July 18, 2018

Wallac Oy, a subsidiary of PerkinElmer
Kay Taylor
VP, Regulatory, Quality, Medical & Scientific Affairs
940 Winter Street
Waltham, MA 02451

Re: K173829
Trade/Device Name: NeoLSD MSMS kit
Regulation Number: 21 CFR 862.1488
Regulation Name: Lysosomal storage disorder newborn screening test system
Regulatory Class: Class II
Product Code: PQW, PQT, PQU, PQV, QCL, QCM
Dated: July 9, 2018
Received: July 10, 2018

Dear Kay Taylor:

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. 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); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR

Part 820); 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 http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). 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 (http://www.fda.gov/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 -S

for Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
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)*
K173829

Device Name

NeoLSD MSMS kit

Indications for Use *(Describe)*

The NeoLSD MSMS Kit is intended for the quantitative measurement of the activity of the enzymes acid-β-glucocerebrosidase (ABG), acid-sphingomyelinase (ASM), acid-α-glucosidase (GAA), β-galactocerebrosidase (GALC), α-galactosidase A (GLA) and α-L-iduronidase (IDUA) in dried blood spots (DBS) from newborn babies. The analysis of the enzymatic activity is intended as an aid in screening newborns for the following lysosomal storage disorders (LSD) respectively; Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease, Fabry Disease, and MPS I Disease.

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 510k Summary information is supplied in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510 (k) number is **K173829**

Date: July 18, 2018

Submitted by: PerkinElmer Inc.
940 Winter Street
Waltham, MA 02451

Wallac Oy, a subsidiary of PerkinElmer Inc.
Mustionkatu 6
Turku, Finland 20750

Contact Person: Kay A. Taylor
Tel: 317 418-1735

Trade Name: NeoLSD MSMS Kit

Common Name: NeoLSD MSMS Kit

Regulation: 21 CFR 862.1488

Classification Name: Lysosomal storage disorder newborn screening test system

Classification: 75 Chemistry

Product Code: PQW, PQT, PQU, PQV, QCL, QCM

Predicate device: Baebies SEEKER 4-Plex Assay (IDUA/GAA/GBA/GLA) (DEN150035)

Device Description:

The NeoLSD MSMS test system uses mass spectrometry to quantitatively measure the activity of six lysosomal enzymes simultaneously from a dried blood spot sample. The NeoLSD MSMS test system is comprised of:

1. NeoLSD MSMS kit, including substrates, internal standards, solutions and controls

The NeoLSD MSMS Kit will contain sufficient reagents and consumables to perform 960 assays (10 x 96-well plates) as listed in the following table.

Component	Description
Kit insert	Instructions for use
QC certificate	QC certificate showing the kit lot specific Kit Control results determined by the manufacturer, and the 1 SD limits.
Internal Standards Substrate Mix	1 vial or several vials of stable-isotope standards and designed substrates, The dried substrates and internal standards are a mixture of the 6 synthetic substrates, the corresponding 6 stable-isotope labeled internal standards, and sodium oleate
DBS controls	C1, C2, C3 control levels on DBS cassettes, manufactured from human blood with a hematocrit value of 45–50%.
Assay buffer	1 bottle of 40 mL buffer, ready-for-use succinate buffered (pH 4.7) salt solution
Extraction Solution	Ethyl acetate
Flow Solvent reconstitution solvent	The ready-for-use Flow Solvent contains acetonitrile, water, and formic acid.
Incubation/Sampling plate	20 x 96-well microplate, U-bottomed
Extraction plate	10 x 96-deep well microplate
Aluminum foil microplate covers	20 adhesive aluminum foil microplate covers
Microplate covers	10 x adhesive microplate covers
Plate barcode labels	30 x plate barcodes

2. Waters TQD MSMS instrument comprised of,
   a. Waters 1525 sample pump
   b. Waters 2777c autosampler
   c. Waters MassLynx v4.1 firmware
   d. Power cables, tubing, syringes, connection cables

3. Waters NeoLynx v4.1 software and computer with monitor
4. PerkinElmer MSMS Workstation Software

The NeoLSD MSMS kit evaluates enzyme activities by measuring the product generated when an enzyme reacts with a synthesized substrate to create a specific end product. The activities of the six lysosomal enzymes present in a 3.2 mm punch from a dried blood spot (DBS) are simultaneously measured by the NeoLSD MSMS kit. The punches are incubated with the assay reagent mixture which contains;

- six substrates, one corresponding to each lysosomal enzyme
- six stable-isotope mass-labeled internal standards (IS) each designed to chemically resemble each product generated
- a buffer to maintain the reaction pH, and to carry inhibitors to limit activity from competing enzymes if present and additives to enhance the targeted enzyme reactions.

