Missouri’s Experience
with Full Population Pilot Screening
for
Pompe, Gaucher, Fabry and MPS-I Disorders

Utilizing Digital Microfluidics Technology

Patrick Hopkins, Chief of Missouri NBS Laboratory
October 29, 2014
Thank You to New York’s NBS Laboratory!

The Krabbe Screening Experts
MO LSD Statewide Pilot Screening

Krabbe (GALC)  →  Testing by New York
Since August 2012
(over 195,000 samples)
(approximately 169,000 births)

Pompe (GAA)
Gaucher (GBA)
Fabry (GLA)
MPS-I (IDUA)  →  Missouri Testing
Since January 11, 2013
(over 163,000 samples)
(approximately 136,000 births)

Krabbe (GALC)
Niemann-Pick (ASM)  →  Missouri to Add-on next
Implementation Process

• Contract procurement (reagent rental)
• Installation and training
• Familiarization
• Validations
• Pre-pilot phase to collect data on de-identified samples for normal ranges and startup cutoffs
• Full population pilot/implementation phase testing with referral and confirmation of positives
• Live testing with reporting on all NBS laboratory reports
2 Work Stations
8 Digital Microfluidics (DMF) Platforms
Open Platform

48 Well Sample Cartridge
2 scientists currently working on 2 work stations of 8 instruments

48 sample wells assayed per instrument

- 10 controls (2 blanks, 4 calibrators, 2 low controls and 2 medium controls)
- 38 patient samples

Sample punch to enzymatic activity results in ~4 hrs

Workflow for LSD Testing in MSPHL

Punch DBS samples
(15 min per 96-well plate)
Single punch for 4-plex assays.

Extraction
(30 min at RT)
Load filler fluid in cartridges. Thaw reagents during extraction.

Loading
(5 min per machine)
Load samples (3.5µL), reagents (12µL) and stop buffer in each cartridge.

Machine run time
(2.5 h for 4-plex assay)
After 2.5h remove the cartridge from the instrument and get ready for next run.
Enzyme Reaction in DMF Method

Artificial Substrate + Enzyme → Product

4MU-α-D-Glucopyranoside + DBS extract (GAA) → 4MU + Glucose

High Fluorescence = normal GAA activity
Low Fluorescence = low GAA

Positive Pompe Screen!
Each Cartridge Has 4 Calibrators

<table>
<thead>
<tr>
<th>Concentration</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0375</td>
<td>104.29</td>
<td>102.23</td>
</tr>
<tr>
<td>0.075</td>
<td>196.57</td>
<td>193.99</td>
</tr>
<tr>
<td>0.15</td>
<td>386.82</td>
<td>380.51</td>
</tr>
<tr>
<td>0.3</td>
<td>763.54</td>
<td>757.61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2506.79</td>
<td>8.18</td>
<td>0.9999</td>
</tr>
</tbody>
</table>
Quality Control Monitor for Run

