Food and Drug Administration
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www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K190266

B Applicant

PerkinElmer Inc.

C Proprietary and Established Names

NeoLSD MSMS Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PQT	Class II	21 CFR 862.1488 - Lysosomal Storage Disorder Newborn Screening Test System	CH - Clinical Chemistry
PQU	Class II	21 CFR 862.1488 - Lysosomal storage disorder newborn screening test system	CH - Clinical Chemistry
PQV	Class II	21 CFR 862.1488 - Lysosomal storage disorder newborn screening test system	CH - Clinical Chemistry
QCL	Class II	21 CFR 862.1488 - Lysosomal storage	CH - Clinical Chemistry

Product Code(s)	Classification	Regulation Section	Panel
		disorder newborn screening test system	
QCM	Class II	21 CFR 862.1488 - Lysosomal storage disorder newborn screening test system	CH - Clinical Chemistry
PQW	Class II	21 CFR 862.1488 - Lysosomal storage disorder newborn screening test system	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Acid-β-glucocerebrosidase (ABG), acid-sphingomyelinase (ASM), acid-α-glucosidase (GAA), β-galactocerebrosidase (GALC), α-galactosidase A (GLA), α-L-iduronidase (IDUA)

C Type of Test:

Quantitative mass spectrometric enzymatic activity assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indictations for use.

B Indication(s) 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 Mucopolyaccharidosis (MPS) Type I Disease.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use only

This test is not intended to diagnose lysosomal storage disorders.
Test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, and clinical evaluation as appropriate.
Known causes for anomalous analytical assay results are:
- Sample not uniformly saturated with blood
- Sample disk punched too close to the edge of the blood spot
- Sample disk punched at the middle of the blood spot
- Poorly collected specimens e.g. excessive milking or squeezing the puncture may cause hemolysis of the specimen or a mixture of tissue fluids with the specimen. Layering successive drops of blood in the specimen may affect the measured results.
- Improperly dried specimens e.g. heating or stacking the specimen collection devices during the drying process
- Humidity and moisture or exposure to direct sunlight are detrimental to the dried blood spot sample
- Non-eluting blood spot due to deterioration of sample
- Contamination of blood spot filter paper e.g. with fecal material
- It has been shown that female patients with the Fabry disorder may show normal GLA enzyme activities at birth but are later symptomatic.
- Hematocrit levels above 65% were found to interfere with the assay by increasing the measured ABG activity that could result in false negatives for Gaucher. Refer to the interference section for complete information about this interference.
- Free (serum) hemoglobin above 15 g/dL was found to interfere with the assay by increasing the measured ABG and ASM that could result in false negative results for Gaucher and Niemann-Pick A/B. Refer to the interference section for complete information about this interference.
- On the QSight galactose levels above 8 mg/dL were found to interfere with the assay by increasing the measured ABG activity, which could result in false negative results for Gaucher. Refer to the interference section for complete information about this interference.

The NeoLSD MSMS kit may result in;
- False negatives by not detecting Fabry disease in females
- False positives by identifying pseudo deficiencies and carriers as affected for MPS I, Gaucher, and Pompe diseases
- False negatives by not detecting certain late onset forms for Pompe disease
- False negatives by not detecting certain late onset forms for Fabry disease
- Increase rate of false positives when the specimen is exposed to high temperature during shipping
- The false negative rate is based on follow-up of subjects up to 4 years of age and on limited data from late onset forms of the disorders since it can take several years to identify a missed late onset case.
- Certain late onset forms for Pompe disorder may have GAA enzymatic activity in the normal range and result in a false negative screening result.

- Heterozygote female Fabry patients may present with normal GLA activity and not be detected by the NeoLSD MSMS kit.

D Special Instrument Requirements:

PerkinElmer QSight 210 MD Mass Spectrometer, QSight HC Autosampler MD Instrument Software, QSight Binary Pump MD, PerkinElmer MSMS Workstation Data Processing Software

IV Device/System Characteristics:

A Device Description:

The NeoLSD MSMS kit consists of substrates, internal standards, and controls. The kit contains sufficient reagents and consumables to perform 960 assays (10 x 96-well plates). The contents of the kit are listed below:

Component	Contents
Internal Standards Substrate Mix	1 vial or several vials of stable-isotope standards and designated 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 Solution	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	10 x adhesive microplate covers
Plate barcode labels	30 x plate barcodes

B Principle of Operation:

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 are simultaneously measured by the NeoLSD MSMS assay. 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

The amount of each product generated is directly proportional to the enzyme activity in the dried blood spot punch. The internal standards are deuterium labeled versions of the corresponding enzymatic products.

