EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
SEEKER System
DECISION SUMMARY

A. DEN Number:

DEN150035

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the SEEKER System

C. Measurand:

α-L-iduronidase (IDUA), α-D-glucosidase (GAA), β-glucocerebrosidase (GBA), and α-D-galactosidase A (GLA)

D. Type of Test:

Quantitative fluorimetric enzymatic activity assay

E. Applicant:

Baebies, Inc.

F. Proprietary and Established Names:

SEEKER System

G. Regulatory Information:

1. Regulation:

   21 CFR 862.1488

2. Classification:

   Class II (special controls)

3. Product code(s):
   PQW
   PQT
   PQU
   PQV

4. Panel:
   Chemistry (75)

H. Indications For Use:

1. Indications for Use:
   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

2. Special conditions for use statement(s):
   - For *in vitro* diagnostic use only.
   - For prescription use only.
   - This test is not intended to diagnose lysosomal storage disorders.
   - Reduced activity for any of the four enzymes should be confirmed by other confirmatory diagnostic methods.
   - 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.
   - Conditions that are known to cause anomalous results are[1]:
     - Specimen spot not uniformly saturated with blood.
     - Specimen spot punched too close to the edge of the blood spot.
     - Poorly collected and improperly dried specimens.
     - Non-eluting blood spot due to deterioration of specimen caused by exposure to heat and humidity.
   - Also, the SEEKER LSD Reagent Kit - IDUA|GAA|GBA|GLA may result in:
     - False negatives by not detecting Fabry disease in females[2].

[1] Clinical and Laboratory Standards Institute (CLSI). Blood Collection on Filter Paper for Newborn Screening Programs: Approved Standard – Sixth Edition. CLSI document NBS01-A6 (ISBN 1-56238-883-5 [Print]; ISBN 1-56238-884-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA. 2013.

[2] Linthorst GE, Poorthuis BJHM, Hollak CEM. Enzyme activity for determination of presence of Fabry disease in women results in 40% false-negative results. *Journal of the American College of Cardiology*. 2008; 51(21):2082.

- False positives by identifying pseudo deficiencies and carriers as affected for MPS I, Gaucher and Pompe diseases[3].
- False negative by not detecting certain late onset forms for Pompe disease[4].
- Increased rate of false positives when the dried blood spot specimen is exposed to high temperature (≥45°C) during shipping.

3. Special instrument requirements:
   SEEKER Instrument

I. Device Description:

The SEEKER System employs digital microfluidic technology to measure multiple lysosomal enzymatic activities quantitatively from newborn dried blood spot specimens. The following components are provided:

1. SEEKER Instrument (including USB and power cables), Desktop PC with monitor, keyboard, mouse, and the Spot Logic software.

2. SEEKER LSD Reagent Kit - IDUA|GAA|GBA|GLA containing enzyme specific substrate reagents, dried blood spot extraction buffer, reaction stop buffer, 4 levels of calibrators and quality control dried blood spots containing 4 levels of quality control (QC) material. Each Reagent Kit contains sufficient consumables for 1440 tests. The contents of the kit are listed below:

Component	Contents	Quantity
Quality control dried blood spots	QC-Base Pool (QCBP)	1x15 spots
	QC-Low (QCL)	1x15 spots
	QC-Medium (QCM)	1x15 spots
	QC-High (QCH)	1x15 spots
Enzyme substrates	IDUA	9x100 µL
	GAA	9x100 µL
	GBA	9x100 µL
	GLA	9x100 µL
Calibrators	Calibrant A (CALA)	9x50 µL
	Calibrant B (CALB)	9x50 µL
	Calibrant C (CALC)	9x50 µL
	Calibrant D (CALD)	9x50 µL

[3] Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J. Lysosomal storage disorder screening implementation: findings from the first six months of full population pilot testing in Missouri. Journal of Pediatrics. 2015; 166(1): 172-177.

[4] Chiang S-C, Hwu W-L, Lee N-C, Hsu L-W, Chien Y-H. Algorithm for Pompe disease newborn screening: Results from the Taiwan screening program. Mol Genet Metab. 2012;106(3):281-286.

Component	Contents	Quantity
Other reagents	Stop Buffer (STB)	9x500 µL

Other components needed to run tests include the following:

Component	Contents	Quantity
Other Reagents	Extraction Buffer (EXT)	9 × 30 mL
	Filler Fluid	9 × 10 mL
Cartridge	SEEKER cartridge	1 × 36

The composition of the enzyme reagents and buffers are summarized below:

Reagent (Description)	Composition
IDUA (α-L-iduronidase substrate)	2 mM 4-MU-α-L-iduronide sodium salt 3mM D-saccharolactone 0.04 M acetate buffer, pH 3.5 20 mM methyl β-cyclodextrin 0.01% Tween20
GAA (α-D-glucosidase substrate)	5 mM 4-MU-α-glucopyranoside 12 µM acarbose 0.04 M acetate buffer, pH 3.8 20 mM methyl β-cyclodextrin 0.01% Tween20
GBA (β-glucocerebrosidase substrate)	16 mM 4-MU-β-glucopyranoside 0.05 M/0.1M citrate phosphate buffer, pH 5.2 0.01% Tween20 1.5% sodium taurocholate
GLA (α-D-galactosidase A substrate)	10 mM 4-MU-α-galactopyranoside 145 µM N-acetyl galactosamine 0.04 M acetate buffer, pH 4.6 20 mM methyl β-cyclodextrin 0.01% Tween20
Stop Buffer STB (reaction stopping buffer)	0.6 M $NaHCO_3$, pH 11.0 in 0.04% Tween 20
Extraction Buffer EXT (dried blood spot extraction buffer)	0.1% Tween 20 in water
Filler Fluid (medium for droplet movement)	0.1% Triton X-15 in 5cSt silicone oil

The Seeker Calibrators are supplied as part of the SEEKER LSD Reagent Kit - IDUA|GAA|GBA|GLA. The calibrators consist of 4 levels of aqueous preparation of 4-methylumbelliferone sodium salt (4-MU) in 0.6M sodium bicarbonate buffer, pH 11.0 with 0.01% Tween 20. The concentration of 4-MU in each of the 4 calibrators is indicated in the table below:

Calibrator Level	Concentration of 4-MU
Calibrant A (CAL A)	0.0375 µM
Calibrant B (CAL B)	0.0750 µM
Calibrant C (CAL C)	0.1500 µM
Calibrant D (CAL D)	0.3000 µM

The quality control dried blood spots include 4 levels of control material: QC Low (QCL), QC Medium (QCM) and QC High (QCH). The composition of the 4 quality control dried blood spot (DBS) is summarized below. QCBP is used to fill empty wells on a cartridge.