48x4v10 QC Report

QCL

QCM
# Results Screen

## Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>IDUA (µmol / L / h)</th>
<th>GAA (µmol / L / h)</th>
<th>GBA (µmol / L / h)</th>
<th>GLA (µmol / L / h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Mean</td>
<td></td>
<td>22.77</td>
<td>23.92</td>
<td>20.80</td>
<td>24.74</td>
</tr>
<tr>
<td>CDCBP281 A02</td>
<td></td>
<td>3.51</td>
<td>1.60</td>
<td>2.39</td>
<td>3.87</td>
</tr>
<tr>
<td>CDCL282 A03</td>
<td></td>
<td>5.13</td>
<td>3.64</td>
<td>2.72</td>
<td>5.49</td>
</tr>
<tr>
<td>CDCM283 A04</td>
<td></td>
<td>24.16</td>
<td>22.73</td>
<td>10.18</td>
<td>37.34</td>
</tr>
<tr>
<td>CDCH284 A05</td>
<td></td>
<td>39.48</td>
<td>33.50</td>
<td>12.92</td>
<td>54.95</td>
</tr>
<tr>
<td>14036 A06</td>
<td></td>
<td>37.31</td>
<td>9.91</td>
<td>35.37</td>
<td>94.67</td>
</tr>
<tr>
<td>14036 A07</td>
<td></td>
<td>28.92</td>
<td>8.33</td>
<td>35.84</td>
<td>86.73</td>
</tr>
<tr>
<td>14036 A08</td>
<td></td>
<td>7.60</td>
<td>8.35</td>
<td>9.88</td>
<td>7.32</td>
</tr>
<tr>
<td>14036 A09</td>
<td></td>
<td>9.09</td>
<td>10.60</td>
<td>11.93</td>
<td>9.46</td>
</tr>
<tr>
<td>QCM A10</td>
<td></td>
<td>12.62</td>
<td>12.22</td>
<td>6.29</td>
<td>31.67</td>
</tr>
<tr>
<td>QCL A11</td>
<td></td>
<td>5.71</td>
<td>5.87</td>
<td>4.00</td>
<td>10.83</td>
</tr>
<tr>
<td>14037 B10</td>
<td></td>
<td>17.84</td>
<td>22.80</td>
<td>20.31</td>
<td>11.81</td>
</tr>
<tr>
<td>14037 B11</td>
<td></td>
<td>23.80</td>
<td>33.63</td>
<td>20.43</td>
<td>14.29</td>
</tr>
<tr>
<td>14037 B12</td>
<td></td>
<td>21.91</td>
<td>33.57</td>
<td>20.52</td>
<td>9.83</td>
</tr>
<tr>
<td>14037 C02</td>
<td></td>
<td>19.67</td>
<td>6.20</td>
<td>18.58</td>
<td>22.28</td>
</tr>
<tr>
<td>14037 C03</td>
<td></td>
<td>18.38</td>
<td>5.55</td>
<td>17.93</td>
<td>20.06</td>
</tr>
<tr>
<td>14037 C04</td>
<td></td>
<td>12.15</td>
<td>12.77</td>
<td>12.39</td>
<td>8.59</td>
</tr>
<tr>
<td>14037 C05</td>
<td></td>
<td>12.08</td>
<td>11.59</td>
<td>10.59</td>
<td>7.90</td>
</tr>
<tr>
<td>14037 C06</td>
<td></td>
<td>21.74</td>
<td>19.23</td>
<td>32.69</td>
<td>11.43</td>
</tr>
<tr>
<td>14037 C07</td>
<td></td>
<td>12.15</td>
<td>14.52</td>
<td>20.72</td>
<td>8.10</td>
</tr>
<tr>
<td>14037 C08</td>
<td></td>
<td>23.08</td>
<td>29.23</td>
<td>25.47</td>
<td>27.50</td>
</tr>
</tbody>
</table>

*Yellow = Instrument Cutoff, Red = Referral Cutoff*
Health Status Effect

Pompe - Full-term vs Premies

FULL TERM
PREMIES
Health Status Effect

Fabry - Full-term vs. Preterm

FULL TERM  PREMIES
Median Enzyme Activities

![Bar chart showing median enzyme activities for GAA, GBA, GLA, and IDUA for male and female subjects. Male: n=27,564, Female: n=26,236.]
MSPHL LSD Pilot/Implementation Screening Algorithm

Assess risk level

High Risk?

