SEEKER™
DEN150035

Executive Summary
Prepared for the
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of the
Clinical Chemistry and Clinical Toxicology Devices Panel
of the
Medical Devices Advisory Committee

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1. EXECUTIVE SUMMARY

1.1. Newborn Screening

Figure 1: Dried spots of blood are collected from each newborn prior to hospital discharge.

Newborn screening (NBS) is regarded as one of the largest and most successful public health programs implemented in the United States. Nearly every newborn is screened for, on average, 43 congenital diseases through biochemical tests that identify affected newborns so that life-saving therapies can be administered before irreversible damage, and in some cases death, occurs. The tests are performed from newborn dried blood spots (DBS; Figure 1), which are typically collected approximately 24 hours after birth and mailed to state public health laboratories for analysis. There are 37 such laboratories (mostly by state with some regional consolidation) in the United States. NBS is typically high throughput testing in a single laboratory, where hundreds to thousands of samples are processed for multiple tests each day. Each state defines its own test menu that is influenced by the U.S. Department of Health and Human Services Secretary's Advisory Committee of Heritable Disorders in Newborns and Children, which recommends the minimum test menu (the Recommended Uniform Screening Panel, or RUSP), and by patient advocacy groups that lobby for state laws related to NBS.

1.2. Newborn Screening for Lysosomal Storage Disorders

Lysosomal storage disorders (LSD) are a group of approximately 50 rare inherited metabolic disorders that are caused by lysosomal dysfunction, usually as a consequence of a deficiency in a single enzyme required for the metabolism of lipids, glycoproteins or mucopolysaccharides. SEEKER™ is designed to screen for 4 LSDs: Mucopolysaccharidosis Type I (MPS I), Pompe, Gaucher and Fabry. MPS I and Pompe were recently added to the RUSP; thus, there is a real need for newborn screening tests for these disorders.

To date, there is no FDA cleared or approved *in vitro* diagnostic (IVD) kit for newborn screening or diagnosis of LSDs. Lack of an IVD kit poses a significant challenge to state public health laboratories that are legislatively mandated to perform LSD NBS for their entire newborn populations. Currently, each laboratory must develop their own site-specific laboratory developed tests (LDTs) to meet medical needs for standard of care newborn testing. Examples of this type of testing are plentiful and include LSD LDT testing by Illinois using reagents provided by Perkin Elmer, Inc.[1,2]; by Kentucky using reagents provided by Perkin Elmer; and by Wisconsin, North Carolina, and Georgia using reagents provided by Perkin Elmer and obtained under NIH-funded contracts[3]. It is important to note that LDTs are not subject to rigorous premarket review of either analytical or clinical performance before entering the market, and under current CLIA regulatory requirements, there is no opportunity to share results of test performance in a public venue.

An FDA approved test platform for NBS of LSDs will provide a validated answer to this critical dilemma. The sponsor's SEEKER™ is simple to use, install, and operate and will meet a growing unmet need to screen newborns for LSDs in public health laboratories. This is particularly important for the growing number of states (Arizona, Illinois, Kentucky, Michigan, Missouri, New Jersey, New Mexico, New York, Ohio, Pennsylvania, and Tennessee) that have legislatively mandated newborn screening for LSDs, and many other states that are considering adding LSD NBS due to recent recommendations from the U.S. Secretary of the Department of Health and Human Services.

Approval of SEEKER™ will assure that platform performance characteristics have been independently analyzed by FDA regulatory scientists, will provide transparency by public disclosure of an FDA decision summary, and will demonstrate that post market controls are in place. These controls include regulatory requirements that assure quality production (i.e. quality system regulations are enforced through FDA on-site manufacturing inspections) and post-market monitoring for adverse events through FDA medical device reports.

1.3. Intended Use

The SEEKER™ System is intended for quantitative measurement of the activity of multiple lysosomal enzymes from newborn dried blood spot samples. Reduced activity of these enzymes may be indicative of a lysosomal storage disorder. The enzymes measured using the SEEKER™ Reagent Kit and their associated lysosomal storage disorder are indicated below.

Enzyme (abbreviation)	*Disorder*
α-L-iduronidase (IDUA)	Mucopolysaccharidosis Type I (MPS I)
α-D-glucosidase (GAA)	Pompe
β-glucocerebrosidase (GBA)	Gaucher
α-D-galactosidase A (GLA)	Fabry

Reduced activity for any of the four enzymes must be confirmed by other confirmatory diagnostic methods.

1.4. SEEKER™ Clinical Summary

A 24-month, full population study was conducted in collaboration with the Missouri State Public Health Laboratory (MSPHL) to screen all newborns for enzymes associated with 4 LSDs (Pompe, Fabry, Gaucher and Mucopolysaccharidosis Type I (MPS I)), representing a very rich data set of 153,697 newborns. This multiplex study was the first of its kind for lysosomal storage disorders in the United States or elsewhere and was conducted under realistic laboratory screening conditions. The results of this study are expected to help newborn screening laboratories establish their expected disease incidence (true positive rate), screen positive rate, false positive rate, false negatives, and retest rate when the platform is used as intended. During the study, 73 newborns were identified as having one of the 4 LSDs. To date, no clinical false negatives have been reported through screening in Missouri; false negatives are the primary driver of safety for a newborn screening device. The period of surveillance for false negatives

includes the 24 month study (January 15, 2013-January 14, 2015) and a 15 month follow up period post study.

The confirmed true positive cases included 1 newborn with MPS I, 17 newborns with Pompe, 3 newborns with Gaucher, and 52 newborns with Fabry. The only confirmed MPS I positive newborn reported as a severe case of MPS I (Hurler disorder).Seventeen newborns were confirmed affected with Pompe disorder during the clinical study. Five of these newborns were confirmed to have infantile onset Pompe disorder and nine newborns were confirmed with late onset Pompe disorder. Three of the newborns were confirmed to have genetic mutations of unknown significance; they will require long term follow up to test for disease onset. Three newborns were confirmed affected with Gaucher disorder. One newborn was confirmed to have Type 1 – non-neuronopathic Gaucher disorder of unknown onset. The other two newborns were found to have mutations of unknown significance; they will also require long term follow up. Fifty-two newborns were confirmed affected with Fabry disorder during the study. The status of 45 of these newborns was reported by the referral center as "Fabry disorder" without a classification of onset. Three newborns were confirmed with mutations consistent with late onset Fabry disorder and four newborns were confirmed to have mutations of unknown significance.

Based on the number of newborns analyzed for this two-year study period (n=153,697, the incidence of each of the disorders was calculated and is summarized along with previously published incidence (Table 1).

Table 1: Incidence rate during clinical study

	Incidence from MSPHL Study	**Published Incidence[4,5]**
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 estimated incidence rates in the literature result in a combined incidence rate of 1:2,500. Based only on confirmed positive cases, data from the current study in Missouri indicate a slightly higher incidence rate of 1:2,105. For Pompe disorder the MSPHL incidence was about 3 times higher than published rates of incidence. For MPS I, Gaucher, and Fabry the MSPHL incidence is comparable to the published rates of incidence. The fact that the combined incidence rate in the study agrees with and is better than published literature suggests that risk related to false negatives is minimal. If all of the approximately 4 million babies born each year in the U.S. are screened for these 4 conditions, it is estimated that 2,000 children annually will be identified with one of these conditions for which effective therapies exist.

1.5. Risks of Testing

For an NBS test, the greatest risk is obtaining false negative findings. False negative NBS tests result in a missed opportunity for early identification and treatment of the condition of interest. Depending on the disorder, false negatives may result in morbidity and mortality with an early onset disorder or, at the very least, morbidity as a result of a late onset disorder. As with all NBS

tests, maximizing test sensitivity to minimize false negatives is a desired goal. The ***absence of testing*** has a much greater negative impact on health outcomes than testing using assays with less than 100% sensitivity. The key to a successful screening program is to minimize the risk of false negatives while balancing the extent of false positives.

False positives are also a concern for a new NBS test. Within the scope of false positives, there is a trade-off with false negatives; state laboratories strive to increase the false positive rate while ensuring no false negatives. Although existing confirmatory testing methods mitigate the health impact of false positives and ensure that no therapies are administered to unaffected children, results from false positive tests can generate greater costs, confusion, and parental anxiety, all of which can negatively impact public acceptance of a new test.

Though there are no consensus targets for a false positive rate, a false positive rate of less than 0.3% has been proposed by a consortium of over 60 newborn screening programs as evidence of adequate tandem mass spectrometry newborn screening test performance[6]. Reported false positive rates from state newborn screening programs for another enzymatic newborn screening assay (galactosemia) vary widely from 0.002% to over 0.8%[7]. A recommended positive predictive value of >20% has been proposed for first tier newborn screening tests[6]. First-tier tests that fall near or below this cutoff are prime candidates for second-tier testing. Sequencing as a second tier test can improve screening specificity by identifying specific pathogenic genetic variant(s) associated with each LSD, including variants of unknown significance. Second tier testing will enable very early identification of pseudodeficiencies and contribute to a lower false positive rate.

1.6. Misdiagnosis Risk Mitigation

A critical challenge in NBS is to identify test-specific cutoff points and define other Risk Assessment methods that correctly identify affected newborns (i.e. no false negatives) while minimizing preliminary over-diagnosis (false positives). Having a standardized framework for Risk Assessment and follow up care of conditions identified through newborn screening can improve newborn healthcare and synchronize NBS across different programs[8]. Diagnostic and clinical follow up services ensure that all screen positive results undergo confirmatory testing and they are an essential part of all newborn screening programs.

In a real world study using SEEKER™ at the Missouri State Public Health Laboratory, the NBS expert laboratory director, in consultation with an LSD task force (comprised of geneticists, genetic counselors, a chemist, NBS follow up staff, and an adult Fabry patient), made multiple adjustments to the cutoff values over time to minimize false negatives and false positives. The number of false positive results was minimized by a system of checks that included a Risk Assessment process and reduction of referrals for positive tests that were identified to be caused by laboratory or clinical features not suggestive of disease. These objective criteria included premature birth, other sample from the same newborn, multiple enzymes below borderline (possibly indicating a poor quality sample), etc. One such example is when an infant is born prematurely, standard clinical practice in newborn screening is to obtain a second sample; therefore the laboratory will typically wait for that second sample before making a presumed positive referral decision.

In Missouri, once a test was presumed positive, the newborn was referred to one of 4 referral centers throughout the state, as well as identified through a NBS follow up center. The referral

centers coordinated additional tests between parents and physicians, and notified the MSPHL about any test results. This coordinated effort ensured that results were communicated to MSPHL and that follow up continued for false negatives. In addition, in keeping with common practice in newborn screening laboratories, the risk of false negatives was minimized by implementing a retesting algorithm for all samples with enzyme activity around levels indicative of disease to account for sample and analytical variability. False negatives were also minimized through an extensive follow up procedure in Missouri for assessing clinical outcomes.

Quality control (QC) materials, including dried blood spots made from newborn cord blood, aid in SEEKER™ QC monitoring and ensure consistent assay performance. These blood spots undergo all of the preparation and analysis steps that the DBS samples are subject to and are included on each assay cartridge (described in Section 4). Appropriate calibrators are also included within each cartridge to normalize for variation between cartridges and analyzers.

Detailed analytical validation was performed to determine precision, linearity, limit of detection, interfering substances, stability, and carryover. Based on the device performance during analytical and clinical validation, cutoff values from these studies will be provided by the sponsor. Consistent with individual state policies, it is recommended that each newborn screening program develop cutoffs based on the regional population and adjust the cutoff values within the limits of the performance of the device to minimize false positives and ensure no false negatives. This is demonstrated in the cutoff value variability for another NBS enzymatic assay for galactosemia, which saw cutoff values vary between 2.4 to 4.3 U/dL in a study of multiple state public health programs that used the same FDA cleared reagent kit[7].

The probable benefits of this device to both the infant and for the public health programs outweigh the probable risks associated with its use, given the robust analytical performance characteristics and thorough clinical validation in a realistic intended use setting. Approval of the SEEKER™ will ensure that a regulated test is available for newborn screening of LSDs. LSD screening for the 4 LSDs (MPS I, Pompe, Gaucher and Fabry) will be standardized across all laboratories, providing an LSD testing solution to meet RUSP and ensure compliance with state legislations.

2. DEFINITIONS AND ABBREVIATIONS

Assay: Quantitative measurement of the activity of a single analyte (enzyme). Each test has four assays.

Borderline Cutoff: Borderline cutoff is set above the high risk cutoff to account for imprecision around the high risk cutoff. Samples above the borderline are presumed normal. Samples below the borderline cutoff are typically repeated to obtain a final average result, based on which further disposition occurs.

Card: A small sheet of filter paper for collection of dried blood spots from a newborn. Each card should contain five (5) full dried blood spots from a single newborn, collected during a standard time frame (24 to 48 hours postnatal age).

Carrier: Indicates the presence of an inherited recessive allele for a genetic trait or a mutation for which there is no clinical presentation of symptoms. Carriers are, however, able to pass the allele onto their offspring, who may then manifest the disorder if they inherit the recessive allele from both parents. Carriers can exhibit lower enzymatic activity than normal population. If identified through newborn screening, carriers are considered as false positives.

Cutoffs: A lab-established quantitative enzymatic activity value below which samples are flagged for further resolution. The common practice in newborn screening programs is to have two cutoffs – a high risk cutoff and a borderline cutoff.

Dried Blood Spot (DBS): One of the five circular dried blood specimens on a newborn screen collection card.

False Negative: A newborn who was presumed normal through the newborn screening process and who later on is reported with a diagnosis of the disease.

False Positive: A newborn who was presumed affected and referred for diagnostic testing, which revealed the absence of disease.

