Pompe Disease 101
Clinical Aspects and Screening Methods

Part 2: Pompe Disease: Newborn Screening Methodology
February 26th, 2014

Pompe Disease 101: Agenda

- **Welcome and Introduction**
  Patricia R. Hunt
  Member, APHL Subcommittee
  Manager, Texas Newborn Metabolic Screening Group

- **Overview of Available Screening Methods for Pompe Disease**
  Dr. Dietrich Matern, MD, PhD
  Co-director, Mayo Clinic Biochemical Genetics Laboratory, Rochester, MN

- **The Missouri Experience**
  Patrick V. Hopkins
  Chair, APHL NBS Quality Assurance/Quality Control Subcommittee
  Chief, Newborn Screening Unit, Missouri State Public Health Laboratory

- **The New York Experience**
  Dr. Joseph Orsini, PhD
  Research Scientist, New York State Department of Health-Wadsworth
  Newborn Screening Program Manager, Wadsworth Center

- **The Illinois Experience**
  Dr. George J. Doliszny, PhD, HCLD/CC(ABB)
  Chief of the Illinois Department of Public Health Newborn Screening Laboratory

- **Proficiency Testing Materials for Pompe Disease**
  Dr. Joanne V. Mei, PhD
  Dr. Hui Zhou, PhD
  Newborn Screening Quality Assurance Program
  Centers for Disease Control and Prevention
Pompe Disease 101: Agenda

- Q&A and Closing Remarks
  Patricia R. Hunt
- Closing Remarks
  Jelili Ojodu, MPH
  Director, Newborn Screening and Genetics
  Association of Public Health Laboratories

Newborn Screening for Pompe Disease in Missouri

Patrick V. Hopkins
Chief, Missouri NBS Laboratory
February 26th, 2014

Utilizing Digital Microfluidics

- Received a legislative mandate for LSD screening.
- Missouri annual birthrate is around 78,000 (91,000 samples/yr with repeat screens).
- Chose this method due to cost, space and time constraints.
- Currently conducting a 4-plex assay: Pompe, Gaucher, Fabry and MPS-I (NY testing Krabbe for us).
- Conducted validations, and pre-pilot in 2012.
- Started Full Population Pilot Screening January 11, 2013 after full IRB review and approval.
2 Work Stations
8 Digital Microfluidics Platforms

Enzyme Reaction in DMF Method

SUBSTRATE + Enzyme → PRODUCT

pH 3.8
4MU-α-D-Glucopyranoside + DBS extract (GAA) → 4MU + Glucose
2 scientists currently working full time in LSD section
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 hours

Workflow for Testing

- Punch DBS samples (15 min per 10-well plate) using punch for a new assay.
- Extraction (50 min at RT) using filter for extraction. Three extractions during extraction.
- Loading (5 min per machine) into each cartridge.
- Machine run time (2.5 h for 96-plex assay)
- Work 3 h; remove the cartridge from the instrument and get ready for next run.

Each Cartridge Has 4 Calibrators

Quality Control Monitor for Run
Results Screen

10 Confirmed Pompe Genotypes in First Year

First Year’s Findings

- We are very pleased with the DMF methodology.
- 33 Positive Pompe were referred in Year 1.
  - 10 Pompe genotypes
  - 6 Pompe Pseudo-deficiencies
  - 6 Pompe Carriers
  - 8 False Positives
  - 3 Pending
- Positive Predictive Value = 30%
- False Positive Rate = 0.026%
- Detection rates so far:
  - Infantile Onset = 1:26,000
  - Late Onset = 1:19,500
Other Important 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. May need more repeat screens.
- 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 can be detected during higher heat and humidity months.