The six enzymatic products and six internal standards are listed in the following table:

Enzymatic product	Molecular weight	Internal Standard (IS)	Molecular weight
ABG P	383.34	ABG IS	390.38
ASM P	397.36	ASM IS	404.40
GALC P	411.37	GALC IS	416.40
IDUA P	425.23	IDUA IS	430.26
GLA P	483.27	GLA IS	488.31
GAA P	497.29	GAA IS	502.32

The blood spot extraction and enzymatic reaction takes place in an aqueous incubation cocktail. The substrates are designed to be hydrophilic and easily soluble in this cocktail. After incubation, the cocktail is vigorously mixed with the NeoLSD Extraction Solution and water, and then allowed to separate into an aqueous layer and an organic layer. The measured cleaved product portion of the substrate is designed to be non-polar so that it will preferentially be dissolved in the organic layer, and the MSMS measurement is performed on an aliquot of this organic phase. A fraction of the uncleaved substrates remains in the aqueous layer and is not introduced to the mass spectrometer. Data is analyzed with the PerkinElmer MSMS Workstation Software.

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☒	☐
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:

PerkinElmer QSight 210 MD Mass Spectrometer
QSight HC Autosampler MD Instrument Software
QSight Binary Pump MD
PerkinElmer MSMS Workstation Data Processing Software

2. Specimen Identification:

Specimens are given unique identifiers.

3. Specimen Sampling and Handling:

Specimens are dried blood spots and should be handled according to the stability claims in the labeling for the device.

4. Calibration:

Instrument is calibrated automatically using mass standards.

5. Quality Control:

Quality control materials are provided with the NeoLSD kit.

V Substantial Equivalence Information:

A Predicate Device Name(s):
NeoLSD MSMS kit

B Predicate 510(k) Number(s):
K173829

C Comparison with Predicate(s):

Device & Predicate Device(s):	K190266	K173829
Device Trade Name	NeoLSD MSMS kit	Same
General Device Characteristic Similarities		
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	Same

	lysosomal storage disorders (LSD) respectively; Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease, Fabry Disease, and Mucopolyaccharidosis (MPS) Type I Disease.	
Measured enzymes	IDUA GAA ABG GLA ASM GALC	Same
Sample Type	Punch from dried blood spot specimen	Same
Test Methodology	Quantitative mass spectrometric enzymatic activity assay	Same
General Device Characteristic Differences		
Instrument / Software Platform	Qsight 210 MD Mass Spectrometer QSight HC Autosampler MD Instrument Software QSight Binary Pump MD	PerkinElmer TQD instrument with MassLynx v4.1 firmware, with PerkinElmer 1525 sample pump, with PerkinElmer 2777c autosampler

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3: Evaluation of precision of quantitative measurement procedures; Approved Guideline - Third Edition

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

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The NeoLSD kit precision was determined based on the recommendations of the CLSI guideline EP5-A3. A 22-day precision study was performed to determine repeatability and also lot-to-lot, within-lab and total variability. Three lots of reagents, three instruments and 15 samples were included in the testing which was performed by 3 operators. On each day of testing, two plates were tested with a minimum of 2 hours between the start of each plate. Each plate contained 2 replicates of each sample. A sampling matrix was established so that each kit lot was tested three times on each instrument. The total number of replicates per sample/instrument/lot was 12 and these were tested over up to 19 non-consecutive days. Within-lab precision was calculated with the repeatability, between-instrument, between-day, and between-plate measurements. Total variability is the within-lab imprecision combined with the between-lot imprecision.