False Positive Rate: False positive rate is calculated by dividing the total number of true positive samples by the total number of newborns screened (minus true positive samples and samples lost to follow up).

GAA: Abbreviation for α-D-glucosidase (EC 3.2.1.20). Pompe disorder is associated with deficient GAA activity.

GBA: Abbreviation for β-glucocerebrosidase (EC 3.2.1.45). Gaucher disorder is associated with deficient GBA activity.

Genotype of Unknown Significance: Presence of a genotype combination that is not currently found in the registry of mutations for a particular disorder.

GLA: Abbreviation for α -D-galactosidase A (EC 3.2.1.22). Fabry disorder is associated with deficient GLA activity.

High Risk Cutoff: High risk cutoff is the clinical decision making level. Samples below the high risk cutoff are at a higher risk of having the disorder.

IDUA: Abbreviation for α -L-iduronidase (EC 3.2.1.76). Mucopolysaccharidosis Type I disorder is associated with deficient IDUA activity.

Infantile Onset: Onset of disorder symptoms within the first few months of life (infancy).

Late Onset: Onset of disorder symptoms from early childhood to adulthood.

Newborn: Generally defined as a child between birth and 30 days of life (inclusive).

Presumed Affected: A newborn who is identified in screening as high-risk for disease and thus requires confirmatory diagnosis.

Presumed Normal: A newborn who is identified in screening as low risk for disease.

Pseudodeficiency: Indicates the presence of a known mutation for a disorder which causes reduced or no enzymatic activity in *in vitro* testing without the presentation of clinical symptoms. If identified through newborn screening, pseudodeficient newborns are considered false positives.

Punch: A single 3.2 mm circle removed from a spot. Up to eight (8) punches can be extracted from a single dried blood spot; given five full spots, up to 40 punches can be extracted from a single screen. Each "test" requires extract from a single punch.

Sample: Can be used interchangeably with "screen" and "specimen"; denotes a single specimen card with five (5) full dried blood spots from a single newborn, collected at the same time. Multiple samples can be collected from a single newborn at different points in time. Missouri guidelines require multiple screens be collected from a newborn in a number of circumstances, including blood transfusion and/or premature birth.

Screen: Can be used interchangeably with "sample" and "specimen"; card for collection of dried blood spots from a newborn. Each card should contain five (5) full dried blood spots from a single newborn, collected at the same time.

Screen Negative: See "presumed normal".

Screen Positive: See "presumed affected".

Specimen: Can be used interchangeably with "sample" and "screen"; card for collection of dried blood spots from a newborn. Each card should contain five (5) full dried blood spots from a single newborn, collected at the same time.

Spot: One of the five circular dried blood specimens on a screen card.

Test: One set of multiplexed assays performed on blood spot extract. Each test requires extract from a single DBS punch. Multiple tests may be performed on a single screen, but each test requires a separate punch.

True Positive: A presumed affected newborn who is confirmed to have the disorder by diagnostic testing.

Unclassified Onset: Confirmed disorder was labeled unclassified if the diagnostic test center did not provide an onset while reporting back to MSPHL.

Unknown Onset: Confirmed disorder by enzymatic and confirmatory diagnosis of which the type of onset (infantile onset, late onset) is unknown.

3. BACKGROUND INFORMATION

3.1. Newborn Screening in the United States

In the U.S., newborn screening in state public health laboratories (PHLs) and/or private companies is performed using newborn heel-stick blood collected and dried on filter paper and is considered a standard of care for pediatric medical practice. The goal of NBS is to identify newborns at risk for developing disabling and potentially fatal conditions as early as possible, thereby providing a window of opportunity for early treatment, often before the onset of clinical symptoms. Early detection and treatment can have a profound impact on the clinical severity of the condition and the long-term prognosis of the affected child. Without prompt diagnosis and treatment, the consequences of many of the targeted disorders are dire and may include irreversible neurological damage, intellectual, developmental and physical disabilities, and death. Although all states require newborn screening for every infant, the number of conditions on a state's screening panel and the types of conditions on the panel vary from state to state.

3.1.1. Current Screening Status

An average of 43 conditions is screened in PHLs. As shown in Figure 2, the number of screening tests in the U.S. has risen substantially since its beginning in the 1960's[9,10]. However, there is good reason to add to the screening panel; there are over 6,000 rare diseases and more than 100 FDA-approved therapies for pediatric diseases that may potentially benefit babies through newborn screening.

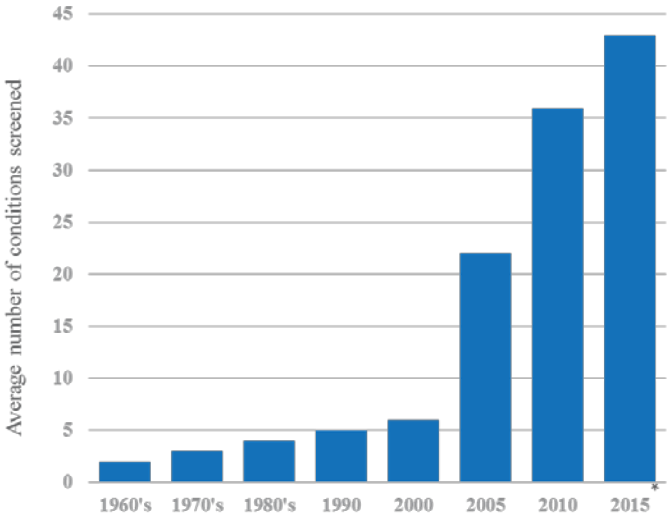


Figure 2: Average number of conditions screened across all U.S. states since the start of NBS in the 1960's.

Thirty-seven of the fifty states conduct NBS in their own state PHL, while the remaining states outsource their screening to other programs (e.g. North and South Dakota send specimens to Iowa for screening). In order to encourage uniform and comprehensive newborn screening across the nation, the U.S. Department of Health and Human Services Secretary has recommended a list of Core Conditions (also known as the Recommended Uniform Screening Panel, or RUSP) that should be included in all newborn screening programs. The majority of state PHLs stipulates that they follow and implement, at a minimum, RUSP guidelines when adding new disorders to their NBS panel[11]. At present, an average of only 30 of the 34 recommended core disorders are screened in each state. Multiple factors contribute to this discrepancy, including the fact that PHLs must have validated testing methods in place to screen for each new disorder and they must have the financial and logistical resources (e.g. personnel and laboratory equipment) necessary to support the addition of new tests.

3.1.2. Considerations for Adding New Tests to State Panels

A robust NBS program is comprised of six key components: education, screening, follow up, diagnostic confirmation, management, and ongoing self-evaluation. When considering onboarding a new NBS assay (test), advisory committees within state public health laboratories must independently review the available scientific evidence related to the disorder of interest and will often seek input from experts and other state newborn screening laboratories. Sometimes the decision-making process might involve a combination of agencies, advisory bodies, and policy makers. Once a decision is made to onboard a new disorder, the state follows College of American Pathologists (CAP), Clinical Laboratory Improvement Amendments (CLIA), and/or Clinical and Laboratory Standards Institute (CLSI) guidelines to identify a vendor and verify and/or validate analytical parameters (such as linearity, precision, accuracy and reporting range) before performing a population study to establish demographic and geographical specific cutoff values.

Typically, a population study will use cutoffs from another program as a starting point[12,13,14]. However, given that factors such as population demographics and environmental conditions (temperature, humidity, etc.), which are known to strongly affect the activity of certain enzymes and can be highly variable between states, it is imperative that each state PHL empirically determine the cutoff values most appropriate for their population[15,16,17]. Once the population study is completed and initial laboratory-specific cutoffs are in place, assay results and cutoffs are closely monitored over the first few months to one year of operation[18]. During this time, program staff, including epidemiologists and medical management physicians, maintains close contact with follow up program officials to determine the correlation between cutoff screen limits and presenting phenotype. These observations help to further refine cutoff values in the state laboratory.

Since most NBS disorders are very rare, it is common to continually revise cutoff values as more data points become available[19,20]. In fact, states often recognize that this is the most arduous part of the process for onboarding a new disorder[21]. The NBS PHL is accustomed to working with medical management physicians and epidemiologists, to refine cutoff values even for mature tests. As all NBS PHLs in the U.S. are at a minimum CLIA certified, and some are even CAP accredited, they are required to have a clinical consultant to help in decision making processes surrounding clinical questions. Furthermore, all NBS PHLs are considered high-complexity labs and therefore must have a lab director possessing a High Capacity Lab Director certificate. As such, this director is tested on typical disease profiles, including those pertaining to newborn screening. The CLIA technical supervisor in each laboratory is required to be a medical doctor, a scientific doctor, or possess scientific training typically represented by the achievement of a bachelor's or master's degree. At minimal, the CLIA technical supervisor should have a B.S. degree. Additionally, they are required to have experience working in high complexity laboratories. This experience enables the technical supervisor to evaluate the technical quality of the data generated and, in conjunction with the lab director, clinical consultant, follow up personnel, and medical management physicians; it allows a team of highly qualified individuals to adjust testing parameters based on analytical data, phenotypic presentation, and demographic and geographical bias.

3.2. Lysosomal Storage Disorders

A lysosome is an organelle found in most human cells except erythrocytes (red blood cells). Lysosomes contain hydrolytic enzymes which are capable of breaking down biomolecules including proteins, nucleic acids, carbohydrates and lipids. Lysosomal storage disorders (LSD) are a group of approximately 50 rare, inherited metabolic disorders that are caused by lysosomal dysfunction, usually as a consequence of a deficiency in a single enzyme required for the metabolism of lipids, glycoproteins or mucopolysaccharides, as shown in Figure 3. FDA-approved enzyme replacement therapy is now available for the following lysosomal storage disorders: Pompe, Fabry, Mucopolysaccharidosis Type I (MPS I), Mucopolysaccharidosis Type II (MPS II), Mucopolysaccharidosis Type VI (MPS VI) and Gaucher[22]. Infants with these disorders will benefit the most from early detection by NBS and immediate treatment because, if not diagnosed and treated early, they often present with irreversible organ damage and death[23,24]. There are currently more than ten additional LSD therapies in various stages of development.

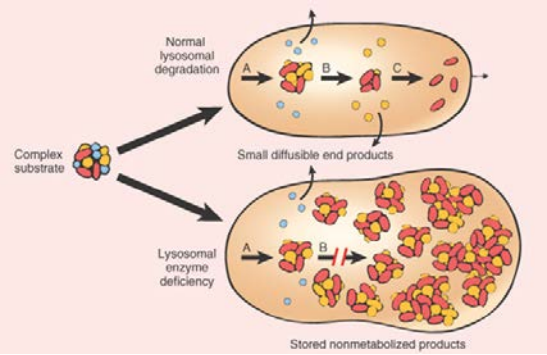


Figure 3: Accumulation of substrate in a patient with lysosomal enzyme deficiency.

3.2.1. Impetus for LSD Newborn Screening

Without newborn screening LSDs are diagnosed through a combination of enzymatic assays, chest x-rays, analysis of glycosaminoglycans, and molecular analysis only after the presentation of symptoms. Pre-symptomatic newborn screening for LSDs and subsequent early treatment prevents the onset of irreversible organ damage and death. There are no FDA approved methods for testing for LSDs.

Despite the lack of an FDA approved test on the market, two factors are leading to an increase in the number of state public health laboratories that are adopting testing for LSDs: 1) state legislatures mandating population-wide newborn screening for LSDs and 2) the U.S. DHHS Secretary's addition of Pompe[25] and MPS I[26] to the RUSP. Initial results of the MSPHL clinical study using SEEKER™ were presented during DHHS evidence review for these conditions and were instrumental in the final decision to add Pompe and MPS I disorders to the RUSP[4,5].

Currently, state laws in Arizona[27], Illinois[28], Kentucky[29], Michigan, Missouri[30], New Jersey[31], New Mexico[32], New York[33], Ohio[34], Pennsylvania[35] and Tennessee[36] have mandated screening for different combinations of LSDs. To meet the state requirements for newborn screening, state laboratories must develop Laboratory Developed Tests (LDTs) which are not currently regulated by the FDA. Implementation of an FDA regulated device, such as SEEKER™, provides consistency in testing methodology, including validated results and stable reagents, for LSD newborn screening.

Eighteen newborns affected with either Pompe or MPS I disorders (RUSP specific LSDs) were identified through the two year clinical study of SEEKER™ in Missouri and a total of 73 newborns were identified with one of the four disorders. The identification of these infants

through NBS has enabled proactive therapies to be pursued, where clinically appropriate, and has thereby increased the chances for these children to lead healthy lives. In doing so, SEEKER™ is fulfilling the vision of the RUSP to improve the long-term health of children affected with screenable disorders. Going forward, the Affordable Care Act requires that health care plans fully cover costs associated with NBS for conditions included in the RUSP. Given these developments, it is expected that most state PHLs will pursue newborn screening for Pompe and MPS I as resources become available. Additionally, several states have taken an interest in testing or have initiated testing, due to state legislative mandates, for two additional LSDs – Fabry and Gaucher disorders.

3.2.2. LDTs: The Only Current Alternative for LSD Newborn Screening

Newborn screening laboratory developed tests (LDTs) for LSDs has been performed by enzyme activity measurements in dried blood spot extracts, using either fluorimetry or tandem mass spectrometry (MS/MS) methodologies[37]. These LDTs are developed and validated at individual laboratories without FDA oversight. LDTs are not subject to rigorous premarket review of either analytical or clinical performance before entering the market, and under current CLIA regulatory requirements, there is no opportunity to share results of test performance in a public venue.