Quality Control Level	Composition
QCBP	Heat inactivated human serum, adjusted to ~50% hematocrit using human red blood cells
QCL	5% cord blood and 95% heat inactivated serum, adjusted to ~50% hematocrit using human red blood cells
QCM	50% cord blood and 50% heat inactivated serum adjusted to ~50% hematocrit using human red blood cells
QCH	Human umbilical cord blood, adjusted to ~50% hematocrit using human red blood cells

The enzymatic activity values for the quality control DBS measured by the manufacturer are given on the lot specific quality control certificate included in each assay kit for all levels except QCBP. Each laboratory should establish its own mean and acceptable ranges for the quality control materials.

3. SEEKER Cartridges

4. Finnipipette Novus 8-channel automatic pipette 1-10 µL

5. Finnipipette Novus 1-channel automatic pipette 10-100 µL

J. Standard/Guidance Documents Referenced:

CLSI EP5-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

CLSI EP6-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

CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

K. Test Principle:

The SEEKER System automates enzymatic analysis for newborn screening using digital microfluidic technology. The Seeker Cartridge integrates and automates all the liquid-handling steps involved in an assay using sub-microliter droplets as reaction vessels. The cartridge manipulates the reagents from the SEEKER LSD Reagent Kit-IDUA|GAA|GBA|GLA and is controlled by the SEEKER Instrument through the Spot Logic software.

The activities of the lysosomal enzymes (IDUA, GAA, GBA and GLA) are measured in DBS extracts by an end point method using synthetic fluorescent substrates. The fluorescent substrates use 4-methylumbelliferone as the fluorophore, which has an excitation peak at 365 nm and emission peak at 460 nm. The synthetic substrates are hydrolyzed by their corresponding enzyme at acidic pH conditions to release free 4-methylumbelliferone (4-MU).

The substrate for IDUA is 4-methylumbelliferyl α-L-iduronide (4-MU-α-IDUA). The chemical D-Saccharolactone is used to selectively inhibit endogenously present β-glucuronidase, which is active for a stereoisomer of 4-MU-α-IDUA (impurity in chemical synthesis)[5]. The reaction is summarized below:

$$\text{4-MU-}\propto\text{-L-iduronide} \xrightarrow{IDUA/pH\ 3.5} \text{4-MU + Iduronic acid}$$

The substrate for GAA is 4-methylumbelliferyl α-D-glucoside (4-MU-α-Gluc). The chemical acarbose is used to selectively inhibit endogenously present maltose glucoamylase which is also active for 4-MU-α-Gluc[6,7,8,9]. The reaction is summarized below:

$$\text{4-MU-}\propto\text{-D-glucoside} \xrightarrow{GAA/pH\ 3.8} \text{4-MU + D-Glucose}$$

[5] Chamoles N, Blanco, M, Gaggioli D. Diagnosis of α-L-iduronidase deficiency in dried blood spots on filter paper: the possibility of newborn diagnosis. *Clinical Chemistry*. 2001; 47:780-781.
[6] Winchester B, Bali D, Bodamer OA, Caillaud C, Christiensen E, Cooper A., *et al*. Methods for a prompt and reliable laboratory diagnosis of Pompe disease: Report from an international consensus meeting. *Molecular Genetics and Metabolism.*2008; 93(3):275-281.
[7] Goldstein JL, Young SP, Changela M, et al. Screening for Pompe disease using a rapid dried blood spot method: experience of a clinical diagnostic laboratory. *Muscle Nerve*. 2009;40(1):32-36.
[8] Li Y, Scott CR, Chamoles NA, Chavami A, Pinto BM, Turecek F, *et al*. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. *Clinical Chemistry*. 2004; 50(10):1785-1796.
[9] Kallwass H, Carr C, Gerrein J, Titlow M, Pomponio R, Bali D, *et al*. Rapid diagnosis of late-onset Pompe disease by fluorometric assay of alpha-glucosidase activities in dried blood spots. *Molecular Genetics and Metabolism*. 2007; 90(4):449-452.

The substrate for GBA is 4-methylumbelliferyl β-D-glucopyranoside (4-MU-β-Gluc). The surfactant sodium taurocholate (NaTC) is present in the buffer and is required to activate the enzyme. The reaction is summarized below:

$$\text{4-MU-β-D-glucopyranoside} \xrightarrow{GBA/pH\ 5.2/NaTC} \text{4-MU + D-Glucose}$$

The substrate for GLA is 4-methylumbelliferyl α-D-galactopyranoside (4-MU-α-Gal). The chemical N-acetyl-D-galacosamine is used to inhibit endogenously present α-N-acetylgalactosaminidase which is also active for 4-MU-α-Gal. The reaction is summarized below:

$$\text{4-MU-}\propto\text{-D-galactopyranoside} \xrightarrow{GLA/pH\ 4.6} \text{4-MU + D-Galactose}$$

For all the enzymatic assays the reaction is stopped using a high pH stop buffer (sodium bicarbonate, pH 11). The high pH is not optimal for the enzymes and effectively stops the substrate turnover. 4-MU is also not fluorescent at the reaction pH (3.5-5.2) and is highly fluorescent at the stopped condition (pH 11) with an excitation at 365 nm and emission at 460 nm.

$$\text{4-MU (acid pH, non-fluorescent)} \xrightarrow{pH\ 11} \text{4-MU (fluorescent, Ex 365nm/Em 460nm)}$$

All enzymatic reactions are performed at 37°C. After a pre-specified incubation time the free 4-MU is measured using a UV fluorimeter on the SEEKER Instrument. The fluorescence value of the 4-MU product is converted to a 4-MU concentration using a 4-MU calibration curve. The amount of 4-MU generated, after correction for substrate background and non-enzymatic hydrolysis, is proportional to the enzyme concentration. Substrate background and non-enzymatic hydrolysis is estimated by substituting the dried blood spot extract in the reaction with extraction buffer. Enzymatic activity is reported as micromoles of 4-MU produced / liter of blood / hour of incubation.