NO

NO

MSPHL notifies Referral Center

MSPHL notifies NBS Follow-up

YES

Retest in duplicate

YES

Value below instrument cut-off

NO

No further action

Average of initial and duplicate values below referral cut-offs

Retest in duplicate

Screening Test
### Missouri LSD Pilot/Implementation Phase Totals

**10/21/14**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Screen Positives</th>
<th>Confirmed Disorders</th>
<th>Conditions of ??? Significance or ??? Onset</th>
<th>Pseudo-deficiencies</th>
<th>Carriers</th>
<th>False Positives</th>
<th>Pending</th>
<th>Lost to Follow-up</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pompe</td>
<td>68</td>
<td>12</td>
<td>3</td>
<td>10</td>
<td>11</td>
<td>16</td>
<td>15</td>
<td>1</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5 infantile, 7 late)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaucher</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>17%</td>
</tr>
<tr>
<td>Fabry</td>
<td>95</td>
<td>35</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>8</td>
<td>6</td>
<td>51%</td>
</tr>
<tr>
<td>MPS-I</td>
<td>65</td>
<td>1</td>
<td>0</td>
<td>23</td>
<td>4</td>
<td>28</td>
<td>8</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Krabbe</td>
<td>32</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25%</td>
</tr>
<tr>
<td>Aggregate</td>
<td>279</td>
<td>49</td>
<td>20</td>
<td>33</td>
<td>41</td>
<td>96</td>
<td>32</td>
<td>8</td>
<td>29%</td>
</tr>
</tbody>
</table>

Total Samples Screened for LSDs in MO NBS lab as of 10/21/14 = 163,528 (~ 136,500 births)

Total Samples Screened for Krabbe via NY as of 10/21/14 = 195,595 (~ 169,000 births)

PPV = CP / TR - Pending & Lost

PPV (Positive Predictive Value)
CP (Confirmed Positive)
TR (Total Referred)
False Positive Rate

- Pompe = 0.02%
- Gaucher = 0.01%
- Fabry = 0.02%
- MPS-I = 0.03%
- Krabbe = 0.01%

Total Samples Screened for LSDs in MO NBS lab as of 10/21/14 = 163,528 (~ 136,500 births)

Total Samples Screened for Krabbe via NY as of 10/21/14 = 195,595 (~ 169,000 births)
Important Laboratory Findings

• Enzyme activities drop slightly during the first 2 weeks of age and then stabilize after 14 days-of-age. Need age-related cutoffs for older babies.
• Premature babies can have altered LSD enzyme levels. The repeat screens may be more reliable on these.
• Multiplexing with other enzyme assays greatly helps assess quality of sample and risk for referral.
• Some seasonal variation is observed with enzyme activities, similar to GALT assay in that more carriers and pseudo-deficiencies will be detected during higher heat and humidity months.
• We are very pleased with the performance of this screening method, the ease at which it can be incorporated into the NBS laboratory, and the ease at which it can conducted.
Missouri’s Follow-up

- Four contracted referral centers
- The designated referral center contacts the primary care physician
- A plan is developed and appointments made with a genetic disease specialist and other related pediatric specialists
- Confirmatory testing is completed and treatment/management started based on developed guidelines
Follow-Up Lessons Learned

- Screening and confirmation for Lysosomal disorders are complex
- Follow-up guidelines may need to be flexible
- Frequent communication between the specialists has been helpful
- Having a close relationship with the appropriate pediatric specialists is key
Follow-Up Challenges

• How to communicate to parents the unknown onset or unknown risk diagnosis
• How to follow patients with unknown onset and unknown risk diagnoses
• What are the implications of testing unaffected siblings for the variants of unknown risk?
• Detection of Pseudo-deficiency and Carriers
• Lost to follow-up
Detected on 2\textsuperscript{nd} Day of Pilot

NBS for Pompe has been recommended by the DACHDNC to be added to the core panel of screening disorders.

Gavin’s Story is on the Save Babies Through Screening Foundation website.
Acknowledgements

• Dr. Joe Orsini and the NY Krabbe screening team.
• Carlene Campbell, Tracy Klug, Darla Eiken, Dennis Schmitz and the Missouri LSD screening team
• Dr. Sharmini Rogers, Julie Raburn-Miller, Jami Kiesling and the Missouri NBS follow-up team
• Dr. Robert Vogt, Dr. Hui Zhou, and the CDC LSD quality assurance support team
• Dr. Dietrich Matern and the Mayo LSD team
• The Baebies Inc. team