It is important to note that approval of SEEKER™ will assure that system performance characteristics have been independently analyzed by FDA regulatory scientists, will provide transparency by public disclosure of a FDA decision summary, and will ensure that post market controls are in place. These controls include sponsor requirements that assure quality production over time (i.e. quality system regulations are enforced through FDA on-site manufacturing inspections) and post-market monitoring for adverse events through FDA medical device reports. Pending panel review, SEEKER™ is poised to become the first and only FDA approved device for LSD newborn screening, and thereby fulfills a critical unmet need in the newborn screening community to standardize LSD enzyme activity measurements.

3.3. Lysosomal Storage Disorders Screened by SEEKER™

3.3.1. Mucopolysaccharidosis Type I (MPS I) Disorder

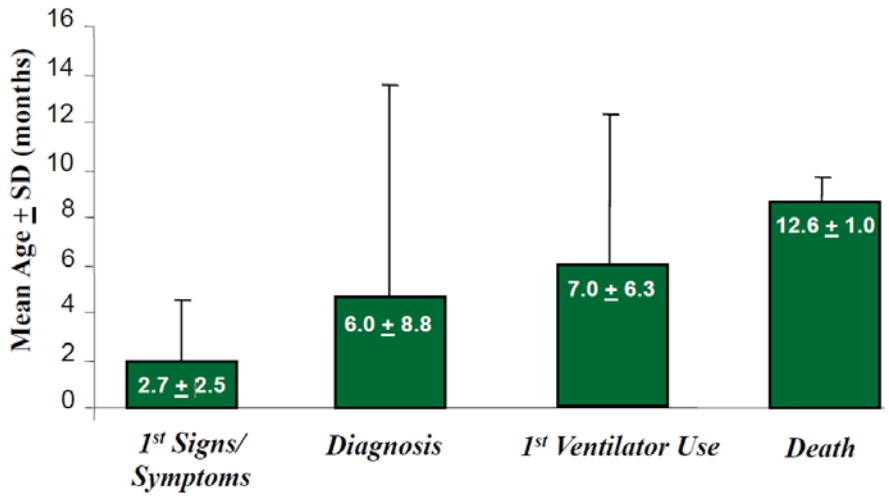
Mucopolysaccharidosis Type I (MPS I) is an autosomal recessive disorder that is caused by a deficiency in the enzyme α-L-iduronidase (IDUA). IDUA participates in the degradation of heparan and dermatan sulfate, two glycosaminoglycans (GAGs) found in nearly all body tissues. Consequently, IDUA deficiency results in a disorder that involves multiple organ systems resulting from the accumulation of un-degradable GAG material throughout the body. The estimated incidence rate is between 1:54,000 and 1:185,000[4]. Three subtypes of MPS I exist: MPS IH (Hurler), MPS IS (Scheie) and MPS IH/S (Hurler-Scheie). The incidence of MPS IS is estimated to be about 1:500,000. Two of the most common DNA mutations for MPS I include W402X and Q70X. Clinical symptoms, if not treated early, include hydrocephalus, corneal clouding, hepatosplenomegaly, and cardiomyopathy. MPS I typically leads to death by age 10 if not detected and treated prior to the onset of symptoms. Onset of symptoms can occur within the first 1-2 years if the disorder is severe (Hurler) and 3-12 years of age for the rest of the spectrum (Hurler-Scheie and Scheie)[38]. Expected lifespan is less than 10 years of age for the severe form (Hurler), with increasing life span (teens -20s) and normal life span for attenuated forms Hurler-Scheie and Scheie respectively. As a general rule, patients with MPS I-H have undetectable

IDUA activity whereas patients with MPS I-HS and MPS I-S have residual IDUA activity[38,39]. For treatment, enzyme replacement therapy (ERT) or hematopoietic stem cell transplantation (HSCT) are indicated. HSCT performed before 24 months of age and before the onset of significant developmental delay has the highest probability of rescuing neurocognitive outcome. MPS I was added to the DHHS Secretary's Recommended Uniform Screening Panel in March 2016.

3.3.2. Pompe Disorder

Pompe disorder (glycogen storage disorder type II) is an autosomal recessive disorder. A deficiency in the enzyme acid α-glucosidase (GAA), which prevents the degradation of lysosomal glycogen, leads to Pompe. Pompe disorder presents as a continuum of disorder severity – at one end of the spectrum is classic infantile Pompe and at the other end is late-onset Pompe. The expected lifespan with infantile onset is less than one year (Figure 4)[40]. Infantile onset patients present with symptoms starting as early as within the first few days and death occurs at a median age of approximately 12 months. The overall estimated incidence rate for Pompe (either infantile or late-onset) is approximately 1:28,000[5]; however, different ethnic populations have varying estimated incidence rates. Glycogen accumulation can lead to cardiomyopathy, respiratory and muscle weakness (hypotonia). Over 300 different mutations for Pompe have been discovered. For newborns diagnosed with Pompe disorder, early initiation of enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) has resulted in significantly improved outcomes. Pompe disorder was added to the DHHS Secretary's Recommended Uniform Screening Panel in March 2015.

Figure 4: Mean age and standard deviation (months) for the onset of symptoms, diagnosis, 1st ventilator use, and even death from untreated infantile onset Pompe.



3.3.3. Gaucher Disorder

Gaucher disorder is an autosomal recessive disorder that results from a deficiency in the enzyme glucocerebrosidase (GBA), or β-glucosidase, in which cerebrosides accumulate. The estimated incidence rate for Gaucher disorder is approximately 1:57,000. Three types of Gaucher disorder have been identified: Type 1 (non-neuronopathic), Type 2 (acute neuronopathic) and Type 3

(sub-acute neuronopathic). The age of onset differs depending on type: Type 1 onset is childhood-adult, Type 2 onset is neonatal-infantile and Type 3 onset is infantile-childhood. Although this categorization facilitates clinical management to a certain degree, it is important to realize that Gaucher, like other lysosomal storage disorders, consists of a continuous spectrum of disease variants with "asymptomatic" and less severely affected Type 1 patients at one end to the severely affected Type 2 and lethal *in utero* forms at the other end of the clinical scale. If not detected early, Gaucher disorder may result in anemia, thrombocytopenia, massive hepatosplenomegaly and bone marrow involvement. Enzyme replacement therapy and substrate reduction therapy are effective treatments for Types 1 and 3. There is no specific therapy for the infantile form, which occurs in 1% of Gaucher cases, because the clinical progression is rapid. However, enzyme replacement therapy is used in these cases to alleviate symptoms.

3.3.4. Fabry Disorder

Fabry disorder is an X-linked disorder characterized by a deficiency in α-galactosidase A (GLA), resulting in glycosphingolipid ceramide trihexoside (GL-3) accumulation. Population pilot studies have estimated that the incidence rate is between 1:1,500 and 1:13,000[41]. Due to random X-chromosome inactivation, enzyme activity in females is highly variable and for some females, enzymatic activity could overlap with the normal range. Therefore, enzyme activity for females should be interpreted with caution, as some female carriers can have enzyme activity in the normal range despite clinical manifestations; these female carriers are not classified as false positives[42]. For females with positive family history identified as obligate carriers by pedigree analysis, particularly if the family mutation was already identified, performing molecular analysis is the most appropriate way to confirm the diagnosis[43]. The accumulation of GL-3 with Fabry disorder may cause cardiomyopathy, hearing loss, cardiac hypertrophy, rhythm disturbances, renal insufficiency, GI involvement, and joint swelling. Fabry disorder manifests as early as 2 years (classical) and is known to disproportionately affect males. Similar to Pompe, Fabry also represents a disorder continuum and can also manifest in adulthood (late-onset). ERT is life-saving in Fabry and has improved outcomes[44]. Early initiation of ERT in babies identified by NBS would further improve outcomes.

4. DEVICE DESCRIPTION

4.1. Intended Use

The SEEKER™ System is intended for quantitative measurement of the activity of multiple lysosomal enzymes from newborn dried blood spot samples. Reduced activity of these enzymes may be indicative of a lysosomal storage disorder. The enzymes measured using the SEEKER™ Reagent Kit and their associated lysosomal storage disorder are indicated below.

Enzyme (abbreviation)	*Disorder*
α-L-iduronidase (IDUA)	Mucopolysaccharidosis Type I (MPS I)
α-D-glucosidase (GAA)	Pompe
β-glucocerebrosidase (GBA)	Gaucher
α-D-galactosidase A (GLA)	Fabry

Reduced activity for any of the four enzymes must be confirmed by other confirmatory diagnostic methods.

4.2. Summary and Explanation of the Device

SEEKER™ uses fluorimetry on a digital microfluidic platform to measure enzymatic activity. Specifically, the system is intended as an aid to screening newborns for a lack of, or reduced activity of, the enzymes related to LSDs: α-D-glucosidase [GAA] (Pompe disorder) , α-L-iduronidase [IDUA] (MPS I), α-D-galactosidase A [GLA] (Fabry disorder), and β-glucocerebrosidase (Gaucher disorder). Briefly, SEEKER™ performs enzymatic analysis for newborn screening by automating all liquid-handling steps involved in an assay using sub-microliter droplets as reaction vessels. The enzymes measured and reported by SEEKER™, the corresponding lysosomal storage disorders associated with deficiency of each enzyme, and the corresponding incidence for each disorder are listed in Table 2. SEEKER™ has been used in the Missouri State Public Health Laboratory to generate the clinical data for the pending submission.

Table 2: Enzymes measured by SEEKER™, corresponding LSD and incidence

Enzyme (abbreviation)	Disorder	Published incidence
α-L-iduronidase (IDUA)	MPS I	1:54,000 – 1:185,000
α-D-glucosidase (GAA)	Pompe	1:28,000
β-glucocerebrosidase (GBA)	Gaucher	1:57,000
α-D-galactosidase A (GLA)	Fabry	1:1,500 – 1:13,000

4.3. Theory of Operations

4.3.1. Digital Microfluidics Technology

SEEKER™ automates dried blood spot enzyme analysis for newborn screening using digital microfluidics. Digital microfluidics is based on the use of electrical fields to directly manipulate discrete droplets in a programmable fashion by the electrowetting effect, whereby activation of an electrode results in a local reduction of the interfacial tension between the droplet and the surface (Figure 5). An array of surface electrodes is layered by another plate to form a chamber, known as the "cartridge", in which the droplets are sandwiched. The remaining space in the cartridge is filled with an immiscible filler fluid to prevent evaporation of the droplets and to facilitate droplet transport. In this platform, droplets can be transferred between any two neighboring electrodes and can be transported anywhere within a network of contiguous electrodes. Because of the discrete nature of the liquid volumes as well as the synchronization and programmability of the fluid manipulations from a specific, fixed program, this technique has been referred to as "digital microfluidics." In addition to transport, other operations such as merging, splitting, dispensing and mixing of droplets can be performed using the same principles.

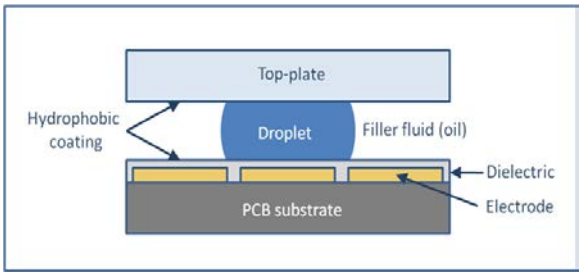


Figure 5: Electrowetting-based digital microfluidics works by turning electrodes on a printed circuit board substrate on and off to manipulate the droplets within an oil-filled chamber.

Other microfluidic technologies pump fluids through small channels using pressure or electro kinetics. Digital microfluidics provides unparalleled flexibility and the ability to perform very complex assays or combinations of assays, because protocols are derived from software and not permanently embedded in a "cartridge". Digital microfluidics is commercialized in Illumina's NeoPrep™ (for research use only) and GenMarks' ePlex™ (CE-IVD).

4.3.2. Test Principle

The activities of the lysosomal enzymes (IDUA, GAA, GBA and GLA) are measured in dried blood spot 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 underlying chemistries used in these fluorimetric assays based on 4-MU have traditionally been used for many years in biochemical testing to aid in the clinical diagnosis of all the four diseases (Pompe, Fabry, Gaucher, MPS-1)[38].

The substrate for IDUA (EC 3.2.1.76) is 4-methylumbelliferyl α-L-iduronide (4-MU-α-IDUA), prepared in an acetate solution at pH 3.5. 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)[45].

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

The substrate for GAA (EC 3.2.1.20) is 4-methylumbelliferyl α-D-glucoside (4-MU-α-Gluc) prepared in an acetate buffer at pH 3.8. The chemical acarbose is used to selectively inhibit endogenously present maltose glucoamylase which is also active for 4-MU-α-Gluc[46,47,48,49].

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

The substrate for GBA (EC 3.2.1.45) is 4-methylumbelliferyl β-D-glucopyranoside (4-MU-β-Gluc) prepared in a citrate phosphate buffer at pH 5.2. The surfactant sodium taurocholate (NaTC) is present in the buffer and is required to activate the enzyme.

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

The substrate for GLA (EC 3.2.1.22) is 4-methylumbelliferyl α-D-galactopyranoside (4-MU-α-Gal) prepared in acetate solution at pH 4.6. The chemical N-acetyl-D-galactosamine is used to inhibit endogenously present α-N-acetylgalactosaminidase which is also active for 4-MU-α-Gal.

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

For all the enzymatic assays the reaction is stopped using a high pH stop solution (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.

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

On SEEKER™, digital microfluidics enables “spatial multiplexing”. Each assay is performed independently; this allows each of the four assays to be executed at an independent, optimized pH. All other multiplexing techniques must compromise on pH because multiple analytes are assayed in the same “pot”.

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 SEEKER™. 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 solution using measurements taken on each cartridge. Enzymatic activity is reported as micromoles of 4-MU produced/liter of blood/hour of incubation.