Comparison Chart:

Comparison of the NeoLSD MSMS Kit with the predicate.

NeoLSD MSMS Kit		
Characteristics	**Proposed Device**	**Predicate (DEN150035)**
Intended Use/Indications for Use	The NeoLSD MSMS Kit is intended for the quantitative measurement of the activity of the enzymes acid-β-glucocerebrosidase (ABG), acid-sphingomyelinase (ASM), acid-α-glucosidase (GAA), β-galactocerebrosidase (GALC), α-galactosidase A (GLA) and α-L-iduronidase (IDUA) in dried blood spots (DBS) from newborn babies. The analysis of the enzymatic activity is intended as an aid in screening newborns for the following lysosomal storage disorders (LSD) respectively; Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease, Fabry Disease, and MPS I Disease.	The SEEKER System, including the SEEKER Instrument and the SEEKER LSD Reagent Kit-IDUA|GAA|GBA|GLA for use on the SEEKER Instrument, is intended for quantitative measurement of the activity of α-L-iduronidase, α-D-glucosidase, β-glucocerebrosidase and α-D-galactosidase A from newborn dried blood spot specimens as an aid in screening newborns for Mucopolysaccharidosis Type I, Pompe, Gaucher and Fabry diseases. Reduced activity of these enzymes may be indicative of these lysosomal storage diseases. The enzymes measured using the SEEKER LSD Reagent Kit-IDUA|GAA|GBA|GLA and their associated lysosomal storage diseases are listed below.. Enzyme (abbreviation) Disease α-L-iduronidase (IDUA) Mucopolysaccharidosis Type I (MPS I) α-D-glucosidase (GAA) Pompe β-glucocerebrosidase (GBA) Gaucher α-D-galactosidase A (GLA) Fabry
Test Methodology	Quantitative mass spectrometric enzymatic activity assay	Quantitative fluorimetric enzymatic activity assay
Instrument / Software Platform	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	Seeker Instrument with Spot Logic Software
Sample Type	Same	Punch from dried blood spot specimen