L. Performance Characteristics:

1. Analytical performance:

a. Reproducibility/Precision:

A study to estimate the imprecision of the IDUA assay was performed using 4 instruments and 3 reagent lots, and performed testing during 21 non-consecutive days, with 2 runs per day and 2 punches from each DBS specimen per run. The samples were prepared by mixing human umbilical cord blood and heat inactivated serum (which has no endogenous lysosomal enzymes) to create samples with different enzyme concentrations. The hematocrit of was adjusted to ~50% to mimic newborn specimens and the samples were spotted onto Ahlstrom 226 grade filter paper. The study was based

on the recommendations in the CLSI EP5-A3 guideline. A total of 336 replicate results for each specimen were tested. There were several invalid test results for each sample for all assays. The results of the study are summarized below. In the following table the repeatability (or within-run precision) estimate includes the instrument run as the component of variability and the reproducibility estimate includes the instrument run, reagent lot, instrument and day as the components of variability.

Results for the IDUA test

Mean µmol/L/h	*N	Repeatability (%CV)	Between Lot (%CV)	Between Instrument (%CV)	Between Day (%CV)	Reproducibility (%CV)
3.53	334	22.7%	15.6%	0%	7.4%	27.2%
6.22	335	26.4%	11.1%	0%	0.0%	28.5%
12.09	334	15.4%	11.0%	0%	0.0%	18.8%
24.06	335	9.0%	9.8%	0%	0.0%	14.2%

*There were a total of 7 invalid replicates obtained in the study resulting in a total N of less than 336 replicates per sample.

Results for the GAA test

Mean µmol/L/h	*N	Repeatability (%CV)	Between Lot (%CV)	Between Instrument (%CV)	Between Day (%CV)	Reproducibility (%CV)
4.29	331	15.6%	5.8%	0%	14%	17.0%
6.27	334	15.2%	4.1%	0%	0.0%	15.8%
9.59	335	9.9%	7.0%	0%	0.0%	12.0%
18.06	334	13.6%	5.9%	0%	0.0%	14.8%
27.37	335	11.3%	6.3%	1.6%	0.0%	12.9%

*There were a total of 11 invalid replicates obtained in the study resulting in a total N of less than 336 replicates per sample.

Results for the GBA test

Mean µmol/L/h	*N	Repeatability (%CV)	Between Lot (%CV)	Between Instrument (%CV)	Between Day (%CV)	Reproducibility (%CV)
2.84	331	34.9%	12.7%	1.8%	2.5%	38.0%
3.47	334	13.5%	10.7%	2.3%	0.0%	18.4%
5.07	335	11.0%	11.2%	0%	0.0%	16.6%
8.55	334	11.6%	10.4%	1.4%	0.0%	15.8%
15.00	335	11.3%	11.4%	2.1%	1.2%	15.7%

*There were a total of 11 invalid replicates obtained in the study resulting in a total N of less than 336 replicates per sample.

Results for the GLA test

Mean µmol/L/h	*N	Repeatability (%CV)	Between Lot (%CV)	Between Instrument (%CV)	Between Day (%CV)	Reproducibility (%CV)
6.94	331	15.4%	5.6%	0%	1.7%	16.3%
9.80	334	10.4%	6.4%	0%	2.9%	13.6%
15.32	335	7.7%	7.2%	0%	3.1%	11.5%
28.76	334	7.8%	4.8%	0%	0.0%	9.4%
52.66	335	8.8%	4.1%	1.3%	2.7%	10.6%

*There were a total of 11 invalid replicates obtained in the study resulting in a total N of less than 336 replicates per sample.

b. *Linearity/assay reportable range:*

Linearity studies were performed following the CLSI EP6-A Guideline using one lot of reagents. The samples used were dried blood spot specimens spotted onto Ahlstrom 226 grade filter paper. For the first study, a high sample pool was prepared by spiking human recombinant enzymes into heat inactivated serum (to remove endogenous lysosomal enzymes), and adjusting hematocrit to ~50% to correspond to the hematocrit of neonates. Eleven intermediate levels were prepared by diluting the high sample with heat inactivated serum and then adjusting hematocrit to ~50%. These 12 samples were tested in replicates of 12 using 4 instruments. A second study was performed using native enzymes. In this study, the high sample was prepared using human umbilical cord blood and the intermediate samples were made by mixing the high sample with heat inactivated serum and adjusting hematocrit to ~50%. A total of 12 samples were tested in replicates of 12 using 4 instruments.

The degree of nonlinearity was assessed by analyzing the second and third order polynomial regression. For any series where the polynomial fit was a better fit statistically, the maximum deviation from linearity was <10%. Acceptable linearity was demonstrated for the following ranges in the different studies:

Analyte	Study 1	Study 2
IDUA	3.02 to 50.75 µmol/L/h	2.61 to 19.40 µmol/L/h
GAA	7.53 to 94.66 µmol/L/h	2.12 to 21.79 µmol/L/h
GBA	7.86 to 73.24 µmol/L/h	2.14 to 10.42 µmol/L/h
GLA	22.12 to 153.74 µmol/L/h	4.38 to 45.34 µmol/L/h

Based on the results of the linearity studies and the limit of quantitation (LoQ) studies, the sponsor claims the following reportable ranges:

Analyte	Reportable range
IDUA	2.77 to 50.75 µmol/L/h
GAA	2.18 to 94.66 µmol/L/h
GBA	2.14 to 73.24 µmol/L/h
GLA	4.88 to 153.74 µmol/L/h

Four statistically high outlier test results were observed in the second study (please refer to section O below). Including these outliers in the analyses did not impact the claimed deviation from linearity. However, these 4 test results are outside the claimed imprecision performance of the test.