4.4. System Description

SEEKER™ (Figure 6) employs digital microfluidic technology and fluorimetry to precisely measure multiple lysosomal enzymatic activities quantitatively from newborn dried blood spot samples. The disposable 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™ 4-Plex Reagent Kit and is controlled by the

SEEKER™ Analyzer through Spot Logic® Software. The Seeker™ Analyzer contains hardware and firmware required to provide the essential system functions such as droplet control, thermal control and fluorescence detection that are required to perform the assay.

Figure 6: A high throughput workstation consists of 4 SEEKER™ analyzers connected to a central computer workstation.

SEEKER™ includes the following provided components:

1. SEEKER™ Analyzer (including USB and power cables)
2. Desktop PC with monitor, keyboard and mouse
3. Spot Logic® Software (on Desktop PC)
4. SEEKER™ 4-Plex Reagent kit containing enzyme specific substrate reagents, dried blood spot extraction solution, reaction stop solution and four levels of calibrators
5. SEEKER™ cartridge
6. Quality Control Dried Blood Spots containing four levels of quality control material
7. Finnipipette™ Novus 8-channel automatic pipette 1-10 µL
8. Finnipipette™ Novus single channel automatic pipette 10-100 µL
9. Uninterruptible Power Supply

4.4.1. Reagents

Each Seeker™ 4-Plex Reagent Kit contains sufficient consumables for 1440 tests per analyte. The reagents in the kit along with the quantity and storage conditions are listed in Table 3. A lot specific quality control certificate with enzymatic activity range for QC samples is provided with each kit.

Table 3: Consumables for SEEKER™

Component	Contents	Storage Conditions
Quality control dried blood spots	QC-Base Pool (QCBP)	-80°C to -70°C
	QC-Low (QCL)	-80°C to -70°C
	QC-Medium (QCM)	-80°C to -70°C
	QC-High (QCH)	-80°C to -70°C
Seeker™ 4-Plex Assay (IDUA|GAA|GBA\GLA) Kit	EZ Reagent IDUA	-80°C to -70°C
	EZ Reagent GAA	-80°C to -70°C
	EZ Reagent GBA	-80°C to -70°C
	EZ Reagent GLA	-80°C to -70°C
	Calibrant A (CALA)	-80°C to -70°C
	Calibrant B (CALB)	-80°C to -70°C
	Calibrant C (CALC)	-80°C to -70°C
	Calibrant D (CALD)	-80°C to -70°C
	Stop Buffer (STB)	-80°C to -70°C
	Extraction Buffer (EXT)	15°C to 25°C
	Filler Fluid	15°C to 25°C
Seeker™ Cartridge	Cartridge	15°C to 25°C

4.4.2. Calibrators

The calibrators consist of 4 levels of aqueous preparation of 4-methylumbelliferone sodium salt (4-MU) in 0.6 M 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 Table 4. The concentrations are chosen to provide a calibration curve around the cutoffs or decision making levels.

Table 4: Concentrations of 4-MU in each Calibrator

Calibrator	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

4.4.3. Quality Control Material

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 specimens is summarized in Table 5. QCL and QCM are used as run controls. QCBP is used a filler sample to fill empty wells. QCH is primarily used for analytical validation.

Table 5: Composition of Quality Control Dried Blood Spots

Quality control specimen	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

4.4.4. Cartridge

All enzymatic assay operations are performed on a SEEKER™ cartridge (Figure 7, left). The SEEKER™ cartridge is single-use and serves as the assay reaction chamber. The cartridge contains 48 wells for samples, controls and calibrators as well as 10 wells for enzyme substrates and stop solutions. All samples, reagents, solutions, calibrants and controls are added to the cartridge prior to the enzymatic reactions. Samples from one 96 well microtiter plate are used to fill two SEEKER™ cartridges (Figure 7, right). Once the cartridge is loaded into SEEKER™, all subsequent reaction steps are automated without further user intervention.

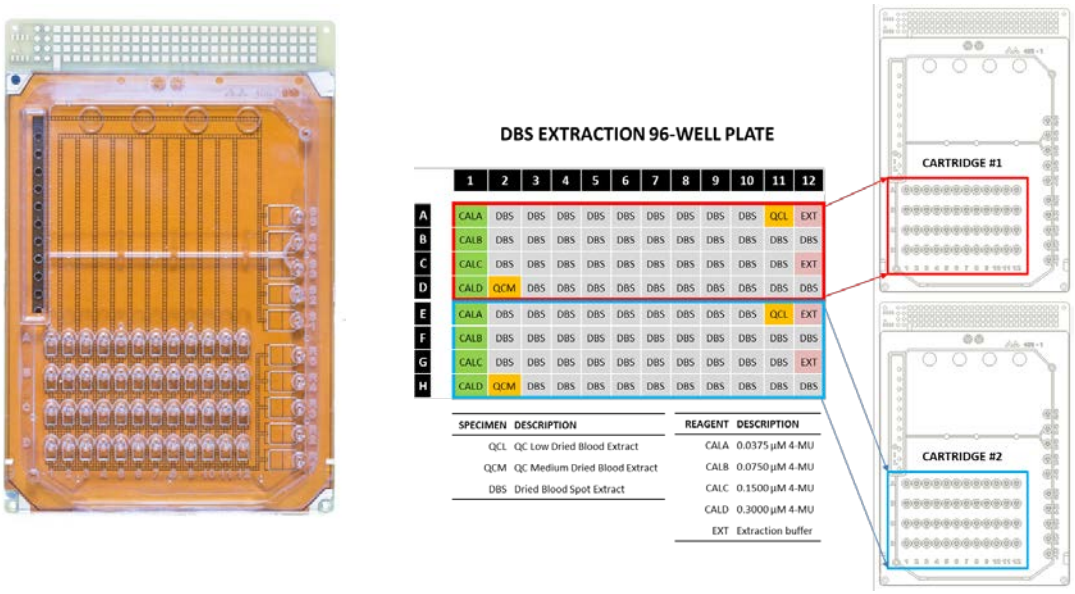


Figure 7: Left – SEEKER™ cartridge with 48 sample wells. Right – DBS extraction on one 96 well microtiter plate is used to fill two SEEKER™ cartridges.

4.5. Test Methodology

4.5.1. Laboratory Workflow

The enzymatic assay workflow on SEEKER™ is summarized in Figure 8.

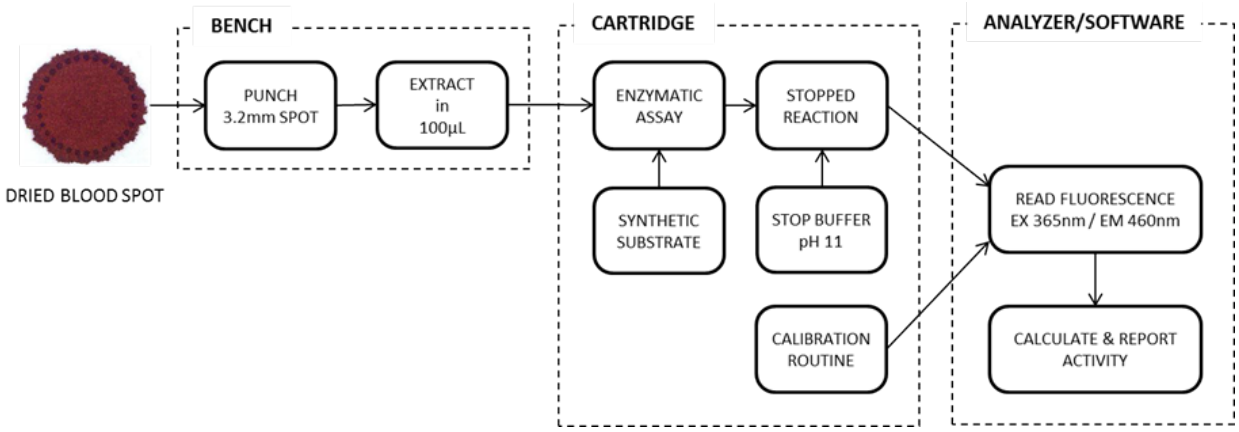


Figure 8: Enzymatic workflow on SEEKER™

The protocol is comprised of the following steps:

1. Dried blood spot extraction:

   In this pre-reaction step, a 3.2 mm sample (~3.1 µL blood) is punched from the dried blood spot. The dried blood punch is then incubated in 100 µl extraction solution for 30 minutes at ambient conditions on a plate shaker.

2. Calibration routine:

   The fluorescence value of the free 4-MU is converted to an equivalent 4-MU concentration using a 4 point calibration curve run in duplicate. This calibration curve is generated using 4 levels of 4-MU at the following concentrations: 0.0375 µM, 0.075 µM, 0.15 µM, 0.3 µM. The calibration curve is generated with every cartridge run on SEEKER™.

3. Assay routine:

   a. Assay reaction: One droplet (~100 nL) of each substrate is mixed with one droplet of the dried blood spot extract (~100 nL) and incubated for a preset amount of time. The substrates are hydrolyzed by their corresponding enzyme to release free 4-MU.

   b. Non-enzymatic hydrolysis reaction: One droplet of each substrate is mixed with one droplet of extraction solution and incubated for the same duration as the assay reaction.

4. Stop reaction:

   A droplet of stop solution (~100 nL) is then added to the reaction droplet to stop the reaction. The sample droplet is effectively diluted threefold at the end of the stop reaction (1 droplet each of sample, substrate and stop solution).

5. Read fluorescence:

The free 4-MU is measured in the stopped assay reaction and non-enzymatic hydrolysis reaction using the UV fluorimeter on SEEKER™.

6. Calculate and report activity:

   The raw fluorescence measurements are corrected for non-enzymatic hydrolysis and converted into moles of 4-MU produced using the 4-point calibration curve obtained at the beginning of the protocol. Enzymatic activity is reported as µmol of 4-MU produced per liter of blood per hour of incubation.

5. ANALYTICAL VALIDATION

5.1. Precision

A precision and reproducibility study was designed based on CLSI EP05-A3. Dried blood spot (DBS) samples representing five enzymatic activity levels (L1-L5) for each enzyme were included in the precision study. These DBS samples were prepared by titrating human umbilical cord blood with heat inactivated serum and hematocrit adjusted to 50%. The study was performed using four SEEKER™ analyzers over 21 non-consecutive days, with two runs per day and two dried blood spot punches of each specimen per run generating a maximum of 336 replicates per enzymatic activity level. The study was conducted using three lots of SEEKER™ reagent kit with each lot balanced between all the runs i.e. 56 cartridges were used with each reagent lot. The study was performed at the sponsor's facility.

Repeatability (within-run precision), between-day precision, between-instrument precision, between reagent lot and overall reproducibility were evaluated for the four lysosomal enzymes (IDUA, GAA, GBA and GLA). The acceptable repeatability was ≤ 1.0 µmol/L/h or 15% CV, whichever is greater. The acceptable reproducibility was ≤ 1.5 µmol/L/h or 20% CV, whichever is greater. Invalid data points were removed from analysis. Precision estimates were calculated by using three-way nested Analysis of Variance technique and are summarized in Table 6 (IDUA), Table 7 (GAA), Table 8 (GBA) and Table 9 (GLA). Precision estimates are reported as standard deviation in µmol/L/hr or as a %CV depending on the mean activity level. For brevity units are omitted in these tables.

Table 6: Summary of precision data for IDUA

Sample	N	Mean	Repeatability	Between Reagent Lot	Between Instrument	Between Day	Reproducibility
L1^	331	2.40	1.79	0.55	0.00	0.83	1.97
L2	334	3.53	0.80	0.55	0.00	0.26	0.96
L3	335/ 333*	6.22/ 6.12*	1.64/ 0.95*	0.69/ 0.71*	0.00/ 0.00*	0.00/ 0.24*	1.77/ 1.21*
L4	333	12.03	12.4%	10.5%	0.0%	0.0%	16.6%
L5	335	24.06	9.0%	9.8%	0.0%	0.0%	14.2%

^L1 is below LoQ

Table 7: Summary of precision data for GAA

Sample	N	Mean	Repeatability	Between Reagent Lot	Between Instrument	Between Day	Reproducibility
L1	331	4.29	0.67	0.25	0.00	0.14	0.73
L2	334	6.27	0.95	0.26	0.00	0.00	0.99
L3	335	9.59	9.9%	7.0%	0.0%	0.0%	12.0%
L4	334	18.06	13.6%	5.9%	0.0%	0.0%	14.8%
L5	335	27.37	9.8%	6.3%	1.6%	0.0%	12.9%

Table 8: Summary of precision data for GBA

Sample	N	Mean	Repeatability	Between Reagent Lot	Between Instrument	Between Day	Reproducibility
L1	331	2.84	0.99	0.36	0.05	0.07	1.08
L2	334	3.47	0.47	0.37	0.08	0.00	0.64
L3	335	5.07	0.56	0.57	0.00	0.00	0.84
L4	334	8.55	11.6%	10.4%	1.4%	0.0%	15.8%
L5	335	15.00	11.3%	11.4%	2.1%	1.2%	15.7%

Table 9: Summary of precision data for GLA

Sample	N	Mean	Repeatability	Between Reagent Lot	Between Instrument	Between Day	Reproducibility
L1	331/ 330*	6.94/ 6.92*	15.4%/ 14.5%*	0.39/ 0.35*	0.00/ 0.00	0.12/ 0.08	1.13/ 1.01*
L2	334	9.80	10.4%	6.4%	0.0%	2.9%	13.6%
L3	335	15.32	7.7%	7.2%	0.0%	3.1%	11.5%
L4	334	28.76	7.8%	4.8%	0.0%	0.0%	9.4%
L5	335	52.66	8.8%	4.1%	1.3%	2.7%	10.6%

* Statistical outlier identified using Grubbs test and removed from analysis

The repeatability and reproducibility of all the assays were within the acceptance criteria for all specimens that were above the LOQ.