<table>
<tr><th>Characteristics</th><th>Proposed Device</th><th>Predicate (K071649)</th></tr>
<tr><td>Reportable Range (µmol/L/h)</td><td>IDUA: 0.34 – 17.2
GAA: 0.44 – 24.2
ABG: 0.69 – 20.1 (identified as GBA in predicate)
GLA: 0.97 – 20.9
ASM: 0.90 – 20.5
GALC: 0.63 – 6.3</td><td>IDUA: 2.77 – 50.75 µmol/L/h
GAA: 2.18 – 94.66 µmol/L/h
GBA: 2.14 – 73.24 µmol/L/h
GLA: 4.88 – 153.74 µmol/L/h
ASM: not detected by predicate
GALC: not detected by predicate</td></tr>
<tr><td>Lower Limits of Measure (µmol/L/h)</td><td>IDUA: LoB=0.059, LoD=0.24, LoQ=0.34
GAA: LoB=0.073, LoD=0.39, LoQ=0.44
ABG: LoB=0.165, LoD=0.63, LoQ=0.69
GLA: LoB=0.476, LoD=0.97, LoQ=0.97
ASM: LoB=0.110, LoD=0.27, LoQ=0.90
GALC: LoB=0.106, LoD=0.34, LoQ=0.63</td><td>IDUA (µmol/L/h): LoB (1.78), LoD (2.77), LoQ (2.77)
GAA (µmol/L/h): LoB (0.50), LoD (2.18), LoQ (2.18)
GBA (µmol/L/h): LoB (0.72), LoD (1.07), LoQ (1.85)
GLA (µmol/L/h): LoB (1.96), LoD (3.18), LoQ (4.88)
ASM: not detected by predicate
GALC: not detected by predicate</td></tr>
<tr><td>Calibrators / Standards</td><td>Molecular Weights & Concentrations of Internal Standards:
IDUA: 430.26 / 15.0 µM
GAA: 502.32 / 24.0 µM
ABG: 390.38 / 20.0 µM
GLA: 488.31 / 24.0 µM
ASM: 404.40 / 15.0 µM
GALC: 416.40 / 10.0 µM</td><td>Calibrant A: (0.0375 µM)
Calibrant B: (0.0750 µM)
Calibrant C: (0.1500 µM)
Calibrant D: (0.3000 µM)</td></tr>
<tr><td>Controls</td><td>3 levels of control material, human blood based</td><td>4 levels of control material, human blood based</td></tr>
<tr><td>Expected Values (µmol/L/h)</td><td>
<table>
<tr><th></th><th>N</th><th>0.1%</th><th>0.2%</th><th>0.3%</th><th>2.5%</th><th>97.5%</th></tr>
<tr><td>IDUA</td><td>5041</td><td>2.06</td><td>2.55</td><td>2.62</td><td>3.82</td><td>13.2</td></tr>
<tr><td>GAA</td><td>5041</td><td>2.33</td><td>2.69</td><td>2.92</td><td>4.28</td><td>17.5</td></tr>
<tr><td>ABG</td><td>5041</td><td>2.85</td><td>3.16</td><td>3.33</td><td>4.74</td><td>20.1</td></tr>
<tr><td>GLA</td><td>5041</td><td>3.04</td><td>3.37</td><td>3.62</td><td>4.81</td><td>25.8</td></tr>
<tr><td>ASM</td><td>5041</td><td>2.12</td><td>2.21</td><td>2.37</td><td>3.15</td><td>12.9</td></tr>
<tr><td>GALC</td><td>5041</td><td>0.43</td><td>0.52</td><td>0.56</td><td>0.95</td><td>9.34</td></tr>
</table>
</td><td>Subjects age 1-6 days
<table>
<tr><th></th><th>N</th><th>0.1%</th><th>0.5%</th><th>50%</th><th>99.5%</th><th>99.9%</th></tr>
<tr><td>IDUA</td><td>102,399</td><td>3.68</td><td>5.63</td><td>18.37</td><td>46.23</td><td>>50.75</td></tr>
<tr><td>GAA</td><td>102,392</td><td>7.61</td><td>10.07</td><td>25.98</td><td>64.56</td><td>77.41</td></tr>
<tr><td>GBA</td><td>102,397</td><td>6.69</td><td>8.20</td><td>20.24</td><td>51.03</td><td>64.53</td></tr>
<tr><td>GLA</td><td>102,371</td><td>7.56</td><td>9.44</td><td>26.73</td><td>123.36</td><td>>153.74</td></tr>
</table>
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<td>
Subjects age 7-31 days
<table>
<tr><th></th><th>N</th><th>0.1%</th><th>0.5%</th><th>50%</th><th>99.5%</th><th>99.9%</th></tr>
<tr><td>IDUA</td><td>7,177</td><td>3.15</td><td>4.82</td><td>18.82</td><td>>50.75</td><td>>50.75</td></tr>
<tr><td>GAA</td><td>7,177</td><td>5.04</td><td>7.75</td><td>21.53</td><td>73.01</td><td>>94.66</td></tr>
<tr><td>GBA</td><td>7,177</td><td>5.16</td><td>6.60</td><td>16.56</td><td>54.83</td><td>>73.24</td></tr>
<tr><td>GLA</td><td>7,176</td><td>5.14</td><td>6.39</td><td>19.70</td><td>96.19</td><td>134.27</td></tr>
</table>
Subjects age >14 days
<table>
<tr><th></th><th>N</th><th>0.1%</th><th>0.5%</th><th>50%</th><th>99.5%</th><th>99.9%</th></tr>
<tr><td>IDUA</td><td>7,447</td><td>2.83</td><td>3.93</td><td>19.41</td><td>>50.75</td><td>>50.75</td></tr>
<tr><td>GAA</td><td>7,447</td><td>4.40</td><td>6.43</td><td>17.76</td><td>54.68</td><td>72.21</td></tr>
<tr><td>GBA</td><td>7,447</td><td>4.31</td><td>5.83</td><td>14.61</td><td>45.21</td><td>64.54</td></tr>
<tr><td>GLA</td><td>7,447</td><td>4.88</td><td>5.36</td><td>14.64</td><td>67.34</td><td>113.61</td></tr>
</table>
ASM: not detected by predicate
GALC: not detected by predicate
</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>
<table>
<tr><th></th><th>Sample Activity Range (µmol/L/h)</th><th>%CV Range</th></tr>
<tr><td>IDUA</td><td>0.59 – 9.11</td><td>6.9 – 10.0</td></tr>
<tr><td>GAA</td><td>1.29 – 10.77</td><td>5.6 – 9.4</td></tr>
<tr><td>ABG</td><td>1.03 – 8.08</td><td>13.0 – 21.0</td></tr>
<tr><td>GLA</td><td>1.04 – 12.49</td><td>8.6 - 15.7</td></tr>
<tr><td>ASM</td><td>2.47 – 9.11</td><td>7.6 – 11.4</td></tr>
<tr><td>GALC</td><td>0.37 – 3.97</td><td>9.3 – 20.7</td></tr>
</table>
</td>
<td>
<table>
<tr><th></th><th>Sample Activity Range (µmol/L/h)</th><th>%CV Range</th></tr>
<tr><td>IDUA</td><td>3.53 – 24.06</td><td>14.2 – 28.5</td></tr>
<tr><td>GAA</td><td>4.29 – 27.37</td><td>12.0 – 17.0</td></tr>
<tr><td>ABG</td><td>2.84 – 15.00</td><td>15.7 – 38.0</td></tr>
<tr><td>GLA</td><td>6.94 – 52.66</td><td>9.4 – 16.3</td></tr>
</table>
ASM: not detected by predicate
GALC: not detected by predicate
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</tr>
</table>