Test results below the LoQ are reported as "<LoQ (value)" with the actual LoQ value in parenthesis. Test results above the reportable range are reported as "> (value)" with the actual upper reportable limit in parenthesis. The following information is provided in the package insert:

> It is recommended to retest specimens with activity above the upper end of the range to ensure that the elevated activity is not due to other pre-analytical causes such as specimen contamination. If the result of the retest is still above the upper end of the linear range, the specimen should be considered presumed normal.

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

Calibrator (traceability and value assignment): Since there is no reference material for 4-methyllumbelliferone, the calibrators are traceable to internal master calibrators. These master calibrators are prepared gravimetrically and value assigned using UV spectrophotometric analysis. Product calibrators are produced in the same manner as master calibrators. Product calibrators are value assigned by testing multiple replicates against the master calibrator lot. Specifications for calibrator target values and linear regression must be met. In addition, calibrators and reagents are tested together using DBS samples and predefined acceptance criteria for bias and precision must be met.

Quality control samples (value assignment): The Seeker Quality Control spots (QCL, QCM and QCH) are analyzed in multiple replicates on multiple cartridges. The sponsor calculates and provides mean enzymatic activity values and standard deviation for the quality control samples with each reagent kit. Each laboratory should establish their own mean and standard deviation values and determine their own acceptable range of enzymatic activity for quality control samples.

Calibrator stability: Accelerated stability studies were performed and supported that the calibrators are stable for 12 months when stored at -80ºC. Real time studies are ongoing to support the claim based on the accelerated stability studies. The protocols were reviewed and found acceptable.

Quality control sample stability: The sponsor performed accelerated stability studies and claims that the controls are stable for 12 months when stored at -80ºC. Real time studies are ongoing to support the claim based on the accelerated stability studies. The protocols were reviewed and found acceptable.

Stability protocols designed to monitor reagent stability (which include the calibrators

and quality control samples) were reviewed and found acceptable.

The reagents (including calibrators and controls) are stable for 90 minutes after removing the aliquots from the freezer. The protocols for determining the in-use stability claim were reviewed and found acceptable.

Sample (DBS) stability: The sponsor provided information to support the following claims:

- Lysosomal enzymes (IDUA, GAA, GBA and GLA) are stable for up to 5 days at 10°C at low humidity (20% RH) and high humidity (80% RH) levels.
- IDUA, GAA and GBA are stable for up to 5 days at 25°C and 50% RH. There is a moderate loss in GLA activity (up to 18%) after 5 days at 25°C and 50% RH.
- At 45°C and low humidity (20% RH), there is a moderate loss in IDUA (up to 32%) and GAA activity (up to 32%), and significant loss in GBA (up to 46%) and GLA activity (up to 66%).
- At 45°C and high humidity (80% RH) there is significant loss in IDUA (up to 81%), GAA (up to 84%), GBA (up to 69%) and GLA activity (up to 90%).
- Lysosomal enzymes (IDUA, GAA, GBA and GLA) (b) (4)

The following limitation is in the labelling:

> The SEEKER LSD Reagent Kit - IDUA|GAA|GBA|GLA may result in: Increased rate of false positives when the dried blood spot specimen is exposed to high temperature (≥45°C) during shipping.

d. Detection limit:

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

To determine the LoB, the reaction blank was used to prepare DBS specimens on Ahlstrom 226 grade filter paper. 80 replicates of the blank were tested using each of 3 reagent lots, 6 cartridges and 1 analyzer. The LoB was determined for each lot using the non-parametric classical approach described in the guideline. The α was set at 5%. The LoB for the worst performing lot is reported in the package insert.

To determine the LoD, low analyte DBS activity specimens were prepared by mixing human umbilical cord blood and heat inactivated serum, adjusting the hematocrit to ~50% and then spotting it onto Ahlstrom 226 grade filter paper. The DBS samples (8 per lot) were tested in 8 replicates using the 3 reagent lots, 6 cartridges and 1 analyzer (for a total of 64 replicates per lot). The LoD was determined for each lot following the parametric classical approach described in the guideline. The β was set at 5%. The sponsor observed 1 statistically high outlier test result in this study (please refer

to section O below) and removed it from the analysis. This test result is outside the claimed imprecision performance of the test. The LoD for the worst performing lot is reported in the package insert.

To determine the LoQ, 8 specimens with varying enzyme activity were prepared by mixing human umbilical cord blood and heat inactivated serum. The hematocrit was then adjusted to ~50% and spotted onto Ahlstrom 226 grade filter paper. The DBS specimens were tested in 16 replicates. 3 reagent lots, 12 cartridges and 4 analyzers were used in the study. The LoQ was determined using the precision profile of each test and defined as the lowest concentration where the total imprecision was ≤ 1.5 µmol/L/h or 20% CV, whichever was greater. Four statistically high outlier test results were observed and removed from the analysis (please refer to section O below). These 4 test results are outside the claimed imprecision performance of the test. The LoQ for the worst performing lot is reported in the package insert. The sponsor also provided the impression observed with the worst performing lot at the claimed LoQ concentration for each enzyme.

The following information is provided in the package insert:

Detection Capability (µmol/L/h)	IDUA	GAA	GBA	GLA
LoB	1.78	0.50	0.72	1.96
LoD	2.77	2.18	1.07	3.18

Detection Capability (µmol/L/h)	IDUA	GAA	GBA	GLA
LoQ	2.77	2.18	1.85	4.88
Standard deviation at LoQ	1.00	0.30	0.48	1.15
%CV at LoQ	36.1%	13.9%	26%	23.6%

e. *Analytical specificity:*

Analytical specificity studies were performed following the recommendations in the CLSI EP7-A2 Guideline. A base pool was prepared to have enzyme concentrations around the cutoffs (high risk or borderline) for each enzyme (≈ 5 µmol/L/h for IDUA, ≈ 6 µmol/L/h for GAA, ≈ 3 µmol/L/h for GBA and ≈ 6 µmol/L/h for GLA) and the hematocrit was adjusted to 50% to mimic neonatal samples. The control pools were prepared by spiking in the appropriate solvent into the base pool. The test pools were prepared by spiking in the interfering substance at an appropriate concentration into the base pool. For the hematocrit interference studies, control and test pools were prepared by adjusting hematocrit to a target of 50% (control pool), 60% (test pool, level 2) and 70% (test pool, level 3). Test and control pools were spotted onto Ahlstrom 226 grade filter paper.