5.2. Analytical Sensitivity

Limit of Blank (LoB) and Limit of Detection (LoD) were determined as per CLSI EP17-A- "Protocols for determination of limits of detection and limits of quantitation-Approved guideline."

For estimating LoB and LoD, SEEKER™ blank extracts and Quality Control Low (QCL) DBS samples were used as surrogate "no analyte" and "low analyte" samples in the study. The study was conducted using three lots of the SEEKER™ 4-plex reagent kit, which includes reagents, cartridges and calibrators. A total of two cartridges were tested for each reagent lot to determine LoB and LoD, resulting in a total of six cartridges for each study. For LoB, a total of 40 replicates were obtained per cartridge resulting in 80 replicates of the no-analyte sample per reagent lot. For LoD, a total of 32 replicates were obtained per cartridge resulting in 64 replicates for the low-analyte sample per reagent lot.

5.2.1. Limit of Blank (LoB)

A non-parametric estimate based on ordered (or ranked) values was used to estimate the LoB. The LoB was estimated as the 95th percentile of the distribution of the blank values, which corresponds to a Type 1 error or false positive rate (α) = 5%. LoB was determined for all three lots individually and the worst of the three lots is reported.

5.2.2. Limit of Detection

Limit of Detection was calculated as LoD = LoB + cβ×SD where $c\beta = 1.645/(1 - 1/4f)$ for Type 2 error or false negatives (β) = 5%, f is the degrees of freedom calculated as the number of observations of low samples – number of low samples. For this study f = 64 – 8 = 56. LoD was calculated for all three reagent lots and the worst of the three lots is reported. Table 10 summarizes the LoB and LoD for each enzymatic assay.

Table 10: Determination of Limit of Blank and Limit of Detection

Detection Capability (µmol/L/hr)	N	IDUA	GAA	GBA	GLA
Limit of Blank (LoB)	80	1.78	0.50	0.72	1.96
Limit of Detection (LoD)	64/63*	2.77	5.36/2.18*	1.07	3.18

*Statistical outlier identified using Grubbs test and removed from analysis.

5.2.3. Limit of Quantitation

Since there is no recognized reference method available, the limit of quantitation was defined using a functional sensitivity study. Functional sensitivity was estimated by determining the precision profile of each assay and calculating the concentration at which the total imprecision was ≤ 20% or ≤1.5 µmol/L/h, whichever is greater. If the calculated LoQ was lower than the LoD the LoQ is set to be equal to the LoD. The study was performed using three different lots of SEEKER™ 4-plex reagent kit.

The precision profile was generated by preparing 8 levels of samples representing low enzymatic activity. The dried blood spot samples were prepared by titrating human umbilical cord blood sample and heat inactivated serum (to remove endogenous lysosomal enzymes) and hematocrit adjusted to ~50%. Four cartridges were tested for each reagent lot resulting in 16 replicates per level for each reagent lot.

A mixed constant / proportional variance function $\sigma^2 = ß0 + ß1\ U^2$ was used to fit the variance against the concentration at each level. LoQ was calculated for all three reagent lots and the worst of the three is reported. Table 11 summarizes the LoQ for each enzymatic assay.

Table 11: Determination of Limit of Quantitation

Detection Capability (µmol/L/hr)	IDUA	GAA	GBA	GLA
Limit of Quantitation (LOQ)	2.77	2.18	1.85	4.88

5.3. Linearity

Linearity was evaluated in accordance with CLSI EP06-A "Evaluation of Linearity of Quantitative Measurement Procedures". Linearity of the assays was evaluated using two separate studies. The first study evaluated linearity around the cutoff values for all four enzymes. A second study was conducted to evaluate the linearity over a broader enzymatic activity range.

5.3.1. Linearity – Study 1

Linearity was evaluated using 12 different levels of dried blood spot samples. Dried blood spot specimens were prepared by titrating human umbilical cord blood and heat inactivated serum (to

remove endogenous lysosomal enzymes) and adjusting hematocrit to ~50%. The study was performed on a total of four SEEKER™ analyzers resulting in a total of 12 replicates per level.

Linearity was evaluated by examining whether a nonlinear polynomial fits the data better than a linear one. First, second and third order polynomial regression were performed to calculate the regression coefficients and a t-test was conducted to determine if the 2nd and 3rd order coefficients were significantly different from zero at a 5% significance level. If the non-linear coefficients were not significantly different from zero the repeatability was checked at each level to verify that the precision was within acceptance criteria. The requirements for precision was ≤ 1.5 µmol/L/hr for concentrations less than the Limit of Quantitation and ≤ 20% for concentrations greater than the Limit of Quantitation. If the non-linear terms showed statistical significance at α=0.05, the acceptable deviation from non-linearity was ±1.0µmol/L/h or ±15%, whichever is greater.

For all four analytes, the 2nd and 3rd order coefficients were not significantly different from zero for the tested range (Table 12). The precision was also within the specified limits for all enzymatic activity levels.

Table 12: Linearity near the clinically relevant linear range

Enzyme	Linear Range (µmol/L/hr)
IDUA	2.77-19.40
GAA	2.18-21.79
GBA	2.14-10.42
GLA	4.88-45.34

5.3.2. Linearity – Study 2

Since the first linearity study did not fully address the complete clinically relevant range, a second linearity study was performed using 12 activity levels representing a broader enzymatic activity range. High enzymatic values are not possible with cord blood, and thus recombinant samples were also used to achieve sample levels covering the entire clinical range.

A high normal sample pool was manufactured by spiking human recombinant lysosomal enzyme into heat inactivated serum and adjusting hematocrit to ~50%. Dried blood spot samples were prepared by titrating high normal sample pool with heat inactivated serum and hematocrit adjusted to ~50%. The study was performed on a total of four SEEKER™ analyzers resulting in a total of 12 replicates per level. Linearity was evaluated using the same analysis method as Study 1. The linearity intervals combined from the two studies is reported in Table 13.

Table 13: Results from linearity study

Enzyme	Linear Range (µmol/L/hr)
IDUA	2.77-50.75
GAA	2.18-94.66
GBA	2.14-73.24
GLA	4.88-153.74

5.4. Carryover

In order to demonstrate that one patient sample would not affect the test results of another patient sample, a carryover study was conducted. Carryover was evaluated by two sample layouts for each assay: one with carryover and another with no carryover. DBS samples with low enzymatic activity for that particular assay while being normal for the other three assays were used as the low sample. DBS samples with four levels of enzymatic activity in the normal range were used as the high sample. The low and high samples were manufactured by spiking human recombinant enzymes into heat inactivated serum and adjusting hematocrit to ~50%. Subsequent levels of the high samples were manufactured by diluting the highest sample with heat inactivated serum and hematocrit adjusted to ~50%. To mimic the sample processing procedure as with the clinical samples, all samples were spotted on Ahlstrom 226 grade filter paper and dried overnight. A brief description of the samples used in this study is provided in Table 14 and Table 15.

Table 14: Composition of "low" samples

Specimen ID	**Composition (All Adjusted to ~50% Hematocrit)**	**Activity (µmol/L/hr)**
IDUA	Low for IDUA and normal for other three enzymes	4.07
GAA	Low for GAA and normal for other three enzymes	8.87
GBA	Low for GBA and normal for other three enzymes	6.37
GLA	Low for GLA and normal for other three enzymes	9.11

Table 15: Composition of samples in the presumed normal activity range

Specimen ID	**Composition (All Adjusted to ~50% Hematocrit)**	**Average activity (µmol/L/hr)**			
		IDUA	**GAA**	**GBA**	**GLA**
N1	25% high normal sample and 75% heat inactivated serum	13.55	16.59	13.62	30.60
N2	50% high normal sample and 50% heat inactivated serum	26.16	30.28	25.11	58.99
N3	75% high normal sample and 25% heat inactivated serum	n/a[1]	n/a[1]	36.18	83.40
N4	High normal sample (target 99.5th percentile)	53.26	58.89	49.57	120.79

[1]These conditions were not tested since the maximum carryover case (using N4) did not result in any carryover.

Carryover studies for each were conducted using a single lot of SEEKER™ 4-plex reagent kit on two SEEKER™ analyzers with two cartridges per analyzer. Number of replicates of each sample for each carry over layout is described in Table 16**.** Carryover was estimated as the percent bias

between the average values of representative affected samples obtained between the carryover and no carryover layout.

Table 16: Number of replicates per sample layout

Sample Layout	Low Samples (IDUA, GAA, GBA, or GLA) (per cartridge)	High Samples (per cartridge)	Note
No Carryover	38 (152 total)	None	Layout represents no carryover
Carryover with N1	16 (64 total)	22 of N1 (88 total)	Layout represents a median baseline.
Carryover with N2	16 (64 total)	22 of N2 (88 total)	-
Carryover with N3	16 (64 total)	8 of N3 (32 total) and 14 of N1 (56 total)	-
Carryover with N4	16 (64 total)	8 of N4 (32 total) and 14 of N1 (56 total)	This layout represents maximum carryover for typical use case

Clinically significant carryover was defined as a bias greater than 15% from the no carryover condition. From the study, it was concluded that there was no clinically significant carryover for IDUA, GAA and GBA. For GLA there is a bias of 21% when a sample with activity 120.79 µmol/L/hr is in the same column as a low sample around the borderline cutoff value. This represents a carryover of 1.95 µmol/L/hr. For GLA we have stated in the instructions for use that a sample with GLA activity within +2 µmol/L/hr of the borderline cutoff should be retested if a sample with GLA activity ≥120 µmol/L/hr is present in the same column (cartridge has 12 columns and 4 rows). In the clinical study, 0.08% of samples met these criteria.

5.5. Interference Study

An interfering substances study was performed to determine and characterize the effects of potential endogenous and exogenous interfering substances on the performance of the 4-plex assay. Study was designed in accordance with CLSI EP07-A2.

A total of nine different substances were tested for interference on the system at the highest recommended concentration as per CLSI EP07-A2. Additional concentrations of the interfering substance were tested if the highest concentration was found to interfere. Interfering substances that were tested include Hemoglobin, D-Glucose, D-Galactose, EDTA, Heparin, Bilirubin (conjugated and unconjugated), total protein and triglycerides. Interference was tested at one enzymatic activity level around the borderline cutoff value. The average enzymatic values for the control specimens are described in Table 17.

Table 17: Average enzymatic values for control specimens

Enzyme	Activity (µmol/L/hr)
IDUA	4.02
GAA	7.43
GBA	4.82
GLA	13.12

Cord blood pool for preparing the dried blood spot sample was prepared by titrating 20% human umbilical cord blood into heat denatured serum (to remove endogenous lysosomal enzymes) and hematocrit adjusted to 50%. Blood was spotted on Ahlstrom 226 grade filter paper and dried overnight. The interfering substances and the concentration levels tested are described in Table 18.

Table 18: Concentration levels of each interfering substance tested

Interfering Substance	Concentration in Whole Blood
Bilirubin-Unconjugated (µM)	342
Bilirubin-Conjugated (µM)	342
D-Galactose (mM)	0.84
D-Glucose (mM)	55
EDTA (µM)	3.4
Heparin (U/L)	3000
Hemoglobin (mg/mL)	200
Protein-Total (mg/mL)	120
Triglyceride (mg/dL)	3000

Dried blood spot (DBS) sample extracts were prepared using the standard extraction procedure and the interfering test substances were spiked into the DBS extracts. Control pools were prepared by adding the same amount of solvent (that was used to prepare the interfering substance) to the DBS extracts. A total of 30 replicates were obtained for each interfering substance.

Percent recovery was calculated by dividing the mean enzymatic activity obtained with the test substance by the mean enzymatic activity obtained with the control pool. Bias in enzymatic activity is obtained by subtracting the mean activity of the control pool from the mean activity of the test pool. Clinically significant interference was defined as percent recovery or bias less than ±15% or ± 1 µmol/L/hr, whichever was greater. Results from the interference study are provided in Table 19.

Table 19: Interference summary

Interfering Substance	CLSI Concentration Guidance (In Blood)	Percent Recovery (%) or Bias (µmol/L/h)			
		IDUA[a]	GAA[b]	GBA[a]	GLA[b]
None (Control)	n/a	4.02	7.43	4.82	13.12
Bilirubin-Unconjugated (µM)	342	0.2	-2.6%	-0.1	2.9%
Bilirubin-Conjugated (µM)	342	0.38	-5.2%	0.08	2.6%
D-Galactose (mM)	0.84	0.52	-0.8%	-0.24	8.1%
D-Glucose (mM)	55	0.65	-0.47	-0.07	9.4%
EDTA (µM)	3.4	0.21	-0.6%	-0.66	2.6%
Heparin (U/L)	3000	0.01	-0.6%	0.17	3.0%
Hemoglobin (mg/mL)	200	0.15	5.5%	-0.12	-10.7%
Protein-Total (mg/mL)	120	1.41	-2.0%	-0.16	2.6%
	75	0.67	3.1%	-0.06	5.7%
	63.75	0.39	-1.5%	-0.03	4.8%
Intralipid (mg/dL)	3000	0.57	8.6%	0.05	5.5%

[a] Bias reported as µmol/L/hr

[b] Percent recovery

Total Protein was found to have a positive bias of 1.41µmol/L/hr for IDUA at a test concentration of 120 mg/mL. Bias decreased with reducing concentration of total protein and was found not to interfere at ≤75 mg/mL. The other substances do not exhibit clinically significant interference.