Summary of Studies:

Reproducibility:

Reproducibility of the NeoLSD MSMS assay was determined on three different TQD MSMS instrument at three sites, two external and one internal. The reproducibility is based on 75 determinations for each level of a six member panel for each enzyme: in each laboratory 5 plates were measured over 5 working days using one kit lot and each plate having 5 replicates per sample per enzyme. The results of reproducibility, between- and within-laboratory precisions are presented below.

Enzyme	Sample	N	Mean	Within Laboratory		Between Laboratory		Reproducibility	
				SD	CV%	SD	CV%	SD	CV%
ABG*	2	75	1.03	0.14	13.8	0.16	15.8	0.22	21.0
	3	75	2.78	0.37	13.3	0.26	9.3	0.45	16.2
	4	75	7.46	0.89	12.0	0.77	10.3	1.18	15.8
	5	75	7.93	0.92	11.6	0.46	5.9	1.03	13.0
	6	75	8.08	1.03	12.8	0.38	4.7	1.10	13.6
ASM*	2	75	2.47	0.21	8.7	0.12	4.8	0.24	9.9
	3	75	3.93	0.43	11.0	0.11	2.9	0.45	11.4
	4	75	5.54	0.44	7.9	0.37	6.6	0.57	10.3
	5	75	7.29	0.56	7.6	0.47	6.4	0.73	10.0
	6	75	9.11	0.67	7.3	0.17	1.8	0.69	7.6
GALC	1	75	0.37	0.07	19.5	0.03	7.0	0.08	20.7
	2	75	0.89	0.11	12.4	0.06	6.9	0.13	14.2
	3	75	1.30	0.20	15.7	0.04	3.2	0.21	16.0
	4	75	1.76	0.25	14.3	0.04	2.1	0.25	14.4
	5	75	3.76	0.34	9.1	0.22	5.9	0.41	10.8
	6	75	3.97	0.31	7.9	0.20	5.0	0.37	9.3
IDUA*	2	75	0.59	0.03	5.8	0.05	8.1	0.06	10.0
	3	75	2.25	0.15	6.9	0.10	4.4	0.18	8.2
	4	75	5.88	0.35	5.9	0.27	4.6	0.44	7.5
	5	75	8.89	0.43	4.8	0.45	5.0	0.62	6.9
	6	75	9.11	0.43	4.7	0.54	5.9	0.69	7.5
GLA	1	75	1.04	0.10	9.5	0.06	5.6	0.12	11.1
	2	75	1.23	0.16	13.3	0.10	8.4	0.19	15.7
	3	75	3.42	0.17	5.0	0.24	7.0	0.30	8.6
	4	75	5.10	0.32	6.2	0.30	6.0	0.44	8.6
	5	75	10.31	0.52	5.1	0.82	8.0	0.97	9.4

	6	75	12.49	0.90	7.2	0.86	6.9	1.25	10.0
GAA*	2	75	1.29	0.07	5.5	0.10	7.6	0.12	9.4
	3	75	2.32	0.10	4.2	0.14	6.1	0.17	7.4
	4	75	5.36	0.26	4.8	0.24	4.5	0.35	6.6
	5	75	7.57	0.36	4.8	0.53	7.0	0.64	8.5
	6	75	10.77	0.46	4.3	0.38	3.5	0.60	5.6

(*) The results of sample 1 for ABG, ASM, IDUA and GAA were outside the measuring range and therefore not included.

Limit of Blank, Detection and Quantification:

The Limits of Blank, Detection and Quantitation were determined in accordance with CLSI document EP17-A2. The Limit of Blank (LoB) for the NeoLSD MSMS kit is defined as the 95th percentile of a distribution of blank samples determined with 120 determinations for the TQD MSMS instrument. The Limit of Detection (LoD) is based on 150 determinations of low level samples using the Waters TQD MSMS instrument.