The control and test pools were tested in replicates of 30 and the means and the imprecision of the results for the test pools were compared to the means and the imprecision of the control pools. Bias estimates were calculated. The following substances at the listed concentrations did not interfere with the performance of the test:

Substance	Concentration	% bias observed
Unconjugated bilirubin	342 µM	≤±10%
Conjugated bilirubin	342 µM	≤±10%
D-galactose	0.84 mM	≤±10%
D-glucose	55 mM	≤±11%
EDTA	3.4 µM	≤±10%
Heparin	3000 U/L	≤±13%
Intralipid	1000 mg/dL	≤±10%

Total protein (at 120 mg/dL) did not interfere with the GAA, GBA, and GLA assay (max bias observed was ± 13%). Hematocrit (at 70%) did not interfere with the GAA, GBA, and GLA assay (max bias observed was ≤± 10%)

The following information is presented in the package insert to describe interferences observed with the IDUA assay:

> For IDUA total protein at 75.8mg/mL did not interfere with the IDUA assay. However total protein at 88 mg/mL resulted in a bias of -16% and total protein at 100.3 mg/mL resulted in a bias of -31%.
>
> For IDUA hematocrit values lower than 59% did not interfere with the test result. However at 66% hematocrit, bias of -38% was observed.
>
> For IDUA, heparin at 3000 U/L may lead to an increased in imprecision.

In this study, 15 statistical outlier test results (5 IDUA, 2 GAA, 6 GBA and 2 GLA) were removed from the analysis (please refer to section O below). These 15 test results are outside the claimed imprecision of the test.

2. Comparison studies:

   a. *Method comparison with predicate device:*
   Not applicable, there is no predicate device.

   b. *Matrix comparison:*
   This device is only intended to be used with DBS specimens collected from neonates.

3. Clinical studies:

An evaluation of the test system was performed over the course of 24 months at a public state health laboratory that performs newborn screening. During the study 275 newborns were referred for confirmatory diagnosis. Of these 73 newborns were confirmed to have one of the 4 diseases. The 73 newborns included 1 newborn with MPS I, 17 newborns with Pompe disease, 3 newborns with Gaucher disease and 52 newborns with Fabry

disease. The incidence from the study is summarized in the table below along with the published incidence.

	Incidence from Study	Published Incidence
MPS I (IDUA)	1:153,697	1:54,000 – 1:185,000
Pompe (GAA)	1:9,041	1:28,000
Gaucher (GBA)	1:51,232	1:57,000
Fabry (GLA)	1:2,956	1:1,500 – 1:13,000

The study consisted of two phases (delineated retrospectively once the study was complete): a pilot study of 6 months that included screening of 48,813 newborns and an 18 month pivotal phase where105,599 newborns were screened. During the first 6 months of testing the device was modified several times and therefore the sponsor defined this period as a pilot phase because of the significant modifications to the device (please refer to the FDA Advisory Panel executive summary prepared for the August 10, 2016 Meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel for information on the device modifications found online at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/ClinicalChemistryandClinicalToxicologyDevicesPanel/ucm511565.htm).

A preliminary study was performed by the public state health laboratory prior to the pilot/pivotal studies to establish initial cutoff values. Approximately 13,000 presumed normal de-identified specimens and 29 known affected specimens were analyzed using the proposed device. The laboratory selected two cutoffs to evaluate test results in the pilot/pivotal studies: a borderline cutoff and a high risk cutoff for each enzyme. The cutoffs were selected to try to ensure that all known affected specimens would be detected and to minimize the false positive rate. The cutoffs used at the start of the study are shown below:

Enzyme	High Risk (µmol/L/hr)	Borderline (µmol/L/hr)
IDUA	4.0	5.0
GAA	8.0	10.0
GBA	4.5	7.0
GLA	5.5	7.0

The cutoff values were adjusted during the course of the study in order to reduce the false negative and false positive rate and also to take into account seasonal changes, i.e., increased heat and humidity that was found to affect the enzymatic activity during transport. The laboratory also instituted an additional set of cutoffs for specimens based on the age of collection as it was observed that enzymatic activity values decreased with the age of the newborn for GAA, GBA and GLA (please refer to FDA's executive summary referenced above for more information on cutoff modifications). The final high risk and borderline cutoff values (in (µmol/L/hr) used at the end of the clinical study period are shown below:

Enzyme	1-6 days of age		7-13 days of age		14+ days of age	
	High Risk	Borderline	High Risk	Borderline	High Risk	Borderline
IDUA	1.5	5.0	1.5	5.0	1.5	5.0
GAA	7.2	10.0	4.5	10.0	4.5	10.0
GBA	5.5	7.0	4.0	7.0	4.0	7.0
GLA	7.0	9.0	5.0	5.0	3.0	5.0

The laboratory's screening procedure is described briefly below. (For detailed information, please refer to FDA's Advisory Panel executive summary at http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/ClinicalChemistryandClinicalToxicologyDevicesPanel/ucm511565.htm.)

The DBS cards from each neonate were tested in singlicate. Results were interpreted as follows:

1. All valid test results above the borderline cutoff (and below an upper threshold set by the laboratory) were considered presumed normal.
2. Test results above the upper threshold were considered invalid. All DBS with invalid test results were retested in singlicate.
3. DBS with at least one enzyme below the borderline cutoff were retested in duplicate and the test results were evaluated by the laboratory for "visual outliers". (Note: If visual outliers were found, additional testing was performed.) If the average of the results (excluding test results considered visual outliers) was below the high risk cutoff a risk assessment was performed by the laboratory. The risk assessment included review of other newborn screening enzyme results, sample quality, transfusion status, health status, transit time, gestational age, family history and age at sample collection. Babies considered to still be at high risk for the screened condition after the risk assessment were referred for confirmatory testing. If the average of the results (excluding test results considered visual outliers) was above the high risk cutoff, then the babies were presumed normal.
4. Specimens collected from premature newborns or newborns aged < 24 hours at the time the sample was collected automatically mandated a repeat screen (i.e., a new DBS card was collected from the baby) and the screening procedure was repeated.

