Newborn Screening for Pompe Disease and other Lysosomal Storage Disorders:  
*CDC Reference Methods and Materials*

Hui Zhou, MD, PhD  
Newborn Screening Translational Research Initiative (NSTRI)  
Newborn Screening and Molecular Biology Branch

*Technical Workshop on Methods to Detect Pompe Disease and other Lysosomal Storage Disorders by Newborn Bloodspot Screening*  
Atlanta, April 16-17, 2015
Disclosures

Dr. Zhou is an NSTRI Senior Scientist who is employed by the CDC Foundation. Her position is supported by donations from Genzyme Corporation.

The CDC Foundation receives donations on behalf of NSTRI from Genzyme Corporation, a Sanofi Company; Biogen-Idec; and The Legacy of Angels Foundation.
QC Blood Pool Production

1. Wash leuko-depleted blood with saline X3
2. Add heat-inactivated serum
3. Base pool 50 ± 2% Hematocrit

- Umbilical Cord blood 50 ± 2% Hematocrit
  - 0% cord blood 100% Base pool (QC BP)
  - 5% cord blood 95% Base pool (QC Low)
  - 50% cord blood 50% Base pool (QC Med)
  - 100% cord blood 0% Base pool (QC High)

(De Jesus et al, Clin Chem 2009; 55(1); 158-164)
LSD Enzyme Function Assays by FIA-MS/MS

One 3.2mm Punch

- Elute in 70 µl
- 37°C, 1 hr

One 3.2mm Punch

- 30 µl GALC
- Substrate Cocktail

Linearity of CDC LSD Reference System

- **GAA Predicted Activity**
  - $r^{2} = 0.99$
  - Slope = $1.0 \pm 0.044$

- **IDUA Predicted Activity**
  - $r^{2} = 0.98$
  - Slope = $1.0 \pm 0.060$

- **GALC Predicted Activity**
  - $r^{2} = 0.99$
  - Slope = $0.95 \pm 0.041$

- **ABG Predicted Activity**
  - $r^{2} = 0.98$
  - Slope = $0.98 \pm 0.049$

- **GLA Predicted Activity**
  - $r^{2} = 0.98$
  - Slope = $0.97 \pm 0.046$

- **ASM Predicted Activity**
  - $r^{2} = 1.0$
  - Slope = $1.0 \pm 0.022$
QC-High DBS-RM Over 4 Years

Enzyme Activity (uM/L/Hr)

Year

2011 2012 2013 2014 2015

GAA

IDUA

ABG

GLA

GALC

ASM
Preparation of Dried Blood Spot Proficiency Testing Materials for Lysosomal Storage Disorders
Preparation of Dried Blood Spot Proficiency Testing Materials for Lysosomal Storage Disorders

1. Wash leuko-depleted blood with saline X3
2. Adjust to 50% hematocrit with heat-inactivated serum
3. Harvest EBV-transformed cell cultures, wash and cell count
4. Condition-specific blood pools (3 ~ 5 x 10^7 cells/ml)
5. Prepare Dried Blood Spots
   - Pompe
   - MPS-I
   - Krabbe
# Inter-Laboratory Evaluation of Condition-specific DBS Reference Material

*June 2012*

<table>
<thead>
<tr>
<th>Condition</th>
<th># Labs</th>
<th>Normal</th>
<th>Pompe</th>
<th>Krabbe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pompe</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Krabbe</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

*Condition-specific DBS are suitable PT material*
In 2013 NSQAP started a pilot proficiency testing (PT) program to detect enzyme deficiencies in dried blood spots for two Lysosomal Storage Disorders:

- Pompe Disease
- Krabbe Disease

US laboratories performing / establishing LSD NBS tests were invited to participate.