5.6. Reagent Stability

5.6.1. Shelf Life

Reagent stability was evaluated using an isochronous study design (in accordance with CLSI EP25-A) in a "staggered start" mode to eliminate the variation from other system components. Reagent aliquots from different lots manufactured at different time points were placed in the recommended storage (-80°C) and tested at the same end time using three different enzymatic activity levels (low, medium and high). Average and standard deviation were calculated for each activity level and reagent lot and compared to the reference reagent lot which was stored in the freezer (at -80°C) for 1 month duration. Stability criteria were percent recovery less than ±15% (recovery between 85% and 115%) or bias less than ± 1µmol/L/hr, whichever was greater. Table 20 shows the average bias for low samples and % recovery for medium and high samples. An * next to the number of samples in Table 20 denotes the exclusion of invalid data during these stability runs. Based on the isochronous stability data, shelf life stability of 14 months was established for the 4-plex reagents for IDUA, GAA, GBA and GLA enzymes.

Table 20: Results of the reagent stability study

Assay	Sample	N (Max = 80)	REF Lot Mean (µmol/L/h)	% Recovery or Bias in Activity			
				Ref Lot	Lot 3- 10 months	Lot 2- 14 months	Lot 1- 22 months
IDUA	L[a]	75*	4.68	0.00	-0.80	0.35	0.46
	M	75	14.87	100%	87%	98%	93%
	H	76	25.97	100%	88%	99%	92%
GAA	L[a]	76	5.81	0.00	0.52	0.13	0.68
	M	75	13.52	100%	101%	99%	109%
	H	76	21.68	100%	102%	95%	111%
GBA	L[a]	80	3.14	0.00	0.22	0.79	0.05
	M[a]	80	4.83	0.00	0.24	0.34	0.07
	H	78*	7.01	100%	105%	105%	103%
GLA	L	80	8.13	100%	93%	97%	96%
	M	80	27.49	100%	87%	94%	100%
	H	80	43.30	100%	95%	96%	112%

[a] Bias is presented in µmol/L/hr

* Invalid data points and statistical outliers removed

5.6.2. Reagent In-Use Stability (Open Vial Stability)

Each reagent vial packaged as part of the SEEKER™ 4-plex reagent kit is designed to be used for four cartridges. Based on reagent in-use stability studies we recommend each reagent vial to be used within 1.5 hours after package opening. Any leftover reagents from an aliquot should be discarded and not used for subsequent runs.

5.7. Sample Shipping Stability

A simulated transport study was conducted to mimic stressful temperature and humidity conditions, and evaluate its effect on enzymatic activity of IDUA, GAA, GBA and GLA in dried blood spots. The dried blood spots were subjected to temperature and humidity conditions indicated in Table 21 in environmental conditioning chambers. For each condition, samples were removed at predetermined time points – 1, 3, and 5 days – and the activity of lysosomal enzymes were measured the same day on SEEKER™ along with unconditioned samples. A total of four different levels of DBS samples were included in the study.

Table 21: Atmospheric conditions for sample shipping stability study

Condition	Temperature	Humidity
1	10°C	20% RH
2	10°C	80% RH
3	45°C	20% RH
4	45°C	80% RH
5	25°C	50% RH

Percent recovery and bias was calculated for each sample by comparing the activity values with the unconditioned samples. From the data analysis we arrive at the following conclusions.

- All lysosomal enzymes are stable for up to 5 days at 10°C between 20% RH and 80% RH.
- 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 (~80% recovery) after 5 days.
- At 45°C and low humidity (20% RH), there is a moderate loss (~70% recovery) in IDUA and GAA activity, and significant loss in GBA (~50% recovery) and GLA activity (~30% recovery).
- At 45°C and high humidity (80% RH) there is significant loss in activity for all enzymes (~10-20% recovery).

Exposure to high temperature during shipping and long transit times may result in an increased rate of false positives. The SEEKER™ Instructions for Use recommend that laboratories monitor the daily patient median to identify any systemic bias in activity due to seasonal weather changes and adjust the cutoff values to minimize the false positive rate.

5.8. Calibrators and Controls

5.8.1. Calibrators

The SEEKER™ calibrators are intended for use with the SEEKER™ analyzer and SEEKER™ cartridge to establish points of reference that are used in the determination of enzymatic activity of α-L-iduronidase [IDUA], α-D-glucosidase [GAA], β-glucocerebrosidase [GBA], and α-D-galactosidase A [GLA] in newborn dried blood spot extracts.

SEEKER™ calibrators are supplied as part of the SEEKER™ 4-plex reagent kit. The calibrators consist of four levels of aqueous preparation of 4-methylumbelliferone sodium salt (4-MU) in 0.6 M sodium bicarbonate solution, pH 11.0 with 0.01% Tween 20. The concentration of 4-MU in each of the four calibrators is 0.0375 µM (CAL A), 0.0750 µM (CAL B), 0.1500 µM (CAL C), 0.3000 µM (CAL D).

There is no international conventional reference material that can be used as the primary calibrator and no reference method available that can be used to assign values. SEEKER™ calibrators are standardized to a Master Reference Calibrator lot and manufactured from a 30 mM stock solution of 4-methylumbelliferone that is prepared gravimetrically using 4-MU Sodium Salt (>98% purity by HPLC). In-process QC checks are incorporated to ensure a much tighter calibration slope between manufacturing lots. An absorbance QC test is performed and compared to a known value at a 4-MU concentration of 75 µM. Concentration is adjusted by changing the dilution factor that is used to prepare 30 µM from 75 µM. An in-process QC check of 30 µM 4-MU is performed by measuring absorbance. Further dilutions to obtain CAL A, CAL B, CAL C and CAL D are performed in calibration solution. Final validation testing of the SEEKER™ reagent kit along with the 4-MU calibrants is performed by testing three levels of dried blood spot samples ranging between LOQ, around cutoff and normal median range. Enzymatic activity values obtained from the reagent lot release process are compared with the assigned values to each sample based on a dried blood spot sample release process.

5.8.1.1. Calibrator Stability

Based on accelerated stability studies performed on 4-MU calibrants, shelf-life stability is 1 year at the recommended storage temperature of -80°C. Real time stability studies using three manufacturing lots are ongoing.

5.8.2. Controls

SEEKER™ quality control dried blood spots (QC spots) are intended for use as assayed multi-analyte quality control material in SEEKER™ for the following lysosomal enzymes – α-L-iduronidase [IDUA], α-D-glucosidase [GAA], β-glucocerebrosidase [GBA], α-D-galactosidase A [GLA].

The composition of the different quality control samples provided as part of SEEKER™ along with typical activity values is summarized in Table 22. The quality control samples have been prepared by titrating cord blood and adult human blood with 50-55% hematocrit to obtain different levels of lysosomal enzyme activity.

The QC spots are used to detect systemic analytical deviations that may arise from reagents or analytical instrument variation and to ensure the consistent performance of the system. Enzymatic activity values for the control samples are determined using the corresponding reagent and calibrator lot and the assigned values will be provided in the package insert along with each shipment. QC spots were produced using human blood specimens that were tested for HIV, Hepatitis B and Hepatitis C using FDA approved or equivalent methods and found to be negative for all of these infectious agents.

Table 22: QC spot descriptions

Quality Control Sample	Composition	Relevance	Median Activity (µmol/L/hr)			
			IDUA	GAA	GBA	GLA
QC-Low (QCL)	5% human umbilical cord blood and 95% heat inactivated serum, ~50% hematocrit	Low Activity	2.38-4.34	3.07-5.16	2.47-3.33	6.27-8.60
QC-Medium (QCM)	50% human umbilical cord blood and 50% heat inactivated serum, adjusted to ~50% hematocrit	Intermediate Activity	9.85-13.33	9.73-14.13	4.6-5.94	18.25-28.43
QC-High (QCH)	Human umbilical cord blood, adjusted to ~50% hematocrit	Normal Activity	17.86-23.26	16.3-23.07	7.31-10.06	30.50-50.98

Control Stability

Based on accelerated stability studies, the shelf-life is 1 year at -80°C.

6. CLINICAL VALIDATION

6.1. Study Objective

The purpose of this prospective clinical study was to demonstrate the clinical performance of SEEKER™ to measure α-D-glucosidase [GAA], α-L-iduronidase [IDUA], α-D-galactosidase A [GLA], β-glucocerebrosidase [GBA] on newborn dried blood spots. To meet this objective, a large, single-site clinical study using the sponsor's SEEKER™ was conducted in the Missouri State Public Health Lab (MSPHL). All newborn dried blood spots received at MSPHL for the other mandatory routine newborn screening tests were used for the four LSD enzymes during the study.

The primary end points of the study included:

- Identification of true positives
- False negatives
- False positive rate
- Retest rate

6.2. Study Design

6.2.1. Study Period

The study was conducted using samples received at MSPHL between January 11, 2013 and January 14, 2015. The testing of these samples occurred from January 15, 2013 and to January 14, 2015, inclusive. In addition, a 15 month period after testing was concluded was used to monitor for false negatives.

The study period was retrospectively separated into "pilot" and "pivotal" phases. Babies born on or before August 26, 2013 were included in the pilot phase and babies born on or after August 27, 2013 were included in the pivotal phase. Of the 153,697 newborns successfully screened during the study..

Prior to the initiation of the pilot phase of the study, pre-pilot testing was completed at MSPHL using de-identified DBS and known affected samples; the results of the pre-pilot testing were used to set preliminary cutoff values for the study. During the pilot phase, several adjustments were made to the cutoff values as the laboratory acquired additional knowledge about the assay performance for different sample subgroups (for example, grouped based on sample age at collection) as well as confirmatory diagnosis on samples that were referred. Most changes to the cutoffs during the study were made during the pilot phase. There were also some device modifications during the pilot phase.

The pivotal phase began with newborns born on or after August 27, 2013; the final version of the SEEKER™ cartridge was used for the entirety of the pivotal phase.

6.2.2. Subject Selection

There were no subject inclusion or exclusion criteria for this study since the intended purpose of this test is population newborn screening. All newborn dried blood spots received at the MSPHL

for routine newborn screening in the study period were screened for the four LSD enzymes during the study period. No newborns were omitted from this study unless their parents had opted-out of routine newborn screening on religious grounds, as per Missouri state law[50].

6.3. Study Protocol

The flowchart in Figure 9 depicts the method for screening and retesting newborns during the pilot and pivotal studies.

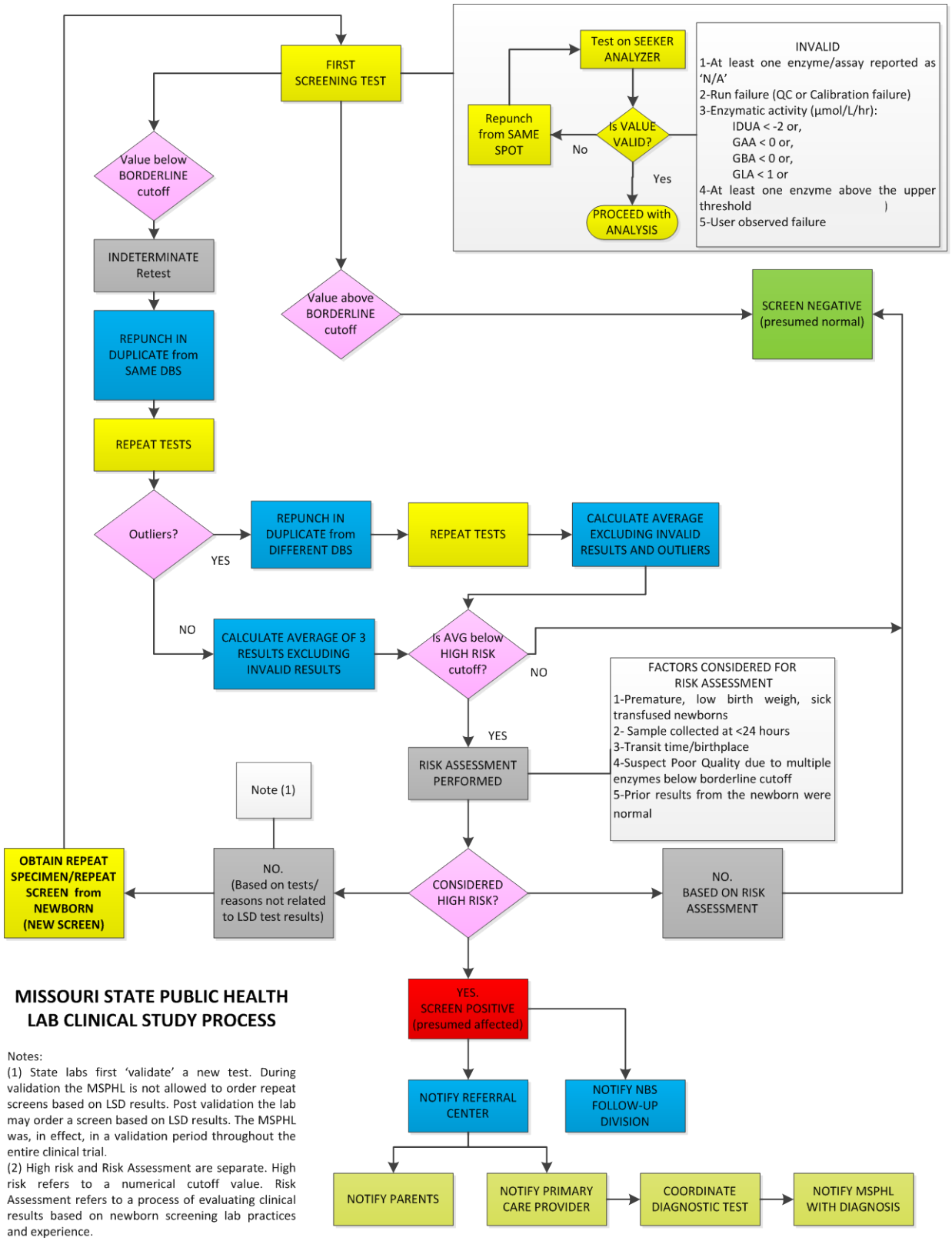


Figure 9: Screening decision flowchart

6.4. Interpretation of Results

6.4.1. Cutoffs

The common practice in newborn screening programs is to utilize two cutoffs – a high risk cutoff and a borderline cutoff. Similarly, this approach is also recommended by CLSI NBS04-A Newborn Screening by Tandem Mass Spectrometry; Approved Guideline. This approach is especially useful for LSDs, where newborns at risk of having a disorder have very low enzymatic activity. The high risk cutoff which is the primary clinical decision making level therefore lies in the lower end of the measurement range of the system.