A total of 105,599 newborns were screened during the pivotal phase. Five hundred ten (510) newborns were excluded from analysis since they had no valid DBS (collected <24 hours, no recorded age at collection, poor quality) or valid test results. A presumptive false positive rate was calculated by applying the cutoffs at the end of the study to the entire pivotal phase results. The pivotal phase study results are summarized below.

	IDUA	GAA	GBA	GLA
Total Newborns	105,089			
1st test below borderline	844	1,398	753	1,419
Average test result below high risk	39	104	52	134
Not referred after risk assessment	10	60	44	79
Presumed Affected and referred for follow-up	29	44	8	55
True Positives	0	7	2	28[a]
Refused/moved	2	0	1	4
Presumptive False Positives (including newborns not referred after risk assessment)	37	97	49	102
Presumptive False Positive Rate	0.035%	0.092%	0.047%	0.097%
Presumptive False Negative Rate	0	0	0	0

[a]The number of true positives for Fabry newborns identified during the study was 30. Two newborns were identified as true positives while the lab was using a cutoff of 8.0µmol/L/hr (compared to the final cutoff of 7.0µmol/L/hr) to account for seasonal variation in the enzyme activity. Using a cutoff of 7.0µmol/L/hr could lead to false negatives.

The pivotal phase study results are summarized according to the age of the newborn at the time of sample collection in the tables below. The analysis is conducted by applying the cutoffs at the end of the study to the entire pivotal phase results:

	IDUA	IDUA	IDUA
Age of newborn at time of sample collection (days)	1 to 6	7 to 13	14+
Total Newborns	92,334	5,328	7,427
1st test below borderline	491	62	159
Average test result below high risk	29	2	6
Not referred after risk assessment	2	1	5
Presumed Affected and referred for follow-up	27	1	1
True Positives	0	0	0
Refused/moved	2	0	0
Presumptive False Positives (including newborns not referred after risk assessment)	27	2	6
Presumptive False Positive Rate	0.029%	0.038%	0.081%
Presumptive False Negatives	0	0	0

	GAA	GAA	GAA
Age of newborn at time of sample collection (days)	1 to 6	7 to 13	14+
Total Newborns	92,334	5,328	7,427
1st test below borderline	615	123	486
Average test result below high risk	68	2	7
Not referred after risk assessment	29	1	2
Presumed Affected and referred for follow-up	39	1	5
True Positives	7	0	0
Refused/moved	0	0	0
Presumptive False Positives (including newborns not referred after risk assessment)	61	2	7
Presumptive False Positive Rate	0.066%	0.038%	0.094%
Presumptive False Negatives	0	0	0

	GBA	GBA	GBA
Age of newborn at time of sample collection (days)	1 to 6	7 to 13	14+
Total Newborns	92,334	5,328	7,427
1st test below borderline	399	66	171
Average test result below high risk	29	1	5
Not referred after risk assessment	19	1	4
Presumed Affected and referred for follow-up	7	0	1
True Positives	2	0	0
Refused/moved	1	0	0
Presumptive False Positives (including newborns not referred after risk assessment)	23	1	5
Presumptive False Positive Rate	0.025%	0.019%	0.067%
Presumptive False Negatives	0	0	0

	GLA	GLA	GLA
Age of newborn at time of sample collection (days)	1 to 6	7 to 13	14+
Total Newborns	92,334	5,328	7,427
1st test below borderline	662	122	508
Average test result below high risk	97	9	5
Not referred after risk assessment	43	8	5
Presumed Affected and referred for follow-up	54	1	0
True Positives	28	0	0

	GLA	GLA	GLA
Refused/moved	4	0	0
Presumptive False Positives (including newborns not referred after risk assessment)	65	9	5
Presumptive False Positive Rate	0.070%	0.169%	0.067%
Presumptive False Negatives	2	0	0

Number of tests performed: A total of 120,118 DBS from 105,089 newborns were tested during the pivotal phase. The number of DBS is higher than the number of newborns since some newborns needed repeat screens and new DBS cards were collected. A total of 137,153 individual tests were performed on the 120,118 specimens. Of these 137,153 individual tests 7785 tests were test results (demonstrating an invalid test rate of 5.7%). However, one of the reasons for invalid test results is when the test is above an upper threshold. The remaining 9,250 tests were due to retesting triggered by activity values below borderline cutoff for one of the assays (as required by the screening protocol).

Note that during the clinical study the upper threshold for each enzyme was set above the reportable range of the assays. Since the completion of the clinical study, the upper thresholds for the assays have been changed to match the top value of the reportable range of the assays. Therefore, the invalid test rate due to a value above the upper threshold may be higher than observed during the clinical study.

The following is a summary of the limitations of the study included in the package insert:

> Risk Assessment: Because test screen positive newborns that were presumed normal following the risk assessment process were not sent for follow up, clinical truth for these test screen positive newborns was not confirmed and false negatives are possible.
>
> Acquiring repeat specimens: During the clinical study, the laboratory was not permitted to rescreen and collect new samples based on the results of the proposed device. Any additional screens were a result of premature infants, sick infants, infant samples collected at age <24 hours or other routine newborn screening results. Therefore, some samples that were suspected to be of poor quality (when for example all of the LSD test results were low) were presumed normal, according the laboratory's risk assessment procedure, and a repeat screen could not be collected.
>
> Cutoffs below LoQ: During the pivotal phase, two of the cutoffs used for the IDUA assay were below the limit of quantitation for IDUA (LOQ=2.77 µmol/L/hr). A total of 49 newborns had IDUA test results between the high risk cutoff at the time of testing and the LOQ and were presumed normal by the laboratory's screening procedure.
>
> Additionally, one of the age specific cutoffs (14+ days of age) used for the GLA

assay was below the limit of quantitation (LOQ=4.88 µmol/L/hr). A total of 50 newborns had GLA test results between the high risk cutoff at the time of testing and the LOQ and were presumed normal by the laboratory's screening procedure.

