CDC's Quality Assurance and Harmonization Activities

Normalization of NBS Laboratory MS/MS Biomarker Results and the Development of a New Generation of Proficiency Testing Materials

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Services Provided by CDC’s Newborn Screening Quality Assurance Program (NSQAP)

The only comprehensive NBS quality assurance program using dried blood spots

- Proficiency testing
- Quality control materials
- Linearity materials
- Filter paper evaluation
- Training and consultation
- NBS translation research
CDC’s Newborn Screening Quality Assurance Program

*By the Numbers*

- **Annual bloodspots:** ≈1,000,000
- **Litters of blood per year:** ≈100
- **Labs participated (2017):** 660
- **Countries participated (2017):** 84
- **Distribution frequency:** Each quarter
- **Years of operation:** 40 years

**PT programs:** 16
- AC, AA, BIOT, GALT, G6PD, HORM, IRT, CAH, CFDNA, Hb, HIV, LSD, TREC, TOXO, XALD, UDOT.

**QC programs:** 13
- 17OHP/TGAL, AAAC, GALT, IRT, T4, TSH, XALD, CAH, GAMT, MSUD-PKU, MMA-HCY, HIV, LSD

**Number of biochemical analytes:** 64
- Excludes Hb phenotypes, CF genotypes etc
NORMALIZATION OF NBS LABORATORY MS/MS BIOMARKER RESULTS
MS/MS Biomarker Measurements and Cutoffs Can Vary Significantly Among Different Labs

>70% (23/32) of RUSP bloodspot disorders can be screened by MS/MS

<table>
<thead>
<tr>
<th>Major Contributors</th>
</tr>
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<tbody>
<tr>
<td>• Extraction methodologies</td>
</tr>
<tr>
<td>• Derivatized vs. non-derivatized</td>
</tr>
<tr>
<td>• Few labs account for analyte recovery, most labs do not</td>
</tr>
<tr>
<td>• Use of additional/different analytes per disorder or second-tier screening</td>
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<table>
<thead>
<tr>
<th>Other Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Population tested</td>
</tr>
<tr>
<td>• Instrumentation</td>
</tr>
<tr>
<td>• Internal standards</td>
</tr>
<tr>
<td>• Calibration techniques</td>
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</tbody>
</table>
Method-specific Variability in C5DC Cutoffs in U.S. NBS Laboratories

C5DC: Glutaryl carnitine, CV: Coefficient of Variation, SD: Standard Deviation
How normalization works:

Simple analogy: Normalization of thermometer results

Liquid-in-Glass Thermometer (°C)

Fever at: 38 °C

Cutoff for Fever

Fever at: 100.4 °F

Platinum Resistance Thermometer (°F)

Freezer

Refrigerator

Room Temperature

Oven

y = 1.8x + 32
How normalization works:

*Use of CDC Quality Control (QC) bloodspot materials to normalize mass spectrometry results*

Same idea as previous slide but instead of:

- Thermometers ... we use Mass Spectrometers
- Four different temperatures ... we use 4 different concentrations of each biomarker in QC Samples

**QC Mass Spectrometry Materials for Amino Acids and Acylcarnitines (AAAC)**

- 29 biomarkers
- 4 concentration levels
- 5 duplicate MS/MS inter-day runs of each level
- Data reported back to CDC

**NBS laboratories could use the QC materials to answer the following questions:**

- What is the variability of each instrument within the same day?
- What is the variability of each instrument between days?
- How similar are the results between instruments?
Addressing Succinylacetone (SUAC) Lab-to-Lab Variability by Normalizing Results

- Use QCs to normalize
- Use PTs to validate the normalization worked

Expectation:
- NBS labs receive the same PT specimens
- PT analytical results should be the same

Methods:
- FIA-MS/MS results
- PT specimens are analyzed only once
- QC and PT results from USQ3 2016 event

Concentrations at µmol/L, SUAC: Succinylacetone, PT: Proficiency Test, FIA: Flow Injection Analysis
Addressing SUAC Lab-to-Lab Variability by Normalizing Results

