Lysosomal Storage Disorder Screening Implementation: Findings from the First Six Months of Full Population Pilot Testing in Missouri

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Objective To evaluate the performance of a statewide full-population pilot study in Missouri on newborn blood spots for screening of lysosomal storage disorders (LSDs) using digital microfluidics.

Study design A full-population pilot study using a multiplexed fluorometric enzymatic assay to detect Pompe disease, Fabry disease, Gaucher disease, and mucopolysaccharidosis type I (MPS I) in the Missouri newborn population is ongoing. Provisional cutoff values were determined during a prepilot study. All newborn dried blood spots received at the Missouri State Public Health Laboratory for routine newborn screening were screened for the 4 LSDs during the pilot study. Newborns determined to be screen-positive were referred for confirmatory testing.

Results The study commenced on January 11, 2013; during the first 6 months, 43 701 specimens were screened, and 27 newborns with a confirmed diagnosis of an LSD genotype (8 with Pompe disease, 1 with Gaucher disease, 15 with Fabry disease, and 3 with MPS I) were identified. These numbers correspond to detection rates of 1:5463 for Pompe disease, 1:43 701 for Gaucher disease, 1:2913 for Fabry disease, and 1:14 567 for MPS I. The positive predictive values were 47% for Pompe disease with 1 lost to follow-up, 10% for Gaucher disease, 58% for Fabry disease with 2 lost to follow-up, and 11% for MPS I with 4 pending.

Conclusion The first 6 months of the Missouri LSD pilot study provided the opportunity to validate the effectiveness of the digital microfluidic screening method, refine the cutoffs for detection of these LSDs, and test the entire system of infant referral, follow-up, confirmation, treatment, and screening program communication. *(J Pediatr 2015;166:172-7)*.

Lysosomal storage disorders (LSDs) are increasingly recognized as diseases for which newborns could greatly benefit from newborn bloodspot screening (NBS).[1] Pompe disease was recently recommended to the US Secretary of Health and Human Services for addition to the recommended uniform screening panel.[2] Interest in newborn screening for additional LSDs continues to expand as new technologies and treatments become available.

The State of Missouri requires screening for LSDs, including Pompe disease (acid α-glucosidase [GAA] deficiency), Fabry disease (acid α-galactosidase [GLA] deficiency), Gaucher disease (deficiency of acid β-D-glucosidase [GBA]), and Krabbe disease.[3] On January 11, 2013, the Missouri State Public Health Laboratory (MSPHL) began screening for Pompe disease, Fabry disease, Gaucher disease, and mucopolysaccharidosis type I (MPS I; deficiency of acid α-L-iduronidase [IDUA]) disorders on all dried blood spot (DBS) specimens collected in the state; screening for Krabbe disease is currently outsourced to the New York newborn screening laboratory.[4,5] The Missouri and New York newborn screening programs are currently the only 2 programs in the US offering statewide screening for any of the LSDs, although 3 other states have passed legislation requiring screening.[6-8] With the pending addition of Pompe disease to the recommended uniform screening panel,[2] more states are likely to initiate NBS for LSDs.

For this LSD screening study, MSPHL set up 2 laboratory work stations, each with 4 digital microfluidics instruments provided by Baebies (Durham, North Carolina) using a multiplex enzymatic assay methodology as described previously.[9] Preliminary enzymatic assay cutoffs were determined statistically by MSPHL during a prepilot study with more than 13 000 deidentified DBS specimens and 29 DBS specimens from patients with a confirmed LSD. In the first 6 months of the

DBS	Dried blood spot
GAA	Acid α-glucosidase
GBA	Acid β-D-glucosidase
GLA	Acid α-galactosidase
IDUA	Acid α-L-iduronidase
LSD	Lysosomal storage disorder
MPS I	Mucopolysaccharidosis type I
MSPHL	Missouri State Public Health Laboratory
NBS	Newborn bloodspot screening
PPV	Positive predictive value

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Baebies, Advanced Liquid Logic Inc, an Illumina company, provided technical support and methodology. The authors declare no conflicts of interest.

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full-population pilot testing phase, 43 701 newborn specimens were screened at MSPHL, and 27 patients were identified and confirmed to have an LSD genotype of some form.

Methods

Residual and deidentified DBS samples from more than 13 000 NBS specimens obtained from Missouri newborns for routine screening and stored in accordance with the state NBS sample storage policy were used for the prepilot enzymatic activity cutoff determinations. These residual specimens were approximately 6 months old at the time of use. The specimens were stored in freezers at −20°C to −30°C in airtight bags with desiccant after the completion of routine screening[10]; at this temperature, the stability of the enzymes is preserved.[11] Fresh DBS samples were punched from routine NBS specimens received at MSPHL on and after January 11, 2013, and were assayed for LSDs following an Institutional Review Board–approved protocol at the same time that the other required NBS tests were performed.

