Development of a new bloodspot screening assay for Duchenne Muscular Dystrophy (DMD)

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DMD Screening programmes

• CK activities increased in asymptomatic boys with DMD

• 1970’s - Development of DBS CK enzyme assay

• 17 programmes in 10 countries
  ~1.8M infants screened

• Wales Experience (1990 – 2011)
  • 343,170 boys screened (PPV 38.6%)
  • 16 False negative cases
  • Screening terminated 2011

Moat SJ et al 2013 Eur J Hum Genet
Renewed interest in newborn screening for DMD

- Molecular & gene therapies on horizon
- Current therapies started earlier improve outcomes
- Change in screening policy to include “Diagnostic Odyssey”
Limitations of the CK enzyme test as a first line screening test

- Issues with reagent stability
- Difficult to automate for high throughput screening
- Lack of assay standardisation
- Poor stability of enzyme activity in DBS
Development of an immunoassay for bloodspot CK-MM isoform

- Enzyme activity – total CK activity
- CK – isoenzyme (MM, MB & BB forms)
- CK-MM (skeletal muscle isoform)

- Cardiff – Developed a two-site chemiluminescent immunoassay to detect CK-MM in DBS
Cardiff – PerkinElmer Collaboration

GSP® CK-MM

Assay cross reactivity to CK isoenzymes
CK-MM 100%, CK-MB <5%, CK-BB 0%

Analytical run time
4hrs 50 mins (26 plates / 13 hours on GSP®)
Perkin Elmer GSP® CK-MM analytical performance

LOB - <1 ng/mL
LOD – 3.1 ng/mL
LOQ – 8 ng/mL
Perkin Elmer GSP® CK-MM

Precision studies – Inter-assay (n=40)

• C1 - 123 ng/mL (CV 5.2%)
• C2 – 410 ng/mL (CV 6.5%)
• C3 – 1780 ng/mL (CV 5.1%)
Population study
(n=8,741 newborn boys)

99.98th Centile 700 ng/ml
## Population study (n=8,741 boys)

### 10 cases >700 ng/ml (703-5490ng/ml)

<table>
<thead>
<tr>
<th>Case</th>
<th>CK-MM (ng/ml)</th>
<th>Serum CK (U/L)</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>5490</td>
<td>3502</td>
<td>DMD</td>
</tr>
<tr>
<td>2</td>
<td>3613</td>
<td>3665</td>
<td>DMD</td>
</tr>
<tr>
<td>3</td>
<td>2875</td>
<td>3885</td>
<td>DMD</td>
</tr>
<tr>
<td>4</td>
<td>1817</td>
<td>229</td>
<td>Not affected</td>
</tr>
<tr>
<td>5</td>
<td>1015</td>
<td>120</td>
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<td>951</td>
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Next steps…

• Retrieval & analysis of 200 DBS from DMD cases & 750 matched controls (PerkinElmer – California Study)

• Retrieval & analysis of DBS from the FN cases from the Wales NBS programme

• EQA Scheme – CDC

• Pilot studies:
  – China
  – US (CA & NYS)
  – Australia
Conclusions

• Development of molecular therapies to treat DMD has renewed interest in NBS for DMD.

• Limitations of the DBS CK enzyme test.

• Development and evaluation of an immuno-assay for DBS CK-MM on a routine analyser.

• Two tier screening protocol (CK-MM – DNA)
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• Wales Newborn Screening Laboratory
• School of Medicine, Cardiff University