

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

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**&**

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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**



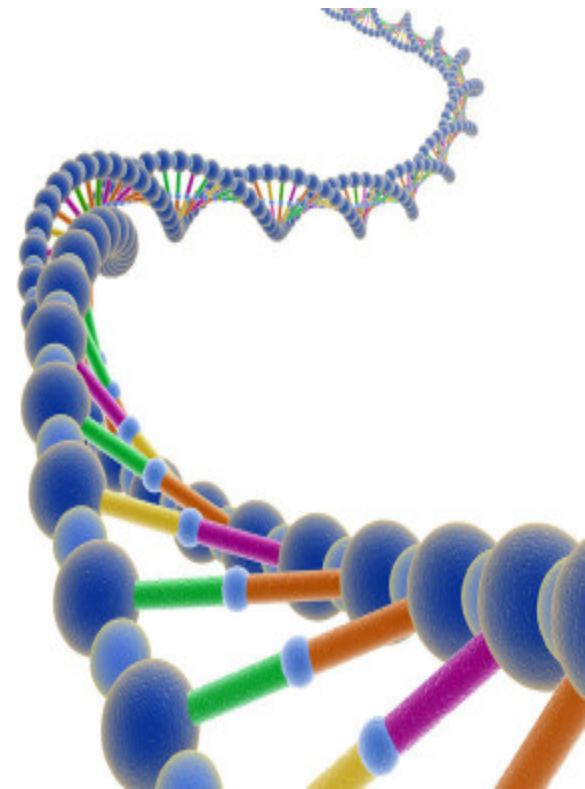
**Ages 6-10**  
**Progressive**  
**weakness**



**Ages 12 and older**  
**Wheelchair dependent**

# Treatment Options for DMD

- **Current**
  - Steroids & Physiotherapy
- **Future**
  - PTC-124 (Translarna)
  - Exon skipping (Eteplirsen)



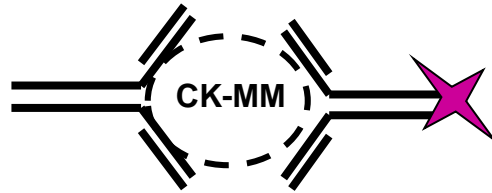
# DMD Screening programmes

- **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

# Limitations of the CK enzyme test

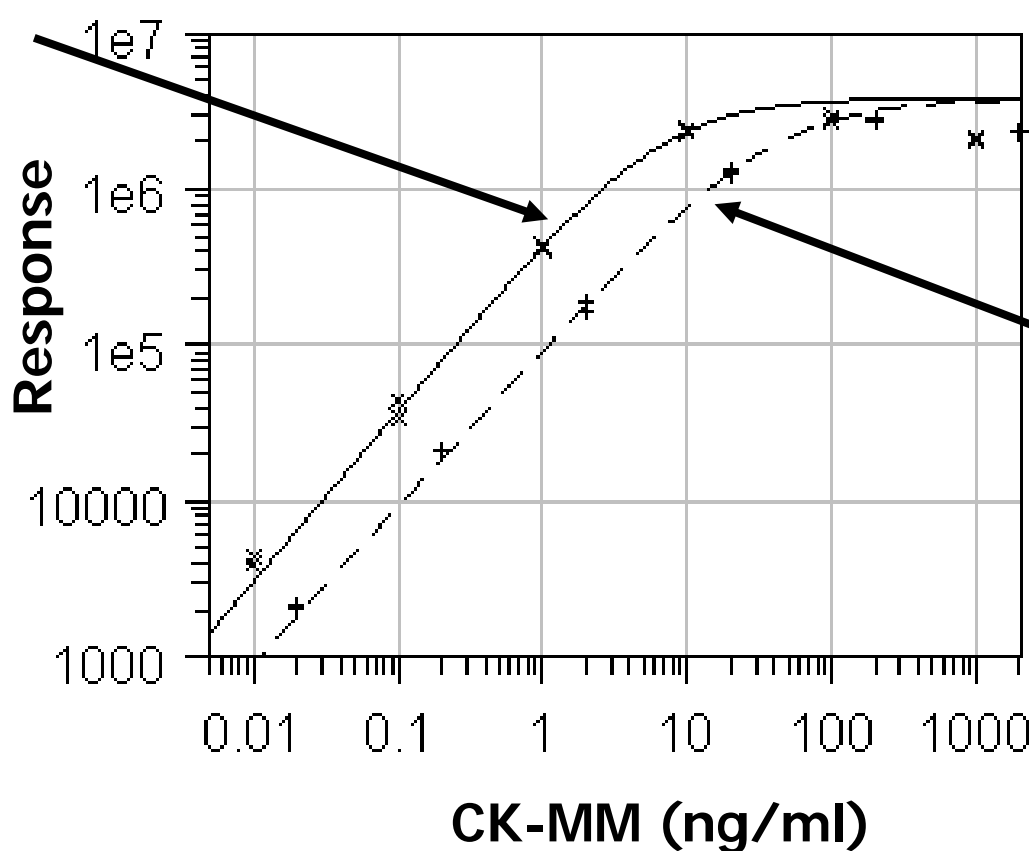
- Issues with reagent stability
  - Lack of assay standardisation
  - Difficult to automate for high throughput screening
  - Poor stability of enzyme activity in DBS
- 
- CK - Marker of disease process
  - Enzyme activity – total CK activity
  - CK – isoenzyme (MM, MB & BB forms)

# Development of an immunoassay for bloodspot CK-MM isoform