Detected on Second Day of Pilot

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

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

Acknowledgements

- 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 Advanced Liquid Logic Team.
Newborn Screening for Pompe Disease in New York State

Joseph J. Orsini, PhD
February 26th, 2014

Varied Method Population Studies: NY

1. NY is using a multiplexed tandem mass spectrometry assay (based on Michael Gelb assay).
2. Why MS/MS? Only method that was available at the time we started screening for Krabbe disease.
3. We modified the Gelb assay so can screen for 6 LSD’s using two 3-mm dried blood spot punches. Can screen for Pompe, Krabbe, Fabry, Niemann-Pick A/B, and MPS-1/X-ALD (Dr. Dieter Matern from Mayo added these).
4. Currently performing a consented Pilot study with Dr. Melissa Wasserstein to screen for Pompe, Fabry, Gaucher, and Niemann Pick A/B.
5. Methods have been validated/approved using NYS Clinical Laboratory Evaluation Program guidelines.
   - requires inter/intra day precision, accuracy, linearity, possible interference studies, as well as reporting methods.

Basic Principal of Method
NYS modified 5-plex method

*Quadruplex solution, time consuming to make. Easy if just doing single assay. Each Substrate/Internal standard pair added separately.

New York State Assay

Punch 3 mm specimen, add assay solution reagent and incubate

19 hours

Quench reaction (50/50 MeOH/EtAc), perform Liquid / liquid extraction (EtAc/H2O), remove organic phase

Dry plates (10 min)

Reconstitute extract in 1:1 EtOAc/MeOH, perform SPE (unnecessary for Pompe only)

Dry plates (30 min)

Re-dissolve in MS suitable solvent (80/20 MeOH/H2O)

Analyze samples, 1.5 minutes per sample

Calculate activity/sample, daily mean activity, % of daily mean activity

Population Studies/Statistics

<table>
<thead>
<tr>
<th>Disease</th>
<th>Population/Mean</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>N</th>
<th>% of mean*</th>
<th>% of mean**</th>
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<tbody>
<tr>
<td>NYS</td>
<td>16.9 ± 7.8 (92%)</td>
<td>15.0</td>
<td>2.6</td>
<td>42.8</td>
<td>131</td>
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<td>16.4 ± 7.2 (92%)</td>
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Pompe

Compared to other newborn screening program results

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Many Things to Consider

1. Pompe only, or other LSDs in future, what disorders may be recommended next (MPS-I, Fabry, ALD ?)
2. Budget/Space
3. Kits or homebrew
4. Staff capabilities
5. Krabbe NP-A/B: currently only can be done by MS/MS
6. Not so obvious considerations:
   a. Planning to run ALD?
   b. Do you use derivatized or underivatized kits for AA/AC?
   c. Succinyl acetone for Tyrosinemia type 1

Conclusions

1. Validations for the MS/MS methods have gone smoothly
2. If had to do this again, would do mostly the same. MS/MS is still the only available method to screen for Krabbe disease.
3. Multiplexed enzyme assays with currently provide materials is complicated as each Substrate/Internal Standard pair is provided for each disease. Makes validations complicated due to “moving target” of diseases that may be screened.

Acknowledgements

• Monica Martin, Amanda Showers (New York State Newborn Screening/Pilot LSD Testing).
• Dr. Melissa Wasserstein, Nicole Kelly and the Mt. Sinai School of Medicine team.
• Dr. Robert Vogt, Dr. Hui Zhou, and the CDC LSD quality assurance support group.
• Dr. Dietrich Matern and Coleman Turgeon (Rochester, MN, Mayo).
• Dr. Micheal Gelb and the University of Washington support team.
Screening for Pompe Disease and other Lysosomal Storage Disorders (LSDs) – Illinois Experience

George J. Dizikes, Ph.D.
Section Chief, Newborn Screening Laboratory
Illinois Department of Public Health (IDPH)

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IDPH
• Khaja Basheeruddin, Ph.D. – Unit Supervisor
• Rong Shao, M.D. – Laboratory Research Scientist
• Fran Balster – Clinical Laboratory Technologist
• Pearlie Gardley – Clinical Laboratory Technologist
• Tamara Simulick – Clinical Laboratory Technologist