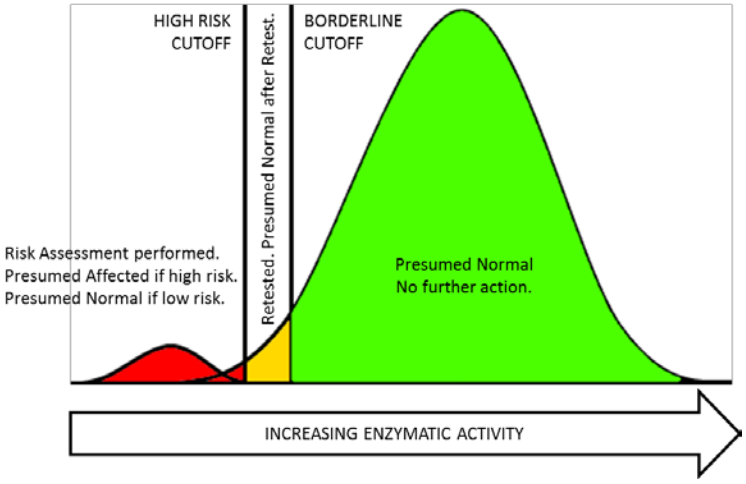


Figure 10: Assessment of cutoff values based on borderline and high risk cutoffs.

This naturally results in higher imprecision around the high risk cutoff. To mitigate this risk a borderline cutoff is set above the high risk cutoff to allow for imprecision around the clinical decision making level. Samples below the borderline cutoff have additional testing (2 minimum for a total of 3 tests per sample) performed on the same specimen.

Samples below the high risk cutoff are carefully assessed using Risk Assessment protocols for diagnostic follow up. This separation of samples by risk level is illustrated in Figure 10.

6.4.2. Risk Assessment

To make a determination regarding referral and confirmatory diagnosis, MSPHL applied a Risk Assessment process to the evaluation of final results. MSPHL applies a number of criteria when assigning risk to samples with test results below the high risk cutoff. Some criteria are reflected in the clinical information collected on the screening card by the clinicians who acquire the blood sample i.e. ‘sick’, ‘transfused’, ‘premature, i.e. gestational age’, age at collection (see Table 23), example of a DBS screening card). This process also includes transit time which can be determined from the information on the card.Table 23 below provides details on the Risk Assessment criteria and a description of their use during the study.

Table 23: Risk Assessment

Risk Assessment Criteria		Description
Additional Samples		Other DBS screen(s) (other DBS specimen cards) from the same newborn that were tested and determined to be presumed normal for LSDs.
Newborn Status	Transfusion status	Samples from transfused babies can have unreliable results and the lab obtains at least one repeat screen; so they did not refer transfused newborns based on the first screen.
	Gestational Age	At least one repeat screen is required from babies that are born significantly premature (<= 34 weeks gestational age). In cases where the birth was premature and another sample was expected, the disposition may be postponed until the repeat sample is received.
	Other Altered Health Status	If the baby is indicated as "Sick" (represented by a category of that designation on the screening card) at the time of collection, the lab requires another screen from the newborn.
	Age at Sample Collection	Initially MSPHL used the newborn age at collection as criterion for Risk Assessment. Later during the study MSPHL instituted age specific cutoffs for samples collected between 7-13 days of life and greater than 14 days of life.
Suspected Poor Quality	Transit Time / Birthplace	MSPHL utilizes a courier system that transports > 95% of newborn samples from the birthing centers to the laboratory. Samples are mailed to the lab in the cases of home births or deliveries at birthing centers that do not participate in the courier program. They have found that in cases where the sample spent significant time in transit, the activity values may be reduced. For samples accessioned 15+ days after collection, the lab will classify the sample as poor quality and request a new screen.
	Other LSD results	MSPHL considered samples where an LSD enzyme is below the high risk cutoff and at least one other enzyme is below the borderline cutoff to be a potentially poor quality sample. Given the population distribution of all four assays and assuming that the activity values of the four assays are expected to be biochemically independent, the estimated likelihood of one assay below high risk and another below borderline is between 1 in 125,000 and 1 in 1,400,000.

6.4.3. Clinical Diagnosis

True clinical status of the referred newborns was determined by the methods summarized in Table 24.

Table 24: Methods for determining true clinical status of referred newborns

Disorder (Enzyme)	Tests	Possible Diagnosis	
		Affected (True Positive)	**Normal (False Positive)**
MPS I (IDUA)	IDUA assay on leukocytes Mutation analysis	• Attenuated • Severe • Genotype of unknown significance	• Normal • Carrier • Pseudodeficiency
Pompe (GAA)	GAA assay on leukocytes Urine HEX4 assay Creatine kinase	• Classical Infantile Onset • Nonclassical Infantile Onset • Late Onset • Unknown Onset • Genotype of Unknown Significance	• Normal • Carrier • Pseudodeficiency
Gaucher (GBA)	GBA assay on leukocytes Mutation analysis.	• Neuronopathic • Non-neuronopathic • Unknown Onset • Genotype of Unknown Significance	• Normal • Carrier
Fabry (GLA)	Male: GLA assay in leukocytes Mutation analysis Female: Mutation analysis	• Classical • Late Onset • Genotype of Unknown Significance	• Normal • Pseudodeficiency

6.5. MSPHL Cutoff Implementation

6.5.1. Initial Cutoffs

Pre-pilot testing was completed at MSPHL using de-identified DBS and known affected samples; the results of the pre-pilot testing were used to set preliminary cutoff values for the study. MSPHL set initial high risk cutoff values for each of the four LSDs by analyzing ~13,000 presumed normal de-identified specimens and 29 known affected specimens on the SEEKER™. Of the affected DBS, 26 of 29 were from pediatric and adult patients (not newborn), because this was the first prospective screening study of newborns and no newborn samples were readily

available. The cutoffs were chosen to ensure that all known affected samples would be detected and to minimize the false positive rate, keeping in mind the expected incidence of the disorders.

MSPHL set their borderline enzymatic cutoff at a slightly higher level than the high risk cutoff values. Initial results below the borderline cutoff were flagged by the analyzer for re-punching and retesting in duplicate. At the start of the clinical study the high risk cutoffs for referral of high risk positive screens were set at the levels described in Table 25.

Table 25: Clinical study high risk and borderline cutoff values

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

6.5.2. Cutoff Changes

The MSPHL utilized an LSD Task Force to evaluate cutoffs and required a review by the task force to adjust high risk cutoff values. The LSD Task Force consisted of geneticists, genetic counselors, newborn screening laboratory staff, newborn screening follow up staff, a chemist, and an adult with Fabry disease. The Task Force met quarterly or as needed and 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 that may affect the enzymatic activity.

6.5.3. Age Specific Cutoffs

During the course of the clinical study it was observed that the enzymatic activity values attenuated with the age of newborn (at time of sample collection) for all assays except IDUA. Based on this observation MSPHL, instituted age specific cutoffs for ages 1-6 days, 7-13 days and 14+ days for GAA, GBA and GLA.

6.5.4. Precision Around Cutoffs

Samples with enzymatic activity below the borderline cutoff are additionally tested in duplicate using 2 additional punches, and the average of all 3 values is used for further disposition. This effectively results in a standard error of the mean equal to standard deviation divided by $\sqrt{3}$. Replication of tests improves the confidence in the enzymatic activity values near the cutoff values and effectively reduces the imprecision.

6.6. Study Limitations

6.6.1. Acquiring New Samples

During the clinical study, MSPHL was not able to obtain a repeat specimen based on the LSD results because the LSD program was in the statewide validation phase. As a result, when the output of the Risk Assessment was to designate the sample as low risk, another sample could not

be collected, and the sample was presumed normal unless another sample was subsequently collected for reasons other than the LSD test results.

The ability to request another sample based on the LSD test results would have removed subjectivity from the screening process during the clinical study. It is recommended that laboratories request an additional sample from the newborn for samples that test below the high risk cutoff but are not determined to be at high risk for disease.

6.6.2. Cutoffs Below LoD

During the clinical study, two of the cutoffs for the IDUA assay were below LoD (2.77). Additionally, one of the age specific cutoffs (14+) for the GLA assay was below the LoD (3.18). MSPHL follow up staff contacted the diagnostic referral centers to confirm that there were no additional newborns diagnosed with MPS I in the clinical study period. The two screens collected from the only prospectively identified MPS I affected newborn identified during the study support this, as the average activity value for both samples was less than the LoD.

6.6.3. Age Specific Cutoffs

Since this was the first prospective clinical study performed for these assays, there was no solid information about the effect of demographics (age, gender, gestational age, etc.) on lysosomal enzyme activity values. During the course of the study, MSPHL determined that three of the four enzymes exhibit a significant decrease in activity in older newborns, and that age specific cutoffs would be required to accurately assess samples from older newborns. Very few affected samples were collected in the 7-13 and 14+ age ranges to perform a receiving operator characteristic (ROC) analysis, but the cutoffs were set near the same percentile rank as the 1-6 day populations.

6.6.4. DBS Variability

Prior studies have been conducted to quantify the variability of specific analytes on DBS[51]; enzyme based assays may be even more sensitive to the causes of variability due to the additional factor of denaturation.

The intrinsic variability within and between blood spots leads to a high coefficient of variability near the cutoffs. The risk of this variability was reduced in several ways:

- Borderline and high risk cutoffs: It is recommended that the borderline cutoff be set above the high risk cutoff by a minimum of two times the assay reproducibility. This accounts for the potential of punch to punch variability causing a false negative result.
- Repeat of samples below borderline cutoffs: All samples that test below the borderline cutoff should be repeated in duplicate. By including additional measurements, the measurement error, including spot variability, is reduced proportionally to the square root of the number of tests.

6.7. Clinical Data Analysis

The clinical study was conducted using specimens received at MSPHL between January 11, 2013 and January 14, 2015. The testing of these specimens occurred from January 15, 2013 and

January 14, 2015, inclusive. The study period was retrospectively separated into "pilot" and "pivotal" phases. The pilot phase included newborns born on or before 8/26/2013. The pivotal phase included newborns born on or after 8/27/2013.

Detailed analysis is provided for the pivotal phase of the study period. This analysis uses the cutoffs that were appropriate at the time of testing and for the specific age ranges. An overall summary of the entire study including both pilot and pivotal phases is also provided.

6.7.1. Specimens Included in Analysis

The total prospective newborn specimen received by MSPHL during the clinical study period was 182,917. When analyzing system performance during both the pilot and pivotal phases, the following samples were excluded from the 182,917 samples received:

- Samples collected at < 24 h of life: Samples collected at less than 24 hours of life trigger a mandatory retest in Missouri. These samples were excluded from the analysis. (n=3,713)
- Samples with no recorded age at collection: If no age at collection was recorded, the sample was excluded from the analysis because age-specific cutoff values could not be applied. (n=580)
- Samples with no valid data point: Some samples did not have a valid test result. These samples were excluded from the analysis. (n=21)
- Samples designated as poor quality on receipt by MSPHL: Reasons for this designation include incomplete saturation, supersaturation, and contamination– in addition to other criteria. These samples were excluded from the analysis. (n=3,055)

The total number of valid samples after these exclusions was 175,548.

6.7.2. Newborns Included in Analysis

From the 175,548 valid specimens, the number of newborns with at least one valid screen was 153,697. The number of specimens is higher than the number of newborns since multiple specimens were collected from the same newborn for a number of reasons, including – but not limited to - premature birth, low birth weight, poor quality initial sample, and sample collection before 24 hours of life (all of these cases require a mandated repeat screen per Missouri state guidelines). Of the 153,697 newborns with at least one valid screen included in the analysis, 48,608 were born during the pilot phase and 105,089 were born during the pivotal phase.

6.7.3. Pivotal Phase Results

The pivotal phase included newborns born on or after 8/27/2013. A total of 105,089 newborns were screened during the pivotal phase. Device performance and clinical results for each assay during the pivotal phase are summarized in 6.7.3.1 through 6.7.3.3. This analysis includes all valid samples from the newborns, including repeat screens. Table 26 lists a description of rows in the analysis tables.