Enzyme	LoB (μmol/L/h)	LoD (μmol/L/h)
ABG	0.165	0.63
ASM	0.110	0.27
GALC	0.106	0.34
IDUA	0.059	0.24
GLA	0.476	0.97
GAA	0.073	0.39

The Limit of Quantitation (LoQ) is defined as the lowest activity fulfilling the total CV% requirement of the assay. For ABG, GLA and IDUA the CV% requirement is < 40%, for ASM and GAA < 30% and for GALC < 50%. If the imprecision criterion was met for activities below LoD, the LoQ was set to be equal to the LoD. The table shows the LoQ results and imprecision observed at these activities:

Enzyme	ABG	ASM	GAA	GALC	GLA	IDUA
LoQ (μmol/L/h)	0.69	0.90	0.63	0.34	0.97	0.44
SD at LoQ	0.15	0.18	0.11	0.07	0.17	0.08
CV% at LoQ	21.7%	20.0%	17.5%	20.6%	17.5%	18.2%

Linearity:

The linearity was determined in accordance with CLSI document EP06-A using the TQD MSMS instrument. For the six enzymes, the method has been demonstrated to be linear as shown in the following table.

Enzyme	Linear Range Lower Limit (μmol/L/h)	Linear Range Upper Limit (μmol/L/h)
ABG	0.69	20.1
ASM	0.90	20.5
GALC	0.63	6.3
IDUA	0.34	17.2
GLA	0.97	20.9
GAA	0.44	24.2

Interference:

The NeoLSD MSMS kit was evaluated for interference in accordance with CLSI document EP07-A2. The substances potentially interfering with the MSMS analysis (mass overlaps ±1 Da of the target analytes) were studied at different concentrations in Neo MSMS Flow Solvent using the assay FIA-MSMS method. The substances were found not to interfere in the MSMS analysis at the concentrations indicated in the following table.

Tested substance	Potential Interference MW	Corresponding NeoLSD analyte	Corresponding NeoLSD exact mass	Concentration of tested substance (μmol/L)
Pantoprazole	383.08	ABG P	383.34	1.1, 3.3 and 10
Meropenem	383.15	ABG P	383.34	
Felodipine	383.07	ABG P	383.34	0.09, 1.1, and 3.3
Cetirizine (M+2 peak)	390.15	ABG IS	390.38	1.1, 3.3 and 10
S-(5'-Adenosyl)-L-methionine	398.14	ASM P	397.36	
PTH-(E-phenylthiocarbamyl) - lysine	398.12	ASM P	397.36	
Sulfasalazine/ sulfadiazine	398.07	ASM P	397.36	3.3, 10 and 754
Perphenazine (M+2 peak)	405.15	ASM IS	404.40	1.1, 3.3 and 10
Lisinopril	405.23	ASM IS	404.40	
Miconazole (M+2 peak)	415.98	GALC IS	416.40	
Spironolactone	416.20	GALC IS	416.40	
Calcitriol	416.33	GALC IS	416.40	
Domperidone	425.16	IDUA P	425.23	
Kanamycin	484.24	GLA P	483.27	3.3, 10 and 124

The substances potentially interfering with the assay were added to whole blood with three LSD enzyme activities (deficient, cut-off and normal). The substances indicated in the table below were found not to interfere with the assay at the concentration indicated.

Substances found not to interfere with NeoLSD assay.

Tested substance	Added concentration of tested substance (in blood)
Bilirubin (Unconjugated)	10 mg/dL
Bilirubin (Conjugated)	15 mg/dL
Albumin (HSA; from human serum)	2.7, 3.2, 4.0 g/dL*
Acetaminophen	5.5 mg/dL
Calcifediol	10.5 µg/dL
Chlorhexidine digluconate	0.04 %
Galactose	15 mg/dL

* For albumin, the concentration range includes endogenous and added albumin levels.

In this study, the following potential interferents (and enzymes affected) were identified.

Glucose interference on GAA: Glucose was found to interfere with the assay by decreasing the measured GAA activity. Glucose at level of 0.50 g/dL decreases GAA activity level of 11.3 µM/h by 17%. Glucose at level of 0.75 g/dL decreases GAA activity levels of 1.44 and 2.87 µM/h by 0.40 µM/h and 23%, respectively. Thus, glucose concentration above 0.25 g/dL with GAA may cause a false positive screening result for a specimen with measured GAA activity close to the cut-off value. However, the observed glucose concentration shown to interfere with GAA is clearly beyond the endogenous reference interval for glucose that has been reported to be for neonates (0-1 months) from 0.055 to 0.115 g/dL in whole blood. Note: Preterm infants typically with very-low birth weight have a high risk of hyperglycemia (blood glucose level > 0.18 g/dL) due to glucose infusion.