Regarding the false negative rate of the test, the state public health laboratory has an active surveillance program to track any reports of false negative results to the contracted metabolic centers they use for confirming diagnosis of any of these LSDs. Based on information from this surveillance program there were no known false negative results due to an incorrect screening result during the 2 year study. It is presumed that the newborns screened during the clinical study with early onset disease would have been reported to one of these metabolic centers.

The package insert includes the following information about the false negative rate:

> Certain late onset forms for Pompe disease may have GAA enzymatic activity in the normal range and result in a false negative[10].
>
> For female Fabry disease patients GLA enzyme activity is highly variable and it could overlap with the normal range. Therefore, GLA enzyme activity for females should be interpreted with caution as some female carriers can have enzyme activity in the normal range and result in a false negative.

On August 10, 2016, a meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel of the Medical Devices Advisory Committee was held to help inform FDA on the safety and effectiveness of the SEEKER System. The panel unanimously agreed that the probable benefits of the device outweighed the probable risks. Please refer to the transcript of the meeting found online at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/ClinicalChemistryandClinicalToxicologyDevicesPanel/ucm511565.htm).

4. Expected Values

The 0.1th, 0.5th, 50th, 99.5th and 99th percentile values from the testing of presumed normal newborns with the proposed device at the state public health laboratory are summarized below for each age group. All values are in µmol/L/hr.

Reference limits for newborns age 1-6 days

	IDUA	GAA	GBA	GLA
%ile	N=102,399	N=102,392	N=102,397	N=102,371
0.1%	3.68	7.61	6.69	7.56
0.5%	5.63	10.07	8.20	9.44

[10] Chiang S-C, Hwu W-L, Lee N-C, Hsu L-W, Chien Y-H. Algorithm for Pompe disease newborn screening: Results from the Taiwan screening program. Mol Genet Metab. 2012;106 (3):281-286.

	IDUA	GAA	GBA	GLA
50.0%	18.37	25.98	20.24	26.73
99.5%	46.23	64.56	51.03	123.36
99.9%	>50.75	77.41	64.53	>153.74

Reference limits for newborns age 7-13days

	IDUA	GAA	GBA	GLA
%ile	N=7,177	N=7,177	N=7,177	N=7,176
0.1%	3.15	5.04	5.16	5.14
0.5%	4.82	7.75	6.60	6.39
50.0%	18.82	21.53	16.56	19.70
99.5%	>50.75	73.01	54.83	96.19
99.9%	>50.75	>94.66	>73.24	134.27

Reference limits for newborns age >14 days

	IDUA	GAA	GBA	GLA
%ile	N=7,447	N=7,447	N=7,447	N=7,447
0.1%	2.83	4.40	4.31	4.88
0.5%	3.93	6.43	5.83	5.36
50.0%	19.41	17.76	14.61	14.64
99.5%	>50.75	54.68	45.21	67.34
99.9%	>50.75	72.21	64.54	113.61

The labeling for the SEEKER System, states that each laboratory should establish their own reference ranges and cutoffs.

M. Instrument Name:

SEEKER Instrument

N. System Description:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device? Yes _X_ or No _____.

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission: Yes ___or No __X____.

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

2. Specimen Identification:
   Specimens are extracted in a 96-well plate and the extracts from one plate are transferred to two SEEKER cartridges. The software will prompt the user to enter the sample and reagent layout information for that cartridge.

3. Specimen Sampling and Handling:
   Specimens are processed according to the package insert instructions.

4. Calibration:
   The 4 calibrators are run with each cartridge. The device software calculates a slope, intercept and R^2 by linear regression for every run. If the calibration for a run is flagged as "failed" no activity values are reported for the entire run and the entire run must be repeated.

5. Quality Control:
   QC samples are provided with the kit. Each cartridge should include at least one punch of two levels of QC (QCL and QCM) to determine the validity of the run. These QC samples are subjected to the same protocol steps as the newborn specimen and the enzymatic activity for all 4 assays is determined. Mean enzymatic activity values and standard deviation is provided with each reagent included in the reagent kit. Each laboratory should establish mean and standard deviation values and determine their own acceptable range of enzymatic activity for QC samples.

   The instrument also checks the non-enzymatic hydrolysis value for each enzyme against a pre-defined range. If the values are outside this range, the activity is not calculated and an "n/a" result is reported for the activity.

   Test results above the upper threshold (which is the upper limit of the reportable range) are highlighted and these samples should be considered invalid and retested.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Outliers: In the analytical studies statistical high outliers were observed at a rate of 0.103% for IDUA and GAA, 0.144% for GBA and 0.092% for GLA. These test results were outside the expected performance of the assays and were excluded from the analysis where noted above. If they occur during routine testing, these incorrectly elevated test results could result in a false negative test. The sponsor stated that because of the low prevalence of the diseases and the low frequency of the observed outliers, the likelihood that an affected neonate would obtain an incorrectly elevated test result is very low.

Carry-over: Carry-over was evaluated using two specimen layouts for each test: one with carry-over and another with no carry-over. The carry-over layout included DBS with concentrations representing affected samples and DBS with concentrations representing normal to high enzyme activity (samples 1 to 4). The no carry-over layout only included DBS with concentrations representing affected samples (no carry-over). Carry-over was estimated as the percent bias between the average values of representative affected specimens obtained between the carry-over and no carry-over layout. One result determined to be a statistical outlier was observed. The results with and without the outlier are summarized below. In the table below the results of the affected samples with and without carry-over are reported in the columns labeled "affected sample" and the results of the samples with normal to high activity are reported in the columns labeled "high sample".

