured with two QSights over 5 working days, each plate having 5 replicates per sample. The results are presented in the table below.

Repeatability, within-laboratory, between-lot, between-instrument and total variation determined for the NeoBase 2 Non-derivatized MSMS kit using QSight:

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	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

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

Analytical Sensitivity:

The analytical sensitivity (the lowest measurable analyte concentrations) has been demonstrated using two QSights. Results are summarized in the table below.

Analytical sensitivity limits for the NeoBase 2 Non-derivatized MSMS kit using QSight:

Analyte	QSight
	Analytical sensitivity limit (µmol/L)
Ala	3.66
Arg	0.64
Asa [1]	0.16
Cit	2.63
Gln\Lys	6.31
Gly	8.61
Leu\Ile\Pro-OH	0.40
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

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

Linearity:

The linearity was determined in accordance with CLSI document EP06-A using one QSight. The assay is demonstrated to be linear as presented in the table below.

Linear ranges determined for the NeoBase 2 Non-derivatized MSMS kit using QSight:

Analyte	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.

Interference:

The NeoBase 2 Non-derivatized MSMS kit was evaluated for interference in accordance with CLSI document EP07 using QSight. The substances potentially interfering with the assay and additional 9 substances that are abundant in the DBS sample matrix and have potential for unspecific effects on the test results were further tested. The substances potentially interfering with the assay were added into whole blood. The interference samples included NeoBase 2 Control analytes at two concentrations (below and above typical cut-off range). The NeoBase 2 surrogate analytes, which are not included in NeoBase 2 Controls, were studied at endogenous concentration level. The following substances were found not to interfere with the assay on QSight 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
Lidocaine	51.2 µmol/L

In the study, the following interferents were identified:

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 theoretically 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 900 µmol/L with Glu or above 1500 µmol/L with Leu may theoretically cause a false positive screening result. The normal expected Creatine level in newborns aged 0–1 months is 107–640 µmol/L in whole blood. Therefore, Creatine is unlikely to interfere with Glu and Leu in routine testing. The proposed cut-off level measured with NeoBase 2 assay for Ala is approximately 600 µmol/L (e.g. 99th percentile, 627 µmol/L). An additional 2619 µmol/L dose of Creatine would be needed to raise the Ala concentration from endogenous level (351

µmol/L) to the cut-off level (600 µmol/L).

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 theoretically 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 [4]. Therefore, L-Asparagine is unlikely to interfere with Orn in routine testing.

L-Lysine:
L-Lysine was found to interfere with the assay by decreasing the measured Arg concentrations and by increasing the measured Gln and Glu concentrations. L-Lysine concentrations above 1000 µmol/L caused a decrease in the measured Arg by 19%. Newborn screening samples with arginine levels close to the cutoff and with suspected hyperlysinemia should be tested using a method that shows no interference by 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 theoretically 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. 99th percentile, 1200 µmol/L). An additional 1059 µmol/L dose of L-Lysine would be needed to raise the Gln concentration from endogenous level (435 µmol/L) to the cut-off area (1200 µmol/L). For Glu, L-Lysine concentrations from 1500 µmol/L may theoretically 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. When considering the possible false positive rate in neonatal screening, it can be expected that any possible effect of endogenous L-Lysine would be incorporated in the users established cut-offs for Gln and Glu (upper population percentiles), and would not therefore cause false positives in routine screening. 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 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 theoretically 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-Methionine sulfone:
Amino acid derivative L-Methionine sulfone was found to interfere with the assay, thus increasing the measured Tyr concentration. L-Methionine sulfone concentrations above 31.3 µmol/L may theoretically 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, the highest concentration of methionine sulfone should not exceed the normal level of methionine 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. 99.0% percentile, 192 µmol/L), L-Methionine sulfone is unlikely to interfere routine testing. In addition, any possible effect of endogenous L-Methionine sulfone would be incorporated in the users established cut-offs (upper population percentiles), and would not therefore cause false positives in routine

screening.

