Screening for three lysosomal storage diseases in a NBS laboratory, and the potential to expand to a nine-plex assay

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1. University of Washington
2. Washington State Newborn Screening Laboratory
Potential candidates for newborn screening of Lysosomal disorders.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Rx</th>
<th>Requires early detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher</td>
<td>ERT/SM</td>
<td></td>
</tr>
<tr>
<td>Fabry</td>
<td>ERT/SM</td>
<td>+/-</td>
</tr>
<tr>
<td>MPS-I</td>
<td>ERT/BMT</td>
<td>+</td>
</tr>
<tr>
<td>Pompe</td>
<td>ERT/SM</td>
<td>+</td>
</tr>
<tr>
<td>MPS-II</td>
<td>ERT</td>
<td>+</td>
</tr>
<tr>
<td>MPS-IVA</td>
<td>ERT</td>
<td>+</td>
</tr>
<tr>
<td>MPS-VI</td>
<td>ERT</td>
<td>+</td>
</tr>
<tr>
<td>Krabbe</td>
<td>BMT</td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick B</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>MLD</td>
<td>BMT</td>
<td>+</td>
</tr>
</tbody>
</table>
Product Description

Substrates and internal standards are available without charge through the CDC.

Each vial contains the optimized ratio of substrate : internal standard for 1200 tests for:
- Gaucher disease
- Pompe disease
- Fabry disease
- Niemann-Pick A/B
- Krabbe disease (600 tests)
- MPS-I

Each box contains 6000 tests.

Reagents for each disorder are packaged separately to allow end-user to choose menu-style.
Anonymous blood spots

Assay plate

Fabric, Pompe, MPS-1

DNA sequencing (GLA, GAA, IdUA) for genotype

Duplicate plate

Triplex Procedure

Normal

Abnormal

Unaffected

Affected

MS/MS
Fabry Disease

- X-linked
- Deficiency of acid $\alpha$-galactosidase
- Shortened life expectancy from:
  - Renal failure at 30-40 years
  - Hypertrophic cardiomyopathy
  - CNS strokes
- Childhood symptoms of:
  - Peripheral pain
  - Fatigue
Fabry Assay

Substrate

Acid alpha-Galactosidase (a-Gal) (Fabry)

Product

Internal standard
\( P_{\text{Fabry}} / I_{\text{Fabry}} = 1.06\% \)

\( P_{\text{Fabry}} / I_{\text{Fabry}} = 95.7\% \)

\( P_{\text{Fabry}} / I_{\text{Fabry}} = 3.5\% \)
GLA activity (Fabry)
mean = 10.7 ; 15% of mean = 1.6

(N = 77,934)
Fabry Disease

- Prevalence: 1/17,000 - 1/50,000
- Taiwan population: 1/16,000 with hypertrophic cardiomyopathy (IVS4 + 919g>a)
- Italian newborn screening: 1/3,100
Table 1: Fabry Disease

<table>
<thead>
<tr>
<th>Cut off at 15% of assay mean: 1.5 µmole/hr/L</th>
<th>Enzyme activity (µmole/hr/L)</th>
<th>% of mean X=10.5</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.72</td>
<td>16.1</td>
<td>p. Ser 334Asn/wt (F)</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>14.0</td>
<td>wt</td>
</tr>
<tr>
<td></td>
<td>1.38</td>
<td>12.9</td>
<td>wt</td>
</tr>
<tr>
<td></td>
<td>1.32</td>
<td>12.3</td>
<td>p.Ala143Thr (M)</td>
</tr>
<tr>
<td></td>
<td>1.12</td>
<td>10.5</td>
<td>wt</td>
</tr>
<tr>
<td></td>
<td>0.89</td>
<td>8.3</td>
<td>p.Asp313Gly (M)</td>
</tr>
<tr>
<td></td>
<td>0.58</td>
<td>5.4</td>
<td>p.Asn215Ser (M)</td>
</tr>
<tr>
<td>known affected (n=5)</td>
<td>0.13 – 0.50</td>
<td>1.2 – 4.7</td>
<td></td>
</tr>
</tbody>
</table>

(M) = male  
(F) = female

Positive predicted Value = 0.42  
clinical prevalence: 1/ 40,000 males

Specificity ~1.0  
NBS prevalence: 1/ 12,000 males

Sensitivity ~ ?
Pompe

Deficiency of lysosomal acid α-glucosidase

Clinical symptoms:

• Progressive muscle weakness with variable onset; 3 mo to adulthood
• Cardiac failure in infancy

In adults:

• Progressive muscle weakness
• Respiratory failure
Infantile-Onset Pompe Disease
Head Lag
Pompe Assay

Acid alpha-Glucosidase (a-Glu) (Pompe)