Others
• Barbara K. Burton, M.D. – Lurie Children’s Hospital
• Michael H. Gelb, Ph.D. – University of Washington
• Joseph J. Orsini, Jr., Ph.D. – Wadsworth Center
• CDC Newborn Screening and Molecular Biology Branch
• PerkinElmer Corporation

Pompe Testing Timeline

• 2007 – Legislative mandate for five LSDs, including Pompe.
• 2010 – Pilot screening for Pompe and two other LSDs using microfluidic platform.
  o 8,012 DBS screened
  o Two had abnormal GAA activities, confirmed negative by second-tier tests
Pompe Testing Timeline (cont'd)

• 2011 – Legislative mandate expanded to seven LSDs, with the following provisions before screening:
  o A method either cleared by the US Food and Drug Administration (FDA) or validated under the Clinical Laboratory Improvement Amendments (CLIA)
  o Availability of quality control and proficiency testing materials
  o Appropriate equipment for high-volume screening
  o Adequate funding

• 2011 – Decision made to switch from microfluidic platform to tandem mass spectrometry (MS/MS).
  o Microfluidic platform did not have substrates for all LSDs.
  o Microfluidic platform lacked throughput for Illinois’ volume (~170,000 newborns per year).
  o Recent developments with multiplex MS/MS promised adequate testing throughput for more disorders and with less staff.
• 2014 – Statewide testing expected to begin July 1.

Multiplex LC-MS/MS Assay

• Modification of method developed at the University of Washington for six LSDs: Pompe, Krabbe, Gaucher, Fabry, Niemann-Pick (A/B), Mucopolysaccharidosis type I (MPS I):
  o Single DBS punch
  o Single buffer
  o In-line chromatographic purification (no solid-phase extraction)
• Three-hour incubation (maintains work flow).
• UPLC column separates product/ISTD pairs and removes salt, detergent, & phospholipids by valving.
• 2.5 minute injection cycle, 500 injections/instrument/day, >10,000 injections/PM.

Acquity TQD Instrument

UPLC Chromatogram

Linearity of Assay

R² = 0.9995

GAA (P/ISTD ratio)

Time (h)

QC Low

R² = 0.9993

GAA (P/ISTD ratio)

Time (h)

QC Medium
Method Validation

• Evaluate different levels of Quality Control samples (Low, Medium, and High).
• Perform precision studies.
• Perform accuracy studies.
• Participate in the CDC pilot Proficiency Testing (PT) program for Pompe (and Krabbe).
• Obtain DBSs from confirmed cases.

Method Validation (cont’d)

• Test de-identified specimens from male, female, low birth weight, and 7+ day-old babies.
• Study the effects of detergents and DBS storage conditions on LSD enzyme activities.
• Determine cut-off values.
• Exchange specimens with a qualified testing laboratory to establish comparability of results.

Assay Results for de-ID DBSs, Confirmed Pompe cases, PTs, and Quality Controls
Lessons Learned

• Many different individuals with a wide range of skills need to work together to successfully develop a complex, high-throughput analytical assay.

• The process will take longer than initially anticipated; regular interactions and good communications are vital.

• MS/MS platform permits expanded test menu and multiplexing with a single injection.

Lessons Learned (cont’d)

• There are many challenges in adapting a research procedure to a high-throughput newborn screening assay (e.g., analytical, personnel, physical plant, and IT). FDA-cleared tests are vastly preferable.