Assay	Affected Sample			High Sample			Carryover µmol/L/hr	Bias
	Carry-over Sample	N	Mean µmol/L/hr	Carry-over Sample	N	Mean µmol/L/hr		
IDUA	No carryover	150	4.07					
	1	64	4.00	1	88	13.55	-0.07	-2%
	2	64	4.23	2	88	26.16	0.16	4%
	4	64	4.34	4	32	53.26	0.27	7%
GAA	No carryover	115*	8.87					
	1	63	8.77	1	88	16.59	-0.10	-1%
	2	60	9.24	2	84	30.28	0.37	4%
	4	64	8.97	4	32	58.89	0.10	1%
GBA	No carryover	149	6.37					
	1	64	6.34	1	88	13.62	-0.03	0%
	2	63	7.04	2	88	25.11	0.67	10%
	3	62	6.72	3	32	36.18	0.35	6%
	4	64	7.23	4	32	49.57	0.86	13%
GLA	No carryover	152	9.11					
	1	64	9.23	1	88	30.60	0.11	1%
	2	64/63*	11.59/10.28*	2	88	58.99	2.48/1.17**	27%/ 13%*
	3	64	10.39	3	32	83.40	1.28	14%
	4	64	11.07	4	32	120.79	1.95	21%

*For the GAA study, one run failed and was not repeated.
**For the GLA study one test result identified as a statistical outlier was removed from the analysis.

Based on the results, the following recommendation is included in the package insert:

A specimen with GLA activity within +2μmol/L/hr of the borderline cutoff should be retested if a specimen with GLA activity ≥120μmol/L/hr is present in the same specimen column (cartridge has 12 columns and 4 rows).

P. Proposed Labeling:

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

Q. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device. However, during the August 10, 2016 meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel of the Medical Devices Advisory Committee for the SEEKER System, multiple patients (or the parents of patients) spoke during the public comment period. All patients (or the parents of patients) were supportive of the authorization for marketing of this device. See the transcript of the meeting found online at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/ClinicalChemistryandClinicalToxicologyDevicesPanel/ucm511565.htm

R. Identified Risks to Health and Required Mitigations:

Identified Risks to Health	Required Mitigations
Inaccurate test results that provide false negative test results could lead to a newborn to not be detected as a possible lysosomal storage disorder case and to be delayed from timely therapy.	Special Controls (1) and (2)
Inaccurate test results that provide false positive test results could lead a newborn to have unnecessary additional confirmatory testing and to add emotional burden to the family of the newborn.	Special Controls (1) and (2)

S. Benefit/Risk Analysis

Summary	
Summary of the Benefit(s)	Newborns may potentially benefit from the use of the device as an aid in screening because it might assist practitioners in making earlier decisions that diagnostic testing is needed for patients with MPSI, Pompe, Gaucher, and Fabry disease which can lead to earlier potentially beneficial treatment.
Summary of the Risk(s)	Associated device risks include erroneous false negative results due to device or user error or false positive results due to device or user error. A false positive result could result in unnecessary additional testing for the newborn and add emotional burden to the family of the newborn. A patient with a false negative result could fail to be detected as a possible LSD case and be delayed from timely therapy.
Summary of Other Factors	On August 10, 2016, a meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel of the Medical Devices Advisory Committee was held to help inform FDA of the safety and effectiveness of the SEEKER System. Clinicians including geneticists and experts in the field of clinical chemistry and biostatistics were members of the panel. The panel unanimously agreed (10 out of 10) that the probable benefits of the device outweighed the probable risks for the proposed intended use and provided recommendations for the types of information that should appear in the labeling for this device to provide a reasonable assurance of the safety and effectiveness of the device. The panel stated that they supported the granting of this de novo given the

	real-world large clinical study performed and the lack of currently available FDA cleared/approved devices for lysosomal storage disease screening in newborns (unmet need), and the potential for standardization in testing between states. Given the results of the pivotal clinical study and the special controls incorporating the panel's recommendations, which include demonstrating the screening performance of the device and including information on test performance including the false positive rate, and the false negative rate observed in the clinical study in the labeling, will mitigate the risk of erroneous false positive and false negative results, the probable benefits of this device outweigh the probable risks for this device.
Conclusions Do the probable benefits outweigh the probable risks?	
Yes, the probable benefits outweigh the probable risks of this device in light of the special controls assigned, along with general controls, including design controls.	

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 862.1488. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. This device is classified under the following:

Product Code:	PQW, PQT, PQU, PQV
Device Type:	Lysosomal storage disorder newborn screening test system
Class:	II (special controls)
Regulation:	21 CFR 862.1488

(a) *Identification*. A lysosomal storage disorder newborn screening test system is intended to measure lysosomal enzyme levels obtained from dried blood spot specimens on filter paper from newborns as an aid in screening newborns for a lysosomal storage disorder.

(b) *Classification*. Class II (special controls). A lysosomal storage disorder newborn screening test system must comply with the following special controls:

1. Premarket notification submissions must include information that demonstrates the performance characteristics of the device, including:
    (i) Study results that adequately demonstrate the clinical validity of the device, which shall include information supporting the link between the analyte being measured and the condition being screened. The clinical validity of the device must be demonstrated in a clinical validation study using either well characterized prospectively or retrospectively obtained clinical specimens from the intended use

population. Testing in the clinical validation study must be performed by operators representative of the types of operators intended to use the test. The study design of the clinical validation study must assess the effects of sample collection and processing steps on test performance. Confirmed positive specimens must have a diagnosis based on confirmatory diagnostic methods or clinically meaningful information regarding the status of the subject must be obtained.

(ii) The reference interval in the normal newborn population for the analyte or analytes measured by the device.

(iii) Study results demonstrating the level of carry-over or drift affecting the device performance.

(iv) Study results demonstrating the concentrations of the limit of blank, limit of detection, and limit of quantitation of the device. Sample concentrations below the limit of quantitation should not be reported by the device.

(v) Study results, which shall be collected using sample panels from at least three reagent lots and at least three instruments over more than 20 testing days, demonstrating the imprecision of the device. The sample panels must consist of blood spot specimens with a range of analyte concentrations that span the reportable range of the device and must include samples with concentrations in the screen positive range, samples with concentrations at each cutoff, and samples with concentrations in the normal range.

2. Your 21 CFR 809.10 compliant labeling for this device must include:

(i) A warning that reads "This test is not intended to diagnose lysosomal storage disorders."

(ii) A warning that reads "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."

(iii) Detailed information on device performance, including the false positive rate and the false negative rate observed in the clinical study.

(iv) Information on device performance in any relevant subgroup (e.g., age of newborn at time of sample collection, birth weight, sex, gestational age, race, ethnicity) observed in the clinical study.