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 may theoretically 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 in blood 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-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 theoretically 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. 99th percentile, 243 µmol/L). An additional 297 µmol/L dose of Orn would be needed to raise Pro concentration from endogenous level (180 µmol/L) to the cut-off area (240 µmol/L). The proposed normal population for Orn measured with NeoBase 2 assay (e.g. 99th percentile, 152 µmol/L) is on much lower level than the needed additional dose. It is unlikely that Orn interferes with Pro in routine testing. In addition, any possible effect of endogenous L-Ornithine would be incorporated in the users established cut-offs for Pro (upper population percentiles), and would not therefore cause false positives in routine screening.

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 (Triglycerides):
Intralipid was found to interfere with the assay. When more than 0.25 g/dL of intralipid 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 theoretically 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 has been 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% and an elevation in Val by 16%. Hemoglobin interference was observed at an SA concentration of 8.7 µmol/L, but not at 0.32 µmol/L. 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 also evaluated. Hematocrit values below 43% increased Arg results by 36%, and hematocrit values above 63% decreased Arg results by 28%. 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 unaffected 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. Therefore, 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 pivalylcarnitine can cause falsely elevated C5 results. Falsely elevated C5 concentrations have been measured in newborns due to pivalylcarnitine interference. Administration of pivalic acid containing prodrugs can lead to carnitine depletion. In addition, falsely elevated C5 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 marker is acylcarnitine C8. Valproic acid is metabolized to valproylcarnitine, which is an 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 samples 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 neighboring 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 measurements, 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 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 Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and Medium-chain ketoacyl-CoA thiolase

deficiency (MCKAT) disorders. 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 C8 isomer, 2-ethylhexacosanoylcarnitine, can lead to false positive C8 test result. In the other neonate sample, also increased level of acylcarnitine C6DC was detected. Interference was likely because of another plasticizer, di-(2-ethylhexyl) adipate (DEHA) metabolite adipic acid, which was 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.

Reproducibility:

Reproducibility of the NeoBase 2 Non-derivatized MSMS assay was determined on QSight across 2 external sites and one internal site. 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.

Reproducibility determined for the NeoBase 2 Non-derivatized MSMS kit using QSight across 2 external sites and one internal site:

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

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

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

Screening Performance:

The screening performance of the NeoBase 2 Non-derivatized MSMS kit on QSight and TQD platforms was compared in a clinical study at one routine screening laboratory in the United States. Newborn population distributions were determined by measuring the analyte concentrations. Using data from routine newborn screening specimens, the cut-offs of the 3044-001U NeoBase 2 Non-derivatized MSMS kit analytes were determined by calculating analyte concentrations corresponding to the 1st, 10th, 99th and 99.5th percentiles. The percentiles and descriptive values calculated from results obtained from 2530 routine screening specimens are presented in the table below.

Descriptive statistics of the study using QSight (n=2530). The values below the measuring range that are not recommended to be considered as accurate are indicated by <LOQ (lower limit of quantitation):

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	98.0	94.9	57.6	71.8	172	207
Met	21.0	20.2	11.6	15.4	38.0	44.1
Orn	74.8	71.2	36.4	49.4	152	166
Phe	54.3	53.0	35.2	42.1	91.1	97.3
Pro	141	137	82.2	103	243	267
Tyr	89.4	83.7	40.5	57.0	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	2.20	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	0.10	0.14	0.65	0.73
C4DC\C5OH	0.25	0.24	0.12	0.16	0.51	0.56
C5	0.10	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	0.10	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	0.10	<LOQ	<LOQ	<LOQ	0.23	0.25
C10	0.10	0.10	0.04	0.06	0.22	0.25
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	0.30

Analyte	Mean (µmol/L)	Median (µmol/L)	Percentiles (µmol/L)			
			1st	10th	99th	99.5th
C12:1	<LOQ	<LOQ	<LOQ	<LOQ	0.25	0.28
C14	0.25	0.24	0.12	0.17	0.46	0.50
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	0.10	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	0.60	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	0.40	0.44
ADO	0.77	0.74	0.38	0.53	1.40	1.47
D-ADO	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C24:0-LPC	0.50	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

The disorders and amount of confirmed positive specimens included in the study are listed in tables below. In total, there were 19 specimens included in the group of amino acid disorders, 12 in the group of fatty acid oxidation disorders, 16 in the group of organic acid conditions and 5 in the group of other conditions. The results for the screening performance data including confirmed positive specimens tested with QSight and TQD are presented in tables below.