Substrate

Product

Internal standard
GAA activity (Pompe)

mean = 17.3 ; 15% of mean = 2.6

(N = 77,934)
Pompe Disease

17q 25.2  25 kb  20 exons

c.IVS1-13t>g  p.Asp645Glu  p.Arg854X

Prevalence:
- Af. American: 1/14,000 (infant)
- US population: 1/40,000 (infant & adult)
- European: 1/100,000 (infant)
- 1/60,000 (adult)
### Table 3: Pompe Disease

<table>
<thead>
<tr>
<th>Cut off at 15% of assay mean: &lt;2.6 µmole/hr/L</th>
<th>Enzyme activity (µmole/hr/L)</th>
<th>% of mean ± X=17.3</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.53</td>
<td>14.4</td>
<td></td>
<td>*IVS1-13t&gt;g/IVS1-13t&gt;g</td>
</tr>
<tr>
<td>2.46</td>
<td>14.0</td>
<td></td>
<td>wt</td>
</tr>
<tr>
<td>2.44</td>
<td>13.9</td>
<td>p.Gly576Ser;p.Glu689Lys/wt</td>
<td></td>
</tr>
<tr>
<td>2.38</td>
<td>13.5</td>
<td>IVS1-13t&gt;g/p.Glu689Lys</td>
<td></td>
</tr>
<tr>
<td>2.21</td>
<td>12.6</td>
<td>IVS1-13t&gt;g/p.Glu689Lys</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>12.5</td>
<td>p.Gly576Ser;p.Glu689Lys/p.Glu945Lys</td>
<td></td>
</tr>
<tr>
<td>1.77</td>
<td>10.1</td>
<td>p.Glu689Lys/?exon9</td>
<td></td>
</tr>
<tr>
<td>1.70</td>
<td>9.7</td>
<td>p.Met122Lys / p.Val642Asp + psdef</td>
<td></td>
</tr>
<tr>
<td>1.70</td>
<td>9.7</td>
<td>unable to sequence</td>
<td></td>
</tr>
<tr>
<td>1.57</td>
<td>8.9</td>
<td>*IVS1-13t&gt;g/IVS1-13t&gt;g</td>
<td></td>
</tr>
<tr>
<td>1.42</td>
<td>8.1</td>
<td>wt (?)</td>
<td></td>
</tr>
</tbody>
</table>

Known affected (n=5) 0.13 – 0.50 1.2 – 4.7

PPV=0.27 Prevalence=1/27,000
MPS-1 disease

Hurler phenotype:
early diagnosis with:
  Coarse features
  Dyostosis multiplex
  Progressive intellectual loss
  Cloudy cornea

‘attenuated’ phenotype:
  Mild somatic changes
  Slow neurological progression
  Stiff joints
  Thickened dura
  Cloudy cornea
MPS-I Assay

IdA-S (m/z 567.2 for M+H⁺)

IdA-P (m/z 391.2 for M+H⁺)

IdA-IS (m/z 377.2 for M+H⁺)
IDUA activity (MPS-I)
mean = 3.6 ; 30% of mean = 1.09

(N = 70,784)
MPS-1 Disease

4p16.3  19 kb  14 exons
p.Gln70X  p.Trp402X
p.Arg89Gln  IVS6-7a>g

Clinical prevalence: ~1/100,000
Table 2: MPS-I

<table>
<thead>
<tr>
<th>Cut off at 30% of assay mean: &lt; 1.07</th>
<th>Enzyme activity (µmole/hr/L)</th>
<th>% of mean ( \bar{X}=3.6 )</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>wt</td>
</tr>
<tr>
<td>1.08</td>
<td></td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>1.06</td>
<td></td>
<td>28.6</td>
<td>p.Asp119Tyr / p.Glu84Ser</td>
</tr>
<tr>
<td>1.05</td>
<td></td>
<td>28.4</td>
<td>p.Gln70X / p.Gln70X</td>
</tr>
<tr>
<td>1.02</td>
<td></td>
<td>27.6</td>
<td>p.Trp402X / wt</td>
</tr>
<tr>
<td>0.94</td>
<td></td>
<td>25.4</td>
<td>wt (poor punch)</td>
</tr>
</tbody>
</table>

| known affected (n=5) | 0.58 – 0.83 | 18.4 – 22.5 |
| known carriers (n=4) | 1.44 – 3.02 | 39 – 81     |

Positive predictive value = 0.4  Clinical prevalence: ~1/ 100,000  NBS prevalence : ~1/ 35,000
Summary

- MS/MS assay can easily be adapted to a NBS screening laboratory
- Multiplexing multiple enzymes simplifies the procedure and is a built-in control for sample integrity
- In the first 70,000+ samples: 8 positive newborns identifies with LSD
- Overall prevalence: ~1/9,600
Potential Diseases for LSD Screening
By Multiplex Analysis

- Fabry
- Pompe
- MPS-I
- Krabbe
- Gaucher
- Niemann-Pick
- MPS-II
- MPS-IVA
- MPS-VI

In queue:
- MPS-IVB, MLD
- CNL1, CNL2
Acknowledgements

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