NeoLSD QSight 22-day precision results

ABG	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	1.05	0.11	10.7	0.24	22.7	0.02	2.4	0.24	22.8
2	108	2.33	0.30	13.0	0.38	16.5	0.11	4.8	0.40	17.2
3	108	6.56	0.71	10.8	0.93	14.1	0.19	2.8	0.95	14.4
4	108	13.0	1.21	9.30	1.73	13.3	0.50	3.9	1.80	13.9
5	108	14.7	2.35	15.9	2.97	20.2	0.29	2.0	2.99	20.3

ASM	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	0.56	0.06	10.0	0.08	14.0	0.02	3.9	0.08	14.5
2	108	1.12	0.09	8.1	0.11	9.6	0.05	4.1	0.12	10.4
3	108	3.47	0.24	6.9	0.27	7.7	0.09	2.5	0.28	8.1
4	108	7.40	0.71	9.6	0.83	11.2	0.25	3.3	0.86	11.7
5	108	12.2	1.13	9.3	1.32	10.9	0.40	3.3	1.38	11.4

GALC	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	0.27	0.03	10.1	0.04	13.2	0.01	2.1	0.04	13.4
2	108	0.56	0.05	8.3	0.07	12.6	0.02	3.6	0.07	13.1
3	108	2.58	0.18	7.1	0.19	7.3	0.02	0.8	0.19	7.4
4	108	4.74	0.24	5.1	0.30	6.4	0.03	0.6	0.30	6.4
5	108	7.75	0.78	10.0	0.82	10.5	0.34	4.4	0.89	11.4

IDUA	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	0.76	0.11	15.1	0.12	15.6	0.02	2.3	0.12	15.8
2	108	1.33	0.12	9.1	0.18	13.5	0.03	2.4	0.18	13.7
3	108	2.71	0.20	7.2	0.25	9.2	0.09	3.3	0.26	9.7
4	108	7.75	0.55	7.0	0.69	8.9	0.04	0.5	0.69	8.9
5	108	16.4	1.78	10.8	2.01	12.2	0.34	2.1	2.04	12.4

GLA	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	1.03	0.11	10.7	0.16	15.9	0.03	2.6	0.17	16.1
2	108	2.46	0.19	7.5	0.26	10.4	0.04	1.7	0.26	10.5
3	108	6.44	0.49	7.6	0.64	9.9	0.01	0.2	0.64	9.9
4	108	12.2	0.58	4.8	0.95	7.8	0.20	1.6	0.97	7.9
5	108	17.6	1.15	6.5	1.27	7.2	0.15	0.8	1.28	7.3

GAA	n	Mean µmol/L/h	Repeatability		Within-lab		Between-lot		Total variation	
			SD	CV %	SD	CV %	SD	CV %	SD	CV %
1	108	0.95	0.08	8.4	0.12	12.7	0.02	1.7	0.12	12.8
2	108	2.80	0.22	7.8	0.24	8.5	0.05	1.7	0.24	8.7
3	108	8.01	0.55	6.9	0.58	7.2	0.11	1.4	0.59	7.3
4	108	17.1	1.76	10.3	1.95	11.4	0.29	1.7	1.97	11.5
5	108	24.7	1.54	6.2	1.81	7.3	0.23	0.9	1.82	7.4

2. Linearity:

Linearity was determined in accordance with the CLSI guideline EP06-A. The linearity samples were prepared by making two blood pools, one with high activity and one with low activity. The high pool was made by mixing buffy coat (WBC layer in centrifuged blood) with whole blood. Recombinant enzymes were then spiked into the final high pool to enhance the activities of certain enzymes to obtain enzyme activities levels around the 95th percentile of the neonatal population. The pools were mixed in different proportions to obtain 13 different pools used to make the linearity DBS samples. The hematocrit was adjusted to 45 – 55%. Each sample was assayed in replicates of four over two runs. The degree of nonlinearity was assessed by polynomial evaluation. For any series where the polynomial fit was a better fit statistically. For any series where the polynomial fit was a better fit statistically, the maximum deviation from linearity was 16.1%.