Hematocrit interference on ABG and GALC: Hematocrit at level of 35% decreases ABG activity levels of 1.10, 2.92 and 8.48 µM/h by 0.49 µM/h, 25% and 18%, respectively. This may cause a false positive screening result for a specimen with measured ABG activity close to the cut-off value. Also, hematocrit at level of 65% increases ABG activity levels of 2.92 and 8.48 µM/h by 29% and 28%, respectively. At hematocrit levels ≥65%, the measured ABG activity levels could be increased as much as by 35%. Therefore, at hematocrit levels of ≥65% the interference could result in misclassification of a patient with an ABG result near the cut-off value as 'normal' when in fact patient should be classified as having presumed ABG deficiency. For samples up to 30% above the ABG cut-off value, with known or suspected high hematocrit levels (≥ 65%); testing by an alternate method that is not subject to hematocrit interference is recommended.

Hematocrit at level of 35% increases GALC activity level of 3.38 µM/h by 22%; while at levels of 65% hematocrit decreases GALC activity levels of 0.75 and 1.02 µM/h by 24% and 20%, respectively. The decrease in GALC activity may cause a false positive screening result for a specimen with measured GALC activity close to the cut-off value. The inhibitory effect of high hematocrit values to GALC activity has been reported.

Hemoglobin interference on ABG, ASM and IDUA:

Hemoglobin was found to interfere with the assay by increasing the measured ABG, ASM and IDUA activity.

ABG: Hemoglobin at level of 16.6 g/dL increases ABG activity level of 2.36 µM/h by 27% when compared to a sample having 15.3 g/dL of hemoglobin. Hemoglobin at level of 18.0 g/dL hemoglobin increases ABG activity level of 0.65 µM/h by 0.34 µM/h when compared to a sample having 15.5 g/dL of hemoglobin. Also, hemoglobin at level of 18.9 g/dL increases ABG activity level of 7.68 µM/h by 30% when compared to a sample having 15.1 g/dL of hemoglobin.

ASM: Hemoglobin at level of 17.8 g/dL hemoglobin increases ASM activity level of 1.57 µM/h by 26% when compared to a sample having 15.3 g/dL of hemoglobin. Hemoglobin at level of 18.0 g/dL increases ASM activity level of 0.41 µM/h by 0.20 µM/h when compared to a sample having 15.5 g/dL of hemoglobin. Also, hemoglobin at level of 20.1 g/dL increases ASM activity level of 4.78 µM/h by 16% when compared to a sample having 15.1 g/dL of hemoglobin.

IDUA: Hemoglobin at level of 18.0 g/dL increases IDUA activity level of 1.18 µM/h by 19% when compared to a sample having 15.5 g/dL of hemoglobin.

Although interference by hemoglobin was observed with concentrations within the newborn reference ranges (12.0 – 22.0 g/dL for hemoglobin), 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.

Triglyceride interference on GAA, GLA and IDUA: Intralipid (Triglyceride) was found to interfere with the assay increasing the measured GAA, GLA and IDUA activity.

GAA: Intralipid at level of 0.23 g/dL increases GAA activity of 2.98 and 7.10 µM/h by 27% and 18%, respectively. Intralipid at level of 0.75 g/dL increases GAA activity level of 1.05 µM/h by 0.52 µM/h.

GLA: Intralipid at level of 0.30 g/dL increases GLA activity level of 7.21 µM/h by 16%; while at level 0.38 g/dL Intralipid increases GLA activity level of 3.63 µM/h by 25%. Intralipid at level of 0.75 g/dL increases GLA activity level of 1.37 µM/h by 0.65 µM/h.

IDUA: Intralipid at level of 0.38 g/dL increases IDUA activity level of 8.18 µM/h by 19% and Intralipid at level of 0.75 g/dL increases IDUA activity levels of 1.17 and 2.67 µM/h by 20%.

Note! High triglyceride concentrations (hypertriglyceridemia) in newborns due to medication effects or pathological conditions may cause a false negative screening result for a specimen with measured GAA, GLA or IDUA activity close to the cut-off value.

EDTA interference on ABG and ASM: EDTA at level 0.10 g/dL increases ABG activity level of 1.80 µM/h by 0.41 µM/h. EDTA at level of 0.04 g/dL decreases ASM activity level of 3.74 µM/h by 20%; while at level of 0.10 g/dL EDTA decreases ASM activity level of 1.18 µM/h by 16%. EDTA at a level of 0.20 g/dL decreases ASM activity level of 0.44 µM/h by 0.17 µM/h.

The concentration of EDTA found to interfere with AGB and ASM were far beyond the typical therapeutic dosage 3.4 µmol/L (0.10 mg/dL) [12]. Also, the direct application of blood from the heel-puncture to the DBS paper is intended for newborn screening eliminating the risk of contamination.

Reference Range:

A prospective clinical study using four-year-old retrospective routine newborn screening samples was conducted with the NeoLSD MSMS test system to screen for the lysosomal enzymes ABG, ASM, GAA, GALC, GLA, and IDUA.