Amino acid disorders (AA)		TQD					
		99th percentile			99.5th percentile		
		Screening positive	Screening negative	Total	Screening positive	Screening negative	Total
QSight	Screening positive	88[1]	12	100	49[2]	9	58
	Screening negative	85	2414	2499	44[3]	2497	2541
	Total	173	2426	2599	93	2506	2599

[1] Includes all 19 specimens confirmed positive for AA detected with both platforms using 99th percentile cut-offs.

[2] Includes 18 specimens confirmed positive for AA detected with both platforms using 99.5th percentile cut-offs.

[3] Includes 1 specimen confirmed positive for OTCD with secondary marker not detected with QSight using 99.5th percentile cut-off, but detected with primary marker Cit with 1st percentile cutoff presented in the next table.

Amino acid disorders (AA)		TQD		
		1st percentile		
		Screening positive	Screening negative	Total
QSight	Screening positive	20[1]	31	51
	Screening negative	12	2519	2531
	Total	32	2550	2582

[1] Includes 2 specimens confirmed positive for OTCD detected with both platforms using 1st percentile cut-offs.

Fatty acid oxidation (FAO)		TQD					
		99th percentile			99.5th percentile		
		Screening positive	Screening negative	Total	Screening positive	Screening negative	Total
QSight	Screening positive	165[1]	52	217	90[1]	33	123
	Screening negative	69	2304[2]	2373	70	2397[2]	2467
	Total	234	2356	2590	160	2430	2590

[1] Includes 9 specimens confirmed positive for FAO detected with both platforms using upper percentile cut-offs.
[2] Includes 1 specimen confirmed positive for CPT-II not detected with either platform using upper percentile cut-offs.Specimen detected with secondary marker in table below.

Fatty acid oxidation (FAO)		TQD		
		Low percentile[1]		
		Screening positive	Screening negative	Total
QSight	Screening positive	283[1]	193	476
	Screening negative	5	2103	2108
	Total	288	2296	2584

[1] "Low Percentile" reflects the positive C0 specimens detected with a 10% cut-off with other carnitine-positive samples at the 1% cut-off
[2] Includes all 4 specimens confirmed positive for FAO detected with both platforms using low percentile cut-offs.

Organic acid condition (OA)		TQD					
		99th percentile			99.5th percentile		
		Screening positive	Screening negative	Total	Screening positive	Screening negative	Total
QSight	Screening positive	86[1]	11	97	51[1]	8	59
	Screening negative	42	2457	2499	33	2504	2537
	Total	128	2468	2596	84	2512	2596

[1] Includes all 16 specimens confirmed positive for OA detected with both platforms using upper percentile cut-offs.

ADA-SCID		TQD					
		99th percentile			99.5th percentile		
		Screening positive	Screening negative	Total	Screening positive	Screening negative	Total
QSight	Screening positive	8[1]	6	14	7[1]	0	7
	Screening negative	40	2528	2568	25	2550	2575
	Total	48	2534	2582	32	2550	2582

[1] Includes 2 specimens confirmed positive for ADA-SCID detected with both platforms using upper percentile cut-offs.

X-ALD		TQD					
		99th percentile			99.5th percentile		
		Screening positive	Screening negative	Total	Screening positive	Screening negative	Total
QSight	Screening positive	7[1]	96	103	3[1]	67	70
	Screening negative	26[2]	2454	2480	15	2498[3]	2513
	Total	33	2550	2583	18	2565	2583

[1] Includes 2 specimens confirmed positive for X-ALD detected with both platforms using upper percentile cut-offs.
[2] Includes one borderline sample with variant of unknown significance (VOUS), not detected with QSight using 99th percentile cut-off and detected with TQD. Primary marker C26-LPC result was 0.45 µmol/l with QSight and 0.42 µmol/l with TQD. Secondary marker C24-LPC result was 0.61 µmol/l with QSight and 0.41 µmol/l with TQD.
[3] Includes one borderline sample with variant of unknown significance (VOUS), not detected with either method using 99.5th percentile cut-off.

Conclusion:

The NeoBase 2 Non-derivatized MSMS kit demonstrates analytical and screening performance that supports its substantial equivalency with the predicate device, NeoBase 2 Non-derivatized MSMS kit (K173568).