NeoLSD linear range

Enzyme	QSight	
	Linear Range Lower Limit (µmol/L/h)	**Linear Range Upper Limit (µmol/L/h)**
ABG	0.39	20.0
ASM	0.09	13.8
GALC	0.18	7.75
IDUA	0.08	22.3
GLA	0.60	20.4
GAA	0.11	25.3

3. Analytical Specificity/Interference:

The potential interfering substances for possible mass overlaps was assessed by searching two mass spectrometry databases, NIST 14 and MassBank, and CLSI EP07-A2 for potential mass overlaps (±1 Da of the target analytes) of the six enzymatic products and six internal standards. These interfering substances were prepared as neat solutions at different concentrations in Neo MSMS Flow Solvent. The study showed that none of the potential interfering substances gave significant signal in the corresponding MRM. The potential interfering substances tested for the mass overlap study are shown in the table below:

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	

Tested substance	Potential Interference MW	Corresponding NeoLSD analyte	Corresponding NeoLSD exact mass	Concentration of tested substance (µmol/L)
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

Analytical specificity studies were performed following the recommendations in the CLSI EP07-A2 guideline. Potential interferents tested included unconjugated bilirubin, conjugated bilirubin, triglycerides (intralipid), free hemoglobin, albumin, acetaminophen, calcifediol, chlorhexidine, glucose, and galactose. Whole blood pools were prepared to three different enzyme activity levels to represent enzyme deficiency, near the cut-off, and normal levels by spiking. The hematocrit value of the blood preparations was adjusted to 45 - 55% to correspond to the hematocrit of neonates. The test pools were prepared by spiking in the interfering substance at an appropriate concentration into the base pool. Control pools were prepared by spiking in the appropriate solvent into the base pool. Test and control pools were spotted onto filter paper. Samples were assayed in replicates of 12. For the hematocrit interference studies, pools were prepared by adjusting hematocrit to a target of 50% for the control pool, and hematocrit targets of 35%, 45%, 50%, 55%, and 65% for the test pools. The limit for significant interference was defined as 15%. The substances indicated in the table below were found not to interfere with the proposed device at the concentration indicated.

Substances found not to interfere with the candidate assay

Tested substance	Added concentration of tested substance (in blood)
Bilirubin (Unconjugated)	10 mg/dL
Bilirubin (Conjugated)	15 mg/dL
Heparin	7500 USP units/dL
Acetaminophen	5.5 mg/dL
Calcifediol	10.5 µg/dL

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

Hematocrit interference on ABG and GALC: Hematocrit at level of 35% decreases ABG activity levels of 1.35 µM/h by 0.55 µM/h when measured on the QSight. This may cause a false positive screening result for a specimen with measured ABG activity close to the cut-off value.

Hematocrit at a level of 65% increases ABG activity levels of 1.35 µM/h by 0.76 µM/h when measured on the QSight.

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 65% 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, when measured on the QSight.

Hematocrit at a level of 65% decreases GALC activity levels of 0.97 µM/h by 21% when measured on the QSight.

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.

For both enzymes mentioned above, there was no observed interference for hematocrit at levels of 45% and 55%.

Hemoglobin interference on ABG, ASM and IDUA: Hemoglobin was found to interfere with the assay by increasing the measured ABG, ASM and IDUA activity, this may result in false negative results for Gaucher, Niemann-Pick A/B, and Mucopolysaccharidosis Type I (MPS I) Disease.

Hemoglobin at level of 19.1 g/dL increases ABG activity level of 2.27 µM/h by 21% as measured on the QSight. For samples up to 30% above the ABG cut-off value, with known or suspected high hemoglobin (>15 g/dL); testing by an alternate method that is not subject to hemoglobin interference is recommended.

Hemoglobin at a level of 19.1 g/dL increases ASM activity level of 1.65 µM/h by 17% when measured on the QSight. For samples up to 30% above the ASM cut-off value, with known or suspected high hemoglobin (>15 g/dL); testing by an alternate method that is not subject to hemoglobin interference is recommended.

Glucose interference on GAA: Glucose was found to interfere with the assay by decreasing the measured GAA activity. Glucose at a level of 0.5 g/dL decreases GAA activity levels of 2.83 µM/h by 17% when measured on the QSight. Glucose at level of 0.75 g/dL decreases GAA activity levels of 2.83 µM/h by 22%, when measured on the QSight.

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.

Glucose does not interfere at concentrations of 0.25 g/dL or below.
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.

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 a level of 0.3 g/dL increases GAA activity of 3.26 µM/h by 22% when measured on the QSight. Intralipid at level of 0.45 g/dL increases GAA activity level of 3.26 µM/h by 30% when measured on the QSight.

Intralipid at level of 0.15 g/dL does not interfere with GAA activity.