MPS-I will be included in the last send-out of 2015.
LSD-NBS PT Sendouts

- DBS with “normal” enzyme activity were prepared from freshly-collected umbilical cord blood
- Normal and condition-specific DBS were randomized into 5-member PT panels
- PT panels were sent nine times between January 2013 and January 2015
# Proficiency Testing Performance Metrics for Pompe and Krabbe in DBS*

*Data compiled from 9 quarters, 5 specimens per proficiency testing panel January 2013- January 2015

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. Labs Reporting</th>
<th>Positive Specimens Assayed (N)</th>
<th>False Negative Errors (%)</th>
<th>Negative Specimens Assayed (N)</th>
<th>False Positive Errors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pompe (GAA)</td>
<td>7</td>
<td>53</td>
<td>0</td>
<td>212</td>
<td>0.94</td>
</tr>
<tr>
<td>Krabbe (GALC)</td>
<td>6</td>
<td>48</td>
<td>0</td>
<td>192</td>
<td>0</td>
</tr>
</tbody>
</table>
### Analytical Summary Data for GAA Activity in Proficiency Testing* DBS (Pompe)

<table>
<thead>
<tr>
<th>Expected Results (GAA Activity µmol/L/hr)</th>
<th>Normal-1</th>
<th>Normal-2</th>
<th>Normal-3</th>
<th>Pompe Abnormal</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19.3</td>
<td>17.2</td>
<td>24.8</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Mean SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Flow Injection Analysis MS/MS Non-Kit (N=28)</td>
<td>18.3</td>
<td>4.2</td>
<td>14.9</td>
<td>4.0</td>
<td>2.9</td>
</tr>
<tr>
<td>LC-MS/MS (N=5)</td>
<td>3.5</td>
<td>0.4</td>
<td>2.3</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Digital Microfluidics (N=6)</td>
<td>31.6</td>
<td>2.7</td>
<td>23.5</td>
<td>1.1</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*Six quarters 2013-2014.
Evaluation of an Alternative LSD-NBS MS/MS Test System in Development

Intra-Lab Evaluation (Instrumentation & Reagents)
Correlation of Enzyme Activity Measured on Two Different MS/MS Instruments

![Graph showing correlation between Xevo TQD MS/MS and API 3200 MS/MS activities.](image)

- **Slope**: $0.99 \pm 0.0073$
- **Y-intercept**: $0.17 \pm 0.14$
- **R-squared**: 0.99

*(n = 228)*
Correlation of Enzyme Activity Measured on Two Different MS/MS Instruments

**IDUA**
- Observations
- Correlation
- Line of Identity
- Slope: 1.1 ± 0.0095
- R square: 0.98

**GALC**
- Observations
- Correlation
- Line of Identity
- Slope: 0.98 ± 0.0063
- R square: 0.99
CDC Lab Results in Condition-Specific DBS-RM

Enzyme Activity uM/L/Hr

- CDC QC Low
- MPS-I Affected
- Pompe Affected
- Krabbe Affected
- CDC QC High

IDUA
Evaluation of an Alternative LSD-NBS MS/MS Test System in Development

Inter-Lab Evaluation
Interlaboratory Results for DBS QC High

- **GAA**: CDC Lab / CDC Ref Method
- **GALC**: CDC Lab / PE Method
- **GLA**: PE Lab / PE Method
- **ABG**: CDC Lab / CDC Ref Method
- **ASM**: CDC Lab / PE Method
- **IDUA**: PE Lab / PE Method

Enzyme Activity (uM/L/Hr)

- 0
- 5
- 10
- 15
- 20
Inter-laboratory Evaluation of QC DBS by Fully-Multiplexed FIA-MS/MS

- **GAA**
  - Enzyme Activity (uM/L/Hr)
  - Base, Low, Medial, High

- **IDUA**
  - Enzyme Activity (uM/L/Hr)
  - Base, Low, Medial, High

- **GALC**
  - Enzyme Activity (uM/L/Hr)
  - Base, Low, Medial, High

- **GLA**
  - Enzyme Activity (uM/L/Hr)
  - Base, Low, Medial, High
Inter-laboratory Evaluation of QC DBS by Fully-Multiplexed FIA-MS/MS

![Graphs showing enzyme activity for ABG and ASM](Image)
Thank you for your attention!

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1600 Clifton Road NE, Atlanta, GA 30333  
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