Over the course of three months 5041 newborn samples were tested at a European (EU) newborn screening laboratory to establish cut-off values. Samples in the reference range study were from newborns ranging 0-30 days of age. The initial cut-off values were based conservatively on 0.1 – 0.3 percentile of enzyme activity distribution and converted to a percentage of population median activity. The retest cut-off values were set 5% lower from the initial cut-off percentage. The cut-off percentages were applied to daily medians established based on the initial routine sample results for the day.

The cut-off percentages were not adjusted during the study. The initial and retest cut-off values used in the clinical study are shown below.

Descriptive statistics for the EU site

Enzyme	n	Enzyme activity (μmol/L/h)						Initial cutoff	Retest cutoff
		Range*	Mean	Median	Lower percentiles				
					0.1%	0.2%	0.3%		
ABG	5041	2.07 - 66.3	10.35	9.60	2.85	3.16	3.33	35%	30%
ASM	5041	1.64 - 35.2	6.38	5.86	2.12	2.21	2.37	40%	35%
GALC	5041	0.22 - 54.9	3.63	3.10	0.43	0.52	0.56	20%	15%
IDUA	5041	0.45 - 35.4	7.55	7.25	2.06	2.55	2.62	30%	25%
GLA	5041	1.48 - 94.8	11.06	9.72	3.04	3.37	3.62	40%	35%
GAA	5041	1.55 - 34.8	9.28	8.74	2.33	2.69	2.92	30%	25%

* Some results were outside the measuring range and cannot be considered accurate

Additional newborn population distributions were determined at two different newborn screening laboratories in the United States. Site A analyzed 5251 and Site B analyzed 5053 newborn dried blood spot specimens submitted for routine testing. Descriptive statistics for the samples are shown below. Samples tested in Site A and Site B were collected from newborns of ≤ 4 days and ≤ 7 days, respectively.

Descriptive statistics for the US Site A

Enzyme	n	Enzyme activity (μmol/L/h)					
		Range*	Mean	Median	Lower percentiles		
					0.1%	0.2%	0.3%
ABG	5251	1.39 – 77.9	10.52	9.67	2.00	2.16	2.36
ASM	5251	1.10 – 24.3	4.70	4.44	1.49	1.60	1.64
GALC	5251	0.52 – 85.0	5.49	4.63	0.86	0.96	1.02
IDUA	5251	0.09 – 18.7	6.31	6.08	0.85	1.40	1.56
GLA	5251	1.69 – 121	16.91	14.39	3.44	4.42	4.89
GAA	5251	0.75 - 43.0	10.21	9.62	2.16	2.38	2.56

* Some results were outside the measuring range and cannot be considered accurate

Descriptive statistics for the US Site B

Enzyme	n	Enzyme activity (μmol/L/h)					
		Range*	Mean	Median	Lower percentiles		
					0.1%	0.2%	0.3%
ABG	5053	1.68 – 76.5	11.4	10.4	2.94	3.23	3.33
ASM	5053	1.02 – 28.4	5.44	5.13	1.71	1.79	1.98
GALC	5053	0.53 – 33.0	4.67	4.20	0.68	0.82	0.86
IDUA	5053	0.9 – 21.8	6.65	6.39	1.59	1.98	2.09
GLA	5053	0.96 – 59.5	13.3	12.0	3.99	4.33	4.43
GAA	5053	1.11 – 36.4	11.0	10.4	2.53	2.88	3.37

* Some results were outside the measuring range and cannot be considered accurate

Screening Performance:

The screening performance of the NeoLSD MSMS kit was determined in a prospective clinical study of four-year-old retrospective routine samples at an EU newborn screening laboratory. Over the two-month study duration 4041 newborn samples were tested. Samples tested were from newborns ≤ 29 days old. All 4011 routine samples were 4 years old enabling the follow-up of clinical status at the age 4 years. Due to the low incidence of LSDs, the study population was enriched with 30 archived confirmed LSD positive newborn DBS specimens obtained from the site's biobank, these samples ranged from 5.8 to 17.6 years of age. Sample inclusion/exclusion criteria were the same as in the reference range study.

The 4011 routine samples were tested according to the defined algorithm to determine the enzyme activities for sample interpretation. Site specific cut-off percentages determined prior to the clinical study were applied as percent of daily median activity. The routine specimens having enzyme activities below the cut-off values in the initial testing were re-tested in duplicate. The final results (normal, presumptive positive, invalid result) were classified after the initial testing for samples above the cut-off values and after the final testing for samples below the initial cut-off level.