GLA: Intralipid at a level of 0.3 g/dL increases GLA activity level of 3.52 µM/h by 23% when measured on the QSight. Intralipid at level of 0.75 g/dL increases GLA activity level of 3.76 µM/h by 2.89 µM/h when measured on the QSight.

Intralipid at level of 0.22 g/dL does not interfere with GLA activity.

IDUA: Intralipid at a level of 1.13 g/dL increases IDUA activity level of 3.26 µM/h by 24% when measured on the QSight.

Intralipid at level of 0.30 g/dL does not interfere with IDUA activity.

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:

ABG: EDTA at a level of 0.2 g/dL increases ABG activity level of 1.59 µM/h by 0.3 µM/h when measured on the QSight.

EDTA at level of 0.05 g/dL does not interfere with ABG activity.

ASM: EDTA at a level of 0.03 g/dL EDTA decreases ASM activity level of 1.14 µM/h by 17% when measured on the QSight.

EDTA at level of 0.02 g/dL does not interfere with ASM activity.

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

Albumin interference on ABG and ASM: Albumin (HSA; from human serum) was found to interfere with the assay, thus increasing the measured ABG and ASM activity.

ABG: Albumin at a level 3.2 g/dL increases ABG activity levels of 1.94 µM/h by 36% when measured on the QSight. Albumin at level of 2.4 g/dL does not interfere with ABG activity.

ASM: Albumin at a level 2.4 g/dL increases ASM activity levels of 1.51 µM/h by 17% as measured on the QSight. Albumin at level of 1.6 g/dL does not interfere with ASM activity.

In infants aged 0-12 months the reference range of albumin concentration in whole blood is approximately 1.4 – 2.2 g/dL. For ABG and ASM, albumin did not show an interference effect within the reference range.

Galactose interference on ABG: Galactose was found to interfere with the assay, thus increasing the measured ABG activity.

ABG: Galactose at a level 11.3 mg/dL increases ABG activity levels of 1.95 µM/h by 40% when measured on the QSight. Galactose at level of 7.5 mg/dL does not interfere with ABG activity.

In infants the reference range for galactose in whole blood is 8 - 10 mg/dL. Therefore, this interference effect is seen outside of the reference range concentration.

Chlorhexidine digluconate interference on ASM: Chlorhexidine digluconate at level 0.036% decreases ASM activity levels of 1.69 µM/h by 16% when analyzed on the QSight. Chlorhexidine digluconate at level of 0.032% does not interfere with ASM activity.

If chlorhexidine gluconate is used for heel skin cleansing in sample collection, allow the skin to thoroughly air dry before puncture to avoid contamination of the sample with the disinfectant.

Filter Paper:
PerkinElmer used DBS spotted onto two legally marketed dried blood spot cards. The sponsor provided information to support that results using the different filter papers were equivalent.

4. Assay Reportable Range:

Enzyme	Measuring Range (µmol/L/h)
ABG	0.79 - 20.0
ASM	0.16 - 13.8
GALC	0.20 - 7.75
IDUA	0.19 - 22.3
GLA	0.80 - 20.4
GAA	0.31 - 25.3

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

Traceability: There are no international reference preparations or reference methods for the activity measurements of lysosomal storage disease enzymes. The device is traceable to in-house reference internal standards determined by gravimetric value assignment.

Sample stability: The influence of storage time, temperature and humidity on LSD activity was studied with several adult whole blood DBS specimens.

- Lysosomal enzymes ASM, GAA, GALC and GLA are stable for up to 18-22 days at 4°C. IDUA is stable for up to 14 days and ABG is stable for up to 7 days.
- GLA and GAA are stable for up to 35 days at 21°C with ambient humidity. ASM, GALC and IDUA are stable for up to 19, 12 and 11 days, respectively. There is a moderate loss of ABG activity (~80% recovery) after 6 days.
- GAA and GLA are stable at 27°C and RH 40% for up to 35 and 17 days, respectively. ASM, GALC and IDUA are stable for up to 3 days. There is a moderate loss in ASM and GALC activity (~80% recovery) after 6 days and a moderate loss in IDUA activity (~80% recovery) after 4 days. Further there is a moderate loss in ABG activity (~80% recovery) after 2 days.
- All lysosomal enzymes studied are unstable at 35°C and RH 80% with a significant loss in activity (~10-60% recovery).