Table 26: Description of rows in assay performance tables

Grouping / Row Title		Description of Row
	Newborns	The total number of newborns included in the pivotal phase analysis.
1st Test	**All screens with first result above borderline**	The number of newborns where the first valid test result for all screens for the newborn were above the borderline cutoff and therefore *presumed normal*.
	At least one screen with first result below borderline	The number of newborns where the first valid test result for at least one screen was below the borderline cutoff and therefore was *repeated.*
Average of all Tests	**All screens w/ avg. above high risk**	After retesting, all screens from the newborn had an average activity value above the high risk cutoff and were therefore *presumed normal.*
	At least one screen w/ avg. below high risk	After retesting, at least one screen from the newborn had an average activity value below the high risk cutoff and *Risk Assessment was performed prior to referral decision.*
	Referred / Not Referred:	After Risk Assessment, the number of newborns that were *presumed affected and referred* or *presumed normal and not referred*.
Referred Sample Summary		The number of newborns in each category of final clinical diagnosis (i.e. true positive, false positive, etc.)
Samples Not Referred Summary		The number of newborns with average activity values below the high risk cutoff that were not referred for each of the reasons provided related to the Risk Assessment.
Performance Summary	**Total Presumed Normal**	The number of newborns *presumed normal* after the first test, repeat testing, or Risk Assessment
	Total Presumed Affected	The number of newborns *presumed affected and referred* after Risk Assessment
	True Positives	The number of newborns *confirmed to have the disorder* after diagnostic and molecular follow up testing
	False Positives	The number of newborns *confirmed to be absent of disease* after follow up testing; this includes carriers and pseudodeficiencies
	Refused/Moved	The number of newborns who *did not receive confirmatory testing* either because it was refused or because the family moved out of Missouri
	Below HR / Not Referred	The number of newborns with activity values below the high risk cutoff that were *not referred* after Risk Assessment
	False Positive Rate (FPR)	The number of newborns with false positive results divided by the total number of newborns – minus true positives and newborns who did not receive follow up testing
	False Positive Rate (FPR) incl. below HR/not referred	The number of newborns with activity values below the high risk cutoff (including those referred and not referred) divided by the total number of newborns – minus true positives and newborns who did not receive follow up testing

6.7.3.1. Presumed Affected

		IDUA	GAA	GBA	GLA
	Newborns	105,089	105,089	105,089	105,089
1st Test	All screens with first result above borderline	104,245	103,691	104,336	103,670
	At least one screen with first result below borderline	844	1,398	753	1,419
Average of all tests	All screens with average above high risk	793	1,288	690	1,219
	At least one screen with average below high risk	51	110	63	200
	Referred	33	45	8	60
	Not Referred	18	65	55	140
Referred Sample Summary	True Positive	0	7	2	30
	Normal, False Positive	9	23	3	26
	Pseudodeficiency, False Positive	20	8	0	0
	Carrier, False Positive	2	7	2	0
	Refused	1	0	1	3
	Moved	1	0	0	1

6.7.3.2. Presumed Normal After Risk Analysis

		IDUA	GAA	GBA	GLA
Samples Not Referred Summary	Other normal sample from newborn	13	42	34	84
	Suspected poor quality sample	2	12	12	29
	Altered health status	0	2	3	6
	Other	3	9	6	21

The Risk Assessment categories in the above table include:

- Other normal sample from newborn: prior sample from newborn (n=131), later sample from newborn (n=42).
- Suspected poor quality sample: other assay below borderline (n=49), spot variability (n=3), contaminated sample (n=1), other assay low normal (n=2).
- Altered health status: transfused (n=11).
- Other: Outliers excluded (n=31), different cutoff applied (n=3), retrospectively referred (n=2), age related enzyme decrease (n=1), and multiple reasons (n=2).

6.7.3.3. Clinical Performance Summary

		IDUA	GAA	GBA	GLA
Performance Summary	Total Presumed Normal	105,056	105,044	105,081	105,029
	Total Presumed Affected	33	45	8	60
	True Positives	0	7	2	30
	False Positives	31	38	5	26
	Refused/Moved	2	--	1	4
	Below HR/Not Referred	18	65	55	140
	False Positive Rate (FPR)	0.029%	0.036%	0.005%	0.025%
	False Positive Rate (FPR) – included below HR/not referred	0.047%	0.098%	0.057%	0.158%

The last line of the above table, False Positive Rate (FPR) – included below HR/not referred, references the number of newborns with activity values below the high risk cutoff (including those referred and not referred) divided by the total number of newborns – minus true positives and newborns who did not receive follow up testing. This would be the worst-case false positive rate if Risk Assessment were not performed.

6.7.3.4. Retest Rate

A total of 120,118 specimens from 105,089 newborns were tested during the pivotal phase. The number of specimens is higher than the number of newborns since many newborns had multiple specimens collected, either due to their health status (low birth weight, premature, transfused) or due to other routine newborn screening results.

A total of 137,153 individual tests were performed on the 120,118 specimens, resulting in an overall retest rate of 1.142 or 14.2% per specimen. Of these 137,153 individual tests 7785 tests were due to invalid data points generated by the device representing an invalid rate of 5.7% (per specimen). The remaining 8.5% were due to retesting triggered by activity values below borderline cutoff for one of the assays (as required by the screening protocol).

Due to the multiplexed nature of the test a retest trigged by one assay will result in all 4 assays being repeated. The average retest rate on a per assay basis is 14.2/4 = 3.55%. The average invalid rate on a per assay basis is 5.7/4 = 1.43%.

6.7.4. Two Year Clinical Study Summary

During the entire study period (both pilot and pivotal), 275 newborns were presumed affected and referred for confirmatory diagnosis. The results of the confirmatory testing are listed in Table 27.

Table 27: Presumed affected individuals – entire study period

	IDUA	GAA	GBA	GLA
Presumed Affected	73	79	19	104
- Refused	1	0	1	4
- Moved	1	1		1
- Normal, False Positive	31	33	13	47
- Carrier, False Positive	4	14	2	0
- Pseudodeficient, False Positive	35	14	0	0
- True Positive	**1**	**17**	**3**	**52**
- Unclassified Onset	1	0	1	45
- Unknown Onset / Genotype of Unknown Significance	0	3	2	4
- Late Onset	0	9	0	3
- Infantile Onset	0	5	0	n/a
- Classical Infantile	0	3	0	n/a
- Non-Classical Infantile	0	2	0	n/a
False positive rate	0.045%	0.039%	0.010%	0.030%

6.7.4.1. Disease Incidence

Based on the number of newborns analyzed for this two-year study period (n=153,697), the incidence of each of the disorders was calculated and is summarized in Table 28.

Table 28: Incidence rate during clinical study

	Incidence from MSPHL Study
MPS I (IDUA)	1 : 153,697
Pompe (GAA)	1 : 9,041
Gaucher (GBA)	1 : 51,232
Fabry (GLA)	1 : 2,956

6.8. Conclusions

6.8.1. True Positives

6.8.1.1. Confirmed Positive Newborns

MPS I: The only confirmed MPS I positive newborn was screened during the pilot phase of the study. This was reported as a severe case of MPS I (Hurler disorder).

Pompe: Seventeen newborns were confirmed affected with Pompe disorder during the clinical study. Five of these newborns were confirmed to have infantile onset Pompe disorder, which results in death at a median age of approximately 12 months. Nine newborns were confirmed with late onset Pompe disorder, which can begin to cause symptoms as early as childhood. Three

of the newborns were confirmed to have genetic mutations of unknown significance; they will require long term follow up to test for disease onset.

Gaucher: Three newborns were confirmed affected with Gaucher disorder. One newborn was confirmed to have Type 1 – non-neuronopathic Gaucher disorder of unknown onset. The other two newborns were found to have mutations of unknown significance; they will also require long term follow up.

Fabry: 52 newborns were confirmed affected with Fabry disorder during the study. The status of 45 of these newborns was reported by the referral center as "Fabry disorder" without a classification of onset. Three newborns were confirmed with mutations consistent with late onset Fabry disorder and four newborns were confirmed to have mutations of unknown significance.

6.8.1.2. Disease Incidence

Based on the number of newborns analyzed for this two-year study period (n=153,697), the incidence of each of the disorders was calculated and is summarized along with previously published incidence (Table 29).

Table 29: Incidence rate during clinical study

	Incidence from MSPHL 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

For Pompe disorder the MSPHL incidence was about 3 times higher than published rates of incidence. For MPS I, Gaucher, and Fabry the MSPHL incidence is comparable to the published rates of incidence.

6.8.2. False Negatives

6.8.2.1. Surveillance

The Missouri Department of Health and Senior Services 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 reported during the 2 year study or in 15 months following the conclusion of the study. Newborns screened during the clinical study with early onset disorders would have been reported to one of these metabolic centers. In March, 2016, the MSPHL received letters from all metabolic centers in the state indicating that no false negatives had been reported or noted clinically since implementation of LSD screening[52].

Newborns screened during the clinical study with early onset disorders would have been reported to one of the metabolic centers. False negatives are based on limited data on late onset forms of the disorders since it would 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[53]. For female Fabry 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.

6.8.2.2. Incidence

The estimated incidence rates in the literature are approximately 1: 28,000 for Pompe, 1: 3,000 for Fabry, 1: 57,000 for Gaucher and 1: 100,000 for MPS I, resulting in a combined incidence rate of 1:2,500. Based only on confirmed positive cases, data from the current study in Missouri indicate a slightly higher incidence rate of 1:2,105. The fact that the individual incidence rates for each disorder in the study agree with or are higher than published literature suggests that risk related to false negatives is minimal.

6.8.2.3. Limitations

- This false negative rate is based on limited data on late onset forms of the disorders since it would take several years to identify an undetected 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[54].
- For female Fabry patients GLA enzyme activity is highly variable and it typically falls in the normal range. Therefore, GLA enzyme activity for females should be interpreted with caution as most female carriers can have enzyme activity in the normal range which results in a normal screen.

6.8.3. False Positives

During the clinical study, several patients with reduced enzymatic activity were confirmed by follow-up testing to be either carriers or pseudodeficient for the referred condition. Carriers were identified for MPS I, Pompe and Gaucher disorders, while pseudodeficiencies were identified for MPS I and Pompe disorders. Carriers and pseudodeficient newborns may exhibit enzymatic activity below the high risk cutoff while remaining asymptomatic for the disorder and are included in the false positive calculations. Table 30 indicates the false positive rate during the pivotal phase and entire study period for each assay.

Table 30: False positive rates during the pivotal phase and entire study period

	IDUA	**GAA**	**GBA**	**GLA**
Pivotal False Positive Rate (FPR)	0.029%	0.036%	0.005%	0.025%
Overall False Positive Rate (FPR)	0.045%	0.039%	0.010%	0.030%

The false positive rates during the pivotal phase and the entire study period for each assay are well below the typical goal of a 0.1% FPR for newborn screening assays.

A number of newborns had average test results below the high risk cutoff during the study but were not referred based on the results of the Risk Assessment. If the Risk Assessment was not performed and these newborns would presumably be false positive results given that the

surveillance program has not identified any false negatives. If these were added as false positives, the adjusted worst-case false positive rate would increase as shown in Table 31.

Table 31: False positive rates including samples below high risk but not referred as false positives

	IDUA	GAA	GBA	GLA
Pivotal False Positive Rate (FPR) including newborns below the high risk cutoff and not referred as false positives *** Note: This does not represent how the study was conducted**	0.047%	0.098%	0.057%	0.158%

It is important to provide some context for evaluating these false positive rates, relative to other newborns screening tests. Summary data for several tests is shown in Table 32[55],[56]. Note that typically additional samples (or screens) are collected from newborns when an initial sample tests below the borderline cutoff.

Table 32: False positive rates for other conditions

Condition	Reported False Positive Rate
Cystic Fibrosis	0.35%
Congenital Hypothyroidism	0.30%
Congenital Adrenal Hyperplasia	0.05%
Biotinidase Deficiency	0.09%
Galactosemia	0.05%

6.8.4. Retest Rate

A total of 120,118 specimens from 105,089 newborns were tested during the pivotal phase. The number of specimens is higher than the number of newborns since many newborns had multiple specimens collected, either due to their health status (low birth weight, premature, transfused) or due to other routine newborn screening results.

A total of 137,153 individual tests were performed on the 120,118 specimens, resulting in an overall retest rate of 1.142 or 14.2% per specimen. Of these 137,153 individual tests 7785 tests were due to invalid data points generated by the device representing an invalid rate of 5.7%. The remaining 8.5% were due to retesting triggered by activity values below borderline cutoff for one of the assays.

Due to the multiplexed nature of the system, a retest triggered by one assay value will result in all 4 assays being repeated. The average retest rate on a per assay basis is therefore 14.2/4 = 3.55%. The average invalid rate on a per assay basis is 5.7/4 = 1.43%.

7. SUMMARY

Nearly every newborn in the United States is screened for, on average, 43 congenital diseases through biochemical tests that identify affected newborns so that life-saving therapies can be administered before irreversible damage, and in some cases death, occurs. The U.S. Department of Health and Human Services Secretary's Advisory Committee of Heritable Disorders in Newborns and Children, which recommends the minimum test menu, has recently recommended that two lysosomal storage disorders be added to this minimum panel. Lysosomal storage disorders are a group of approximately 50 rare inherited metabolic disorders that are caused by lysosomal dysfunction, usually as a consequence of a deficiency in a single enzyme required for the metabolism of lipids, glycoproteins or mucopolysaccharides. The company developed SEEKER™ to screen for 4 LSDs: Mucopolysaccharidosis Type I (MPS I), Pompe, Gaucher and Fabry.

The SEEKER™ System uses a novel liquid handling technology called digital microfluidics, combining this with conventional fluorescent measurement techniques and well-known chemistry. The SEEKER™ System is intended for quantitative measurement of the activity of multiple lysosomal enzymes from newborn dried blood spot samples. Reduced activity of these enzymes may be indicative of a lysosomal storage disorder. The SEEKER™ System and its panel of four LSD's were evaluated analytically and clinically. Over the course of 24 months, 153,697 newborns (with valid screens) were screened at the Missouri State Public Health Laboratory in pilot and pivotal studies. Screening was conducted in a manner where all valid initial tests were compared to a borderline cutoff value. If a newborn tested below the borderline cutoff for any of the four conditions, retesting and Risk Assessment were conducted to further assess the newborn.

A total of 275 newborns were ultimately referred for further diagnosis, and 73 newborns were confirmed positive for one of the 4 LSDs. The clinical data demonstrates that SEEKER™ accurately measures and reports enzymatic activity for the stated lysosomal storage disorders. The use of SEEKER™ in combination with a program to properly train operators and staff, and to appropriately establish and manage cutoffs is effective for successful population screening of newborns.

8. APPENDICES

1. SEEKER™ Clinical Study Report

9. REFERENCES

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