• If at all possible for mandated testing, have legislation or administrative rules written to permit adequate preparation and milestones (e.g., method validation, QC and PT availability, acquisition of high volume equipment, & funding).
Thank you

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312-793-4745

Proficiency Testing Materials for Pompe Disease

Hui Zhou, PhD
Newborn Screening Translation Research Initiative
Joanne Mei, PhD
Team Lead, Newborn Screening Quality Assurance Program

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National Center for Environmental Health
Division of Laboratory Sciences

Wash leuko-depleted blood with saline X3
Adjust to 50% hematocrit with heat-inactivated serum
Base pool
Harvest EBV-transformed Cell cultures, wash And cell count
Condition-specific blood pools (3 ~ 5 x 10^7 cells/ml)
Prepare Dried Blood Spots

Krabbe  Pompe  MPS-I

Preparation of Dried Blood Spot Reference Materials for Lysosomal Storage Disorders
NSQAP Pilot Proficiency Testing For LSD

- Available for Pompe and Krabbe disease
- 4 times per year for US programs
- Information collected:
  - Analytical data
  - Clinical assessments
  - Methods
  - Cutoffs
- One-month data turnaround
- Summary reports issued two weeks after deadline

Performance of Methods that Detect GAA Enzyme Deficiency in DBS Proficiency Testing Specimens

<table>
<thead>
<tr>
<th>Type of Proficiency Testing Specimen (DBS)</th>
<th>Flow Injection Analysis</th>
<th>MS/MS Non-Kit (pM)</th>
<th>MS/MS Kit (pM)</th>
<th>Digital Microfluidics (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Specimen</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
</tr>
<tr>
<td>Pompe Abnormal Specimen</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
<td>Error bars ± 1 SD</td>
</tr>
</tbody>
</table>
Analytical Summary Data for GAA Activity in Proficiency Testing DBS

<table>
<thead>
<tr>
<th>Expected Results (GAA Activity µmol/hr/L)</th>
<th>Normal-1</th>
<th>Normal-2</th>
<th>Normal-3</th>
<th>Pompe Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>New Injection Analysis (N=19) (µmol/hr/L)</td>
<td>18.04</td>
<td>4.15</td>
<td>14.88</td>
<td>4.14</td>
</tr>
<tr>
<td>LC-MS/MS (N=4)</td>
<td>18.64</td>
<td>9.51</td>
<td>9.56</td>
<td>7.66</td>
</tr>
<tr>
<td>Digital Microfluidics (N=4)</td>
<td>30.98</td>
<td>2.02</td>
<td>23.18</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Proficiency Testing for Pompe (GAA) in DBS Results for 2013*

<table>
<thead>
<tr>
<th>Condition (GAA)</th>
<th>No. Labs Reporting</th>
<th>Positive Assayed (N)</th>
<th>False Negative Errors (%)</th>
<th>Negative Assayed (N)</th>
<th>False Positive Errors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pompe (GAA)</td>
<td>8</td>
<td>27</td>
<td>0</td>
<td>108</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data compiled from 4 quarters, 5 specimens per proficiency testing panel.

Summary

- CDC LSD Reference Materials can be used for assay development and validation for all methods in current use
- EBV-transformed lymphoblast cells derived from LSD patients provide a sustainable resource for condition-specific reference materials
- NSQAP’s Pilot PT Program for Pompe (GAA)
  - No false positive results reported
  - No false negative results reported
CDC Contacts
DBS Reference Materials for LSDs

- DBS reference materials, certain reagents, training
  - Dr. Hui Zhou (HZhou2@cdc.gov) or 770.488.4861

- Proficiency testing materials (US only)
  - Dr. Hui Zhou (HZhou2@cdc.gov or 770.488.4861)
  - Dr. Joanne Mei (JMei@cdc.gov or 770.488.7945)

- Proficiency testing instructions and data reporting
  - Ms. Irene Williams (IWilliams1@cdc.gov or 770.488.7024)

For more information please contact Centers for Disease Control and Prevention
1600 Clifton Road, NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Clinical Aspects and Screening Methods

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- Victor DeJesus, PhD (CDC)
- Ruhiyyih Degeberg, MPH (APHL)

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APHL 2014 Newborn Screening and Genetic Testing Symposium

- October 27-30, 2014
- Anaheim, California
- Theme: *Newborn Screening: Reassessing Business as Usual*