In the labeling, the sponsor provides the following information:

- Care should be taken in the storage and transport of the dried blood spots (DBS). Storage of specimens in an environment with elevated temperature and humidity increases the risks of false positive screening results. For long-term storage the specimens should preferably be stored at -20°C and protected from moisture. Sample exposure to high temperature and high humidity during shipping may result in increased rates of false positives.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit and Quantitation (LoQ) for the test system was determined. The analysis of the LoB, LoD, and LoQ were performed in accordance with the CLSI guideline EP17-A2.

To determine the LoB, four enzyme-free pools were made by washing red blood cell concentrate with saline solution and adjusting the hematocrit to 40 - 55%. The samples were assayed in replicates of 6 over five working days using two reagent lots. The LoB was determined for each lot using the non-parametric classical approach described in the guideline. The LoB for the worst performing lot is reported in the package insert.

To determine the LoD, five sample pools were prepared by mixing a high enzyme activity pool with a low enzyme activity pool and adjusting the hematocrit to 40 - 55%. The samples were assayed in replicates of 6 over five working days using two reagent lots. The LoD was determined using the parametric approach described in the guideline. The LoD for the worst performing lot is reported in the package insert.

Enzyme	LoB	LoD
ABG	0.114	0.79
ASM	0.046	0.16
GALC	0.120	0.20
IDUA	0.044	0.13
GLA	0.519	0.80
GAA	0.080	0.31

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:

NeoLSD Limit of Quantitation

Enzyme	LoQ (µmol/L/h)	SD at LoQ	CV% at LoQ
ABG	0.79	0.17	21.5%
ASM	0.16	0.03	18.8%
GALC	0.20	0.04	20.0%
IDUA	0.19	0.04	21.1%
GLA	0.80	0.15	18.8%
GAA	0.31	0.05	16.1%

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

See C3 below for screening performance.

2. Matrix Comparison:

Not applicable. This device is only intended to be used with DBS specimens collected from neonates.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

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

The screening performance of the candidate device was evaluated in a clinical study at a US newborn screening laboratory. In the study 2489 routine newborn samples were tested. Samples tested were from newborns ≤ 48 hours old. The study population was enriched with 12 archived confirmed LSD positive newborn DBS specimens.

The 12 confirmed LSD specimens consisted of 2 cases of Gaucher, 1 case of Niemann-Pick A/B, 1 case of Pompe, 3 cases of Krabbe, 4 cases of Fabry, and 1 case of MPS I disease. The 2489 routine samples were tested according to the testing algorithm described in the package insert. Briefly, these specimens were first tested in singlicate for all six enzymes. The specimens having at least one enzyme result below the initial cut-off value were retested in duplicate to confirm the low enzyme result and the result was compared to the final cut-off (see Expected Values/Reference Range for information on cut-offs). The 12 confirmed positives were tested once (not according to the testing algorithm). The confirmed positives were evaluated against the final cut-off. Two of the confirmed positives (Krabbe) and one routine sample had three enzyme activity levels below the initial cut-off and were deemed invalid, per the instructions in the labeling. Invalid results are excluded from the tabulations. During routine the user is instructed to obtain a new DBS for any invalid sample.

Routine	**ABG**	**ASM**	**GALC**	**IDUA**	**GLA**	**GAA**
Screened samples	2489	2489	2489	2489	2489	2489
Above initial cutoff	2479	2482	2477	2483	2483	2486
Below initial cutoff	10	7	12	6	6	3
Retest rate	0.40%	0.28%	0.48%	0.24%	0.24%	0.12%
Screen negative	2488	2488	2486	2484	2485	2489
Screen positive	3	2	4	6	6	1
False positive rate	0.04%	0.04%	0.12%	0.20%	0.16%	0.00%

Screening performance (samples with invalid results are excluded).