Prepackaged reagents for GAA, GBA, GLA, and IDUA; cartridge filler oil; calibrants; and stop and extraction buffers were all procured directly from Baebies. Reagent formulations for Pompe disease, Fabry disease, Gaucher disease, and MPS I have been described previously.[9,12]

For each DBS sample used in the prepilot and pilot studies, one 3-mm sample was punched into a deep-well 96-well microtiter plate, and extraction buffer was added to obtain DBS extracts.[9] In brief, 100 μL of extraction buffer (0.1% [wt/vol] Tween 20 in water) was added to the DBS punches in a microtiter plate. The DBSs were allowed to incubate for 30 minutes at room temperature on an orbital shaker. Before each assay, 1.6 μL of DBS extract was transferred to a well on a digital microfluidic cartridge using a multichannel pipette; stop buffer, reagents, and calibrators were also transferred to appropriate wells on the cartridge.[9]

Prepilot Phase: Determination of Preliminary Enzymatic Assay Cutoff Values

The initial cutoff values for each of the 4 LSDs were determined using normal patient percentile data obtained from the deidentified prepilot samples along with DBS enzyme-level data from 29 known clinical cases provided by our contracted genetic referral centers. All of these samples were analyzed on the Baebies digital microfluidic instruments using the multiplex method as described previously.[9,12] Pilot cutoffs for referral of positive screens were set at the following levels: GAA, 8.0 μmol/L/hour (0.17 percentile); GBA, 4.5 μmol/L/hour (0.4 percentile); GLA, 5.5 μmol/L/hour (0.23 percentile); IDUA, 4.0 μmol/L/hour (0.8 percentile). These cutoffs for referring positive screens were chosen to ensure that the known clinical case control samples could be detected without provoking too many referred cases from the newborn screening process, taking into account the expected incidence of each of the 4 disorders. Provisional enzymatic cutoffs were set at slightly higher levels than the final referral values. Initial results below the provisional cutoffs were flagged by the instrument for repunching and retesting in duplicate. The average enzyme activity from all 3 runs was then applied to final referral cutoffs to trigger referral action. If the initial assay value or any other single value was significantly different from the other 2 values, then another set of duplicate punches was obtained from different blood spot circles on the same DBS specimen and then assayed to further investigate spot-to-spot variation.

Pilot/Implementation Phase

The full-population pilot included all Missouri newborn specimens received on and after January 11, 2013, with subsequent referral and confirmation of all screen-positives (**Figure 1**). During the first 6 months of the pilot, 43 701 newborn specimens were screened using the multiplex enzymatic assay on the digital microfluidic platform. Those samples that tested positive (ie, with average triplicate screening values breaching our referral cutoff) for Pompe disease, Fabry disease, Gaucher disease, or MPS I were then assessed for risk. Risk level was assessed by reviewing other LSD enzyme results from the multiplex assay along with the infant's gestational age, age at specimen collection, and health status. All Missouri NBS specimens collected from premature infants, sick infants, or infants aged <24 hours automatically mandate a repeat screen, because these circumstances can produce false-positive, false-negative, and unreliable NBS results.

During our pilot study phase, if none of these conditions apply to the positive screen, and if the quality of the specimen is not considered poor owing to multiple low enzyme levels, then the screen-positive result is considered high risk and is referred to 1 of 4 contracted genetic referral centers for evaluation, confirmatory testing and diagnosis (**Figure 1**). Genetic center referrals are based on geographic regional coverage and the location of the patient's residence.

Results

Prepilot Phase: Determination of Enzymatic Assay Cutoff Values

Initial cutoffs determined during the prepilot phase were found to be effective. Cutoff values of 8.0 μmol/L/hour for GAA, 4.5 μmol/L/hour for GBA, 5.5 μmol/L/hour for GLA, and 4.0 μmol/L/hour for IDUA were established. Many of the published newborn screening feasibility studies using tandem mass spectrometry were performed on anonymous specimens.[13-16] Fluorometric methods were used on pilot studies with identified newborns.[17-20] The results generated in our study were obtained from newborns from the entire state with reporting and follow-up procedures, unlike studies on anonymous specimens where no reporting is done, which required us to set the cutoff values rather conservatively to minimize the possibility of false-negatives. These initial startup cutoff values were applied to DBS samples from 3 clinically diagnosed infants who had been identified by the