Confirmed LSD positive newborn specimens were tested once, and assessed using the retest cut-off values for the enzymes. Given the known status of the confirmed LSD positive newborn specimens, processing the samples through the full testing algorithm provided no additional information nor changed the sample interpretation.

Clinical outcome was used as a comparator for all samples, including the 4011 routine screening samples, as derived from the civil registry status and national hospital registry. Subject´s survival at 4 years of age without LSD diagnosis or clinical signs suggestive of an LSD was used as clinical confirmation of an unaffected newborn. Subjects who did not have follow-up information available up to 4 years were classified as lost to follow-up.

Results

A total of 4041 specimens (4011 routine newborn specimens and 30 specimens from confirmed positive LSD cases) were tested in the clinical study. The following is a summary of the samples tested.

Summary of the samples tested in the pivotal study.

Routine	ABG	ASM	GALC	IDUA	GLA	GAA	Total
Screened samples	4011	4011	4011	4011	4011	4011	4011
Below initial cutoff	15	11	12	33	12	10	88
Screen positive	3	0	10	5	5	4	26
Unaffected	3	0	10	5	5	3	25

Lost to follow up	0	0	0	0	0	1	1
Retest rate	0.40%	0.27%	0.30%	0.17%	0.85%	0.30%	2.2%
False positive rate	0.07%	0.00%	0.10%	0.07%	0.27%	0.10%	0.6%

Confirmed Positive	ABG	ASM	GALC	IDUA	GLA	GAA	Total
Screened samples	5	1	10	5	5	4	30
Below initial cutoff	5	1	10	5	3	4	28
Screen positive	3	1	10	5	3	4	26
Invalid	2	0	0	0	0	0	2
Screen negative	0	0	0	0	2	0	2

Two confirmed positive Gaucher specimens were categorized as “invalid result”. In routine newborn screening, if a specimen result is categorized as invalid result, a new dried blood spot specimen should be obtained and retesting performed using age-specific cut-off values.

In total 86 subjects had emigrated before the age of 4 years and therefore their clinical status could not be confirmed. None of the subjects in the lost to follow-up cohort had LSD diagnosis or showed signs and symptoms of any of the screened LSDs at the time of being lost to follow up.

The screening performance results for the 4041 specimens, including the 30 confirmed LSD positive specimens, tested with the NeoLSD MSMS kit are shown below.

Screening performance of NeoLSD MSMS kit

		Outcome, all 6 enzymes			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	26	25	1	52
	Negative	2*	3900	85	3987
	Invalid	2	0	0	2
	Total	30	3925	86	4041

Performance estimates (invalid and lost-to-follow-up excluded)			
Sensitivity	**False-negative rate**	**Specificity**	**False-positive rate**
92.9 % (76.5%-99.1%)	7.1%* (0.9% - 23.5%)	99.4 % (99.1% - 99.6%)	0.6 % (0.4% - 0.9%)

* includes 2 Fabry females

	Outcome, Gaucher (ABG)

Screening Results		Affected	Unaffected	Lost-to-follow-up	Total
NeoLSD MSMS kit	**Positive**	3	3	0	6
	Negative	0	3947	86	4033
	Invalid	2	0	0	2
	Total	5	3950	86	4041

		Outcome, Niemann-Pick A/B (ASM)			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	1	0	0	1
	Negative	0	3952	86	4038
	Invalid	0	2	0	2
	Total	1	3954	86	4041

		Outcome, Krabbe (GALC)			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	10	4	0	14
	Negative	0	3939	86	4025
	Invalid	0	2	0	2
	Total	10	3945	86	4041

		Outcome, MPS I (IDUA)			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	5	3	0	8
	Negative	0	3945	86	4031
	Invalid	0	2	0	2
	Total	5	3950	86	4041

		Outcome, Fabry (GLA)			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	3	11	0	14
	Negative	2	3937	86	4025
	Invalid	0	2	0	2
	Total	5	3950	86	4041

		Outcome, Pompe (GAA)			
Screening Results		**Affected**	**Unaffected**	**Lost-to-follow-up**	**Total**
NeoLSD MSMS kit	**Positive**	4	4	1	9
	Negative	0	3945	85	4030
	Invalid	0	2	0	2
	Total	4	3951	86	4041

With the female Fabry subjects excluded the NeoLSD MSMS test system has no false negative results for any of the enzymes; and a false positive range of ABG (0.07%), ASM (0%), GALC (0.10%), IDUA (0.07 %), GLA (0.27 %), GAA (0.10%). . The retest rate was 6.6%, of which only 2.2% was due to test failure (e.g. quality control exceeded limits). Remaining retests (4.4%) were due to the screening algorithm, which requires results below initial cut-off value for any or the six enzymes to be repeated in duplicate.