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

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What is Duchenne Muscular Dystrophy (DMD)?

- Fatal X-linked neuromuscular disorder
- Incidence 1:5000 males
- Serum CK/Genetics/Muscle Biopsy
- Mean age of diagnosis ~5 years of age
DMD Disease Progression

Ages 3-5
Early signs of weakness

Ages 6-10
Progressive weakness

Ages 12 and older
Wheelchair dependent
Treatment Options for DMD

• **Current**
  - Steroids & Physiotherapy

• **Future**
  - PTC-124 (Translarna)
  - Exon skipping (Eteplirsen)
DMD Screening programmes

• 17 pilot programmes in 10 countries

• Wales Experience (1990 – 2011)
  • 343,170 boys screened
  • 145 screen positives
    66 confirmed elevated CK (56 DMD)
  • 17 False negative cases

Moat SJ et al 2013 Eur J Hum Genet
Limitations of the CK enzyme test

• Issues with reagent stability
• Lack of assay standardisation
• Difficult to automate for high throughput screening
• Poor stability of enzyme activity in DBS

• CK - Marker of disease process
• Enzyme activity – total CK activity
• CK – isoenzyme (MM, MB & BB forms)
Development of an immunoassay for bloodspot CK-MM isoform

Recombinant

Purified Human

CK-MM (ng/ml)

Response

1e7

1e6

1e5

10000

1000

0.01 0.1 1 10 100 1000

CK-MM (ng/ml)
DMD patient bloodspot samples

![Graph showing CK Activity U/L vs. CK-MM (ng/ml)](image-url)
Cardiff – PerkinElmer Collaboration

GSP® CK-MM

Assay cross reactivity to CK isoenzymes
CK-MM 100%, CK-MB 18-25%, CK-BB 0%

Analytical run time
3hrs 50 mins – 26 plates / 13 hours on GSP
Perkin Elmer GSP® CK-MM analytical performance

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<td>Y-axis</td>
<td>LogB</td>
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PerkinElmer GSP CK-MM

Precision studies – Inter-assay (n=40)

- C1 - 145ng/ml (CV 8.9%)
- C2 - 530ng/ml (CV 5.4%)
- C3 - 2150ng/ml (CV 10.2%)
PerkinElmer GSP CK-MM

Log CK-MM Concentration (ng/ml)

<table>
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<tr>
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<th>Normal DBS (n=296)</th>
<th>DMD Cases (n=10)</th>
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<tr>
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<td>5458ng/ml</td>
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Hook effect of the GSP CK-MM assay
Next steps...

- Assess stability of CK-MM bloodspots
- Retrieval & analysis of 200 DBS from DMD cases & 750 matched controls
- EQA Scheme – CDC
- Pilot studies:
  - US (CA & NYS)
  - China
  - Australia
Conclusions

• Development of molecular therapies to treat DMD
• Limitations of the bloodspot 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)
• CK – marker of disease process and therefore risk of false negatives.
Acknowledgements

• PerkinElmer

• Wales Newborn Screening Laboratory

• School of Medicine, Cardiff University
Wales DMD Screening Protocol
‘Opt in test’

Day 5-8 blood spot sample: CK Test

Test in singlicate

CK ≥ 200U/L

Yes

Repeat CK test in duplicate

Mean of CK triplicates
CK ≥ 250U/L

No

Yes

6-8 week follow-up serum CK

Serum CK Raised

Yes

DMD - Suspected

No

DMD - Not suspected
Overview of results of the DMD NBS program in Wales
July 1990 - November 2011

Number of boys 369,780

Boys tested 343,170 (92.8%)
Test’s declined 21,942 (5.9%)
Defaults 4,668 (1.3%)

Blood spot CK ≥250
145 (0.04%)

Blood spot CK <250
343,025

Serum CK at 6 weeks of age

Confirmed elevated CK
66

Transient raised CK
79 (0.023%)

56 - DMD's
5 - BMDs
5 - other dystrophies

17 - False Negatives
2 - DMD's
Distribution of serum CK iso-enzymes in DMD patients and normal controls

Relationship between serum CK-MM and age of DMD patient