Incidental G-6-PD Identification Using the PerkinElmer GSP Neonatal GALT Kit
Project Initiation
Instrumentation

- PerkinElmer AutoDelfia
  - T4, TSH, 17-OHP and IRT
- Introduction of Genetic Specimen Processor (GSP)
  - Transfer of AutoDelfia tests
  - Expansion capability to add GALT and Biotinidase
- Decision made to add GALT to GSP using the PerkinElmer Neonatal GALT kit
Beutler Assay to GSP GALT
Screening for Galactosemia

- 1964 to February 2012 – Beutler assay
- Detection of galactose-1-uridyltransferase (GALT) enzyme
- Presence or absence of fluorescence
- Results read visually using a UV light box

[Image of a UV light box with normal and abnormal samples]
Beutler Assay

- Manual method
- In-house reagents
- Visually read
- Qualitative
- Subjective
PE GSP GALT Assay

- Automated method
- Manufactured reagents
  » Adaptation of Beutler/Baluda assay
- Instrument read
- Quantitative
- Objective
Validation

- Beutler to GSP Neonatal GALT Kit comparisons

- 2,800 blood spot specimens
  - 1,411 first screen specimens (zero to 7 days of age)
  - 1,359 second screen specimens (>7 days of age)
  - All Beutler normal

- CDC PT samples
  - Beutler normal, reduced or no fluorescence
Validation Results

- First specimens - zero through 7 days of age
  » Mean 12.58 U/dL
  » Standard deviation 3.92
- Second specimens - > 7 days of age
  » Mean of 12.43 U/dL
  » Standard deviation of 11.30
  » Higher variability
- CDC specimens – Interpretation matched Beutler GALT test results
Validation Results

• Classic galactosemia specimens
  » GSP GALT values < 1.5 U/dL

• Reduced fluorescence specimens
  » GSP GALT values ≤ 5.50 U/dL

• Proposed cutoffs
  » Abnormal < 2.5 U/dL
  » Borderline 2.5 to 3.49 U/dL
  » Normal ≥ 3.50 U/dL
Validation Results

• False Positive Predictions
  » < 2.50 U/dL would produce 11 false positives in addition to the true positives

• Borderline range
  » < 3.00 U/dL – 15 false positives
  » < 4.00 U/dL – 1 false positive
  » Selected cutoff of < 3.50 U/dL – 8 more borderline positives
Assay Implementation
Screening Results

• Increased number of presumptive positives with the GSP GALT
  » 120 in 2011 (Beutler assay)
  » 681 in 2012

• Mainly reduced fluorescence values

• Majority from Hawaii

• Asian and Pacific Islander population
Diagnostic Results

- Galactosemia false positives
- Confirmation of glucose-6-phosphate dehydrogenase deficiency (G-6-PD)
- Already well known to be common in the Hawaiian population
- Testing routinely performed in the state
• Deficiency in glucose-6-phosphate dehydrogenase
• Insufficient amounts – red blood cell destruction
• Triggered by infections, severe stress, certain foods or drugs
Assay Evaluation
Cutoff Analysis and Revision

- 6 month data review
- Evaluation of confirmed cases
  - GALT deficient
  - D/G
  - Duartes
  - Carrier
  - Other variant
Cutoff Analysis and Revision

- **Beutler assay classic Galactosemia specimens**
  - GSP GALT values < 1.5 U/dL

- **Beutler assay reduced fluorescence specimens**
  - GSP GALT values ≤ 5.50 U/dL

- **Revised cutoffs**
  - Abnormal < 2.5 U/dL → < 2.0 U/dL
  - Borderline 2.5 to 3.49 U/dL → 2.0 to 3.49 U/dL
  - Normal > 3.50 U/dL → No change
Beutler Assay to GSP GALT
Statistics
# Beutler vs. GSP GALT Cases

<table>
<thead>
<tr>
<th></th>
<th>Beutler</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>As of 10/2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
<td>2011</td>
<td>2012</td>
<td>2013</td>
<td></td>
</tr>
<tr>
<td>Presumptive Positive</td>
<td>189</td>
<td>108</td>
<td>120</td>
<td>681</td>
<td>922</td>
<td>660</td>
</tr>
<tr>
<td>Classic Galactosemia</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>G-6-PD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Duarte Variant</td>
<td>43</td>
<td>14</td>
<td>9</td>
<td>12</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>G/N Heterozygote</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Specimens Screened</td>
<td>315,670</td>
<td>301,897</td>
<td>293,405</td>
<td>292,372</td>
<td>291,946</td>
<td>216,480</td>
</tr>
<tr>
<td>Average PP Frequency</td>
<td>1:1,094</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:197</td>
</tr>
</tbody>
</table>
Summary
Summary

- GSP Neonatal GALT Kit
  - Greater sensitivity than Beutler in G-6-PD detection
  - Unexpected increase in presumptive positives with population bias
  - Increased short-term follow-up
  - Increased diagnostic costs – GALT and G-6-PD analysis vs. GALT alone
Incidental vs. Supplemental Findings
• **incidental**

  » *adjective*

  \( \text{\textls[80]{in\text{-}t\text{-}s\text{-}'den\text{-}t}} \): happening as a minor part or result of something else

• **supplemental**

  » *adjective*

  \( \text{\textls[80]{s\text{-}pl\text{-}'men\text{-}t}} \): added to something else to make it complete
Questions

Contact Info:
cheryl.a.hermerath@state.or.us
503-693-4172