### SUAC PT Normalization

<table>
<thead>
<tr>
<th>Lab</th>
<th>Raw Value</th>
<th>Normalized Value</th>
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</thead>
<tbody>
<tr>
<td>State Lab A</td>
<td>62.7</td>
<td>25.7</td>
</tr>
<tr>
<td>State Lab B</td>
<td>44.3</td>
<td>25.6</td>
</tr>
<tr>
<td>State Lab C</td>
<td>10.7</td>
<td>22.9</td>
</tr>
<tr>
<td>CDC</td>
<td>27.3</td>
<td>27.3</td>
</tr>
<tr>
<td>CV</td>
<td>62%</td>
<td>7%</td>
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</tbody>
</table>

### SUAC Cut-off Normalization

<table>
<thead>
<tr>
<th>Lab</th>
<th>Raw Value</th>
<th>Normalized Value</th>
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<tr>
<td>State Lab A</td>
<td>5.4</td>
<td>2.7</td>
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<tr>
<td>State Lab B</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>State Lab C</td>
<td>1.0</td>
<td>2.1</td>
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<tr>
<td>CDC</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>CV</td>
<td>64%</td>
<td>15%</td>
</tr>
</tbody>
</table>

**Times Difference between Lab A and Lab C**

- **Normalization Before**: 5.9
- **Normalization After**: 1.12

**Normalization Before**: 5.4
- **Normalization After**: 1.28

**CDC cut-off**: The mean of all US State laboratories non-normalized cut-offs
Does Normalization Work?

Orthogonal validation using the PT results

Glutaryl carnitine (C5DC) PT results

Without normalization … CV: 32.8%

After normalization … CV: 14.6%

CV: Coefficient of Variation, PT: Proficiency Test
Does Normalization Work?
Orthogonal validation using the PT results

Citrulline (Cit) PT results
Without normalization ... CV: 15.4%

After normalization ... CV: 6.6%

CV: Coefficient of Variation, PT: Proficiency Test
Does Normalization Work?

Orthogonal validation using the PT results

Malonylcarnitine (C3DC) PT results

Without normalization … CV: 56.7%

After normalization … CV: 18.7%

CV: Coefficient of Variation, PT: Proficiency Test
Does Normalization Work?

Orthogonal validation using the PT results

US and International NBS labs
15 different methods (most non-MS/MS)
+: US Labs, ●: International Labs

Phenylalanine PT results
Without normalization … CV: 20.8%

Phenylalanine PT results
After normalization … CV: 10.1%
NBS labs LC-MS/MS biomarker CV results before and after normalization

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<td>56.7%</td>
<td>18.7%</td>
<td>54.5%</td>
<td>24.3%</td>
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<tr>
<td>SUAC</td>
<td>50.8%</td>
<td>24.5%</td>
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<td>31.7%</td>
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<tr>
<td>Arg</td>
<td>34.7%</td>
<td>18.2%</td>
<td>37.3%</td>
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<tr>
<td>C5DC</td>
<td>32.8%</td>
<td>14.6%</td>
<td>39.5%</td>
<td>19.5%</td>
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<tr>
<td>C16OH</td>
<td>23.1%</td>
<td>14.9%</td>
<td>70.5%</td>
<td>21.4%</td>
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<tr>
<td>Val</td>
<td>19.9%</td>
<td>15.0%</td>
<td>22.9%</td>
<td>12.6%</td>
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<tr>
<td>C5OH</td>
<td>19.8%</td>
<td>16.2%</td>
<td>36.0%</td>
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<tr>
<td>C0(L)</td>
<td>19.0%</td>
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<tr>
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<tr>
<td>Cit</td>
<td>15.4%</td>
<td>6.6%</td>
<td>22.6%</td>
<td>13.0%</td>
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<tr>
<td>C18</td>
<td>15.3%</td>
<td>11.6%</td>
<td>20.2%</td>
<td>16.8%</td>
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<tbody>
<tr>
<td>Met</td>
<td>14.8%</td>
<td>9.5%</td>
<td>22.9%</td>
<td>12.4%</td>
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<tr>
<td>C4</td>
<td>14.3%</td>
<td>10.0%</td>
<td>18.0%</td>
<td>12.0%</td>
</tr>
<tr>
<td>C18OH</td>
<td>13.3%</td>
<td>11.7%</td>
<td>49.4%</td>
<td>21.9%</td>
</tr>
<tr>
<td>Phe</td>
<td>13.1%</td>
<td>7.9%</td>
<td>20.8%</td>
<td>10.1%</td>
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<tr>
<td>Tyr</td>
<td>12.7%</td>
<td>9.0%</td>
<td>19.0%</td>
<td>15.7%</td>
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<tr>
<td>C8</td>
<td>12.2%</td>
<td>11.9%</td>
<td>19.7%</td>
<td>15.1%</td>
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<tr>
<td>C3</td>
<td>12.1%</td>
<td>9.7%</td>
<td>20.4%</td>
<td>16.6%</td>
</tr>
<tr>
<td>C6</td>
<td>10.9%</td>
<td>10.7%</td>
<td>20.9%</td>
<td>15.5%</td>
</tr>
<tr>
<td>Leu</td>
<td>10.8%</td>
<td>8.2%</td>
<td>18.2%</td>
<td>10.1%</td>
</tr>
<tr>
<td>C14</td>
<td>8.5%</td>
<td>8.2%</td>
<td>19.1%</td>
<td>15.2%</td>
</tr>
<tr>
<td>C16</td>
<td>7.2%</td>
<td>7.1%</td>
<td>14.9%</td>
<td>13.3%</td>
</tr>
</tbody>
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Note: The represents data from CDC's NSQAP Proficiency Testing Program in the 3rd Quarter of 2016
Normalization of MS/MS results allows the normalization of cutoffs