Gaucher (ABG)		TQD		
		Screen Positive	**Screen Negative**	**Total**
QSight	**Screening Positive**	3*	0	3
	Screening Negative	0	2487	2487
	Total	3	2487	2490

*Includes 2 retrospective confirmed positive samples

Niemann-Pick A/B (ASM)		TQD		
		Screen Positive	**Screen Negative**	**Total**
QSight	**Screening Positive**	2*	0	2
	Screening Negative	0	2488	2488
	Total	2	2488	2490

*Includes 1 retrospective confirmed positive sample

Krabbe (GALC)		TQD		
		Screen Positive	**Screen Negative**	**Total**
QSight	**Screening Positive**	4*	0	4
	Screening Negative	0	2486	2486
	Total	4	2486	2490

*Includes 1 retrospective confirmed positive sample

There were two “invalid” samples for Krabbe that were not included in the analysis. These samples were screen positive for multiple enzyme activities including GALC, triggering an invalid sample alert. The sample storage conditions for those cards were unknown. The labeling recommends obtaining a fresh sample following an invalid result.

MPS I (IDUA)		TQD		
		Screen Positive	**Screen Negative**	**Total**
QSight	**Screening Positive**	6*	0	6
	Screening Negative	0	2484	2484
	Total	6	2484	2490

*Includes 1 retrospective confirmed positive sample

Fabry (GLA)		TQD		
		Screen Positive	Screen Negative	Total
QSight	Screening Positive	6*	0	6
	Screening Negative	0	2484**	2484
	Total	6	2484	2490

* Includes 2 retrospective confirmed positive samples

**Two screen negative results were from female babies with Fabry. It has been shown that female patients with the Fabry disorder may show normal GLA enzyme activities at birth but are later symptomatic

Pompe (GAA)		TQD		
		Screen Positive	Screen Negative	Total
QSight	Screening Positive	1*	0	1
	Screening Negative	0	2489	2489
	Total	1	2489	2490

*Includes 1 retrospective confirmed positive sample

D Clinical Cut-Off:

See expected values.

E Expected Values/Reference Range:

The labeling states that each laboratory should establish its own reference range and cut-off values.

Descriptive statistics for the 2489 presumed unaffected newborns in the clinical study on the QSight with the NeoLSD MSMS assay are below. All values are in µmol/L/hour.

Enzyme	Median	Mean	Min	Max	0.1%	0.2%	0.3%	2.5%	97.5%
ABG	8.83	9.47	1.06	102.0	1.40	1.78	1.96	3.66	18.94
ASM	4.30	4.55	1.09	23.70	1.32	1.43	1.57	2.32	8.01
GAA	9.53	10.07	1.87	31.80	2.32	2.54	2.79	4.23	19.54
GALC	4.70	5.57	0.48	50.00	0.80	1.01	1.07	1.62	14.90
GLA	15.30	17.53	2.21	133.0	3.83	4.74	5.44	7.59	41.99
IDUA	7.05	7.30	0.34	21.00	1.36	1.51	1.86	3.31	12.54

The initial cut-off values were based on 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 following cut-offs were used in the clinical validation study in section 3c above.

QSight								Initial cut-off	Retest cut-off
Enzyme	n	Enzyme activity (µmol/L/h)							
		Range	Mean	Median	Lower percentiles				
					0.1%	0.2%	0.3%		
ABG	2489	1.06 – 102	9.47	8.83	1.40	1.78	1.96	25%	20%
ASM	2489	1.09 – 23.7	4.55	4.30	1.32	1.43	1.57	35%	30%
GALC	2489	0.48 – 50.0	5.57	4.70	0.80	1.01	1.07	25%	20%
IDUA	2489	0.34 – 21.0	7.30	7.05	1.36	1.51	1.86	25%	20%
GLA	2489	2.21 – 133	17.5	15.3	3.83	4.74	5.44	35%	30%
GAA	2489	1.87 – 31.8	10.1	9.53	2.32	2.54	2.79	25%	20%

F Other Supportive Instrument Performance Characteristics Data:

Carry-over: Carry-over was evaluated using one lot of the NeoLSD MSMS kit. Carry-over was defined as the average amount of analytes transferred from the high sample to the low sample calculated as the mean concentration difference between the first and second replicates of the low sample. Multiple enzyme concentrations were included in the study and there was no significant carry-over demonstrated by the study.

Plate Drift: The sponsor evaluated plate drift to ensure the on-board stability of processed samples for 12 and 24 hours. Two separate controls were subject to repeated testing over the course of 24 hours and showed no significant change in results over that period.

VIII Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable and the special controls for this device type under 21 CFR 862.1488.

IX Conclusion:

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