**Glutaryl carnitine (C5DC) US labs cutoffs**

Without normalization ... CV: 45.55%

After normalization ... CV: 30.22%

NBS labs with high biomarker normalized cutoffs could reevaluate them

Method Platform

C5DC: Glutaryl carnitine, CV: Coefficient of Variation, PT: Proficiency Test
DEVELOPMENT OF A NEW GENERATION OF PROFICIENCY TESTING MATERIALS
High Accuracy Multi-Analyte Dried Blood Spot Enrichment Method

Breakthrough: Enrichment within 5% of desired concentration

+ Ability to normalize MS/MS data

+ Confirmed cases MS/MS data from NBS labs with quarter + year info

↓ Proficiency testing materials that are “biochemical carbon copies” of babies that were diagnosed with the disorder
New Generation of PT materials

**When:** July Shipment (Q3-2018 PT event)

**What:** Proficiency testing materials that are “biochemical carbon copies” of babies that were diagnosed with the disorder for the analytes and ratios of interest

**Which ones:** Amino acid, Fatty Acid Oxidation and Organic Acid Disorders

**Where:** From MS/MS data submitted to CDC from US state labs that contained quarter and year of specimen collection information

**How:** Report as usual, working on updating NSQAP website

**Interpretive algorithms:** Q3 2018 PTs should work with any workflow, including reflex to biochemical second-tier screening

Looking forward to feedback from NBS labs!
Future Directions

- CDC will continue to improve normalization and visualization of the results and will expand the number of analytes in QC materials.
- High accuracy multi-analyte bloodspot enrichment will allow the creation of borderline materials for educational purposes.
- CDC will be creating reference materials for MS/MS kits to use for difference applications, including:
  - Changes of instrumentation, method, kit lots
  - Abnormal and borderline specimens to assess cut-offs
  - Linearity materials for method performance
  - Provide assistance for method development, validation
- CDC is redesigning the data reporting website to improve QC and PT data submission and to accommodate expanded programs.
Conclusions

- Based on preliminary results: It seems possible to normalize MS/MS analyte results by using the CDC's QC materials.
- The CV of all PT analytes improved after normalization.
- CDC will be reporting de-identified normalized cutoffs to NBS laboratories to help them compare their cutoffs to their peers.
- CDC has begun the development of new PT and borderline materials that more closely mimic the pattern and concentrations of biochemical analytes as screened in babies diagnosed with the disease.
- CDC is developing a repository of artificial blood spot specimens to be used as kits for verification/validation or program evaluation. Samples will be distributed upon request.
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Patrice Held, Wisconsin
Adrienne Manning, Connecticut
Sonal Bhakta, Arizona
Mark Morrissey, New York

Many thanks to all NBS laboratories that submitted de-identified confirmed cases data to CDC
Thank you for your attention!

For more information please contact Centers for Disease Control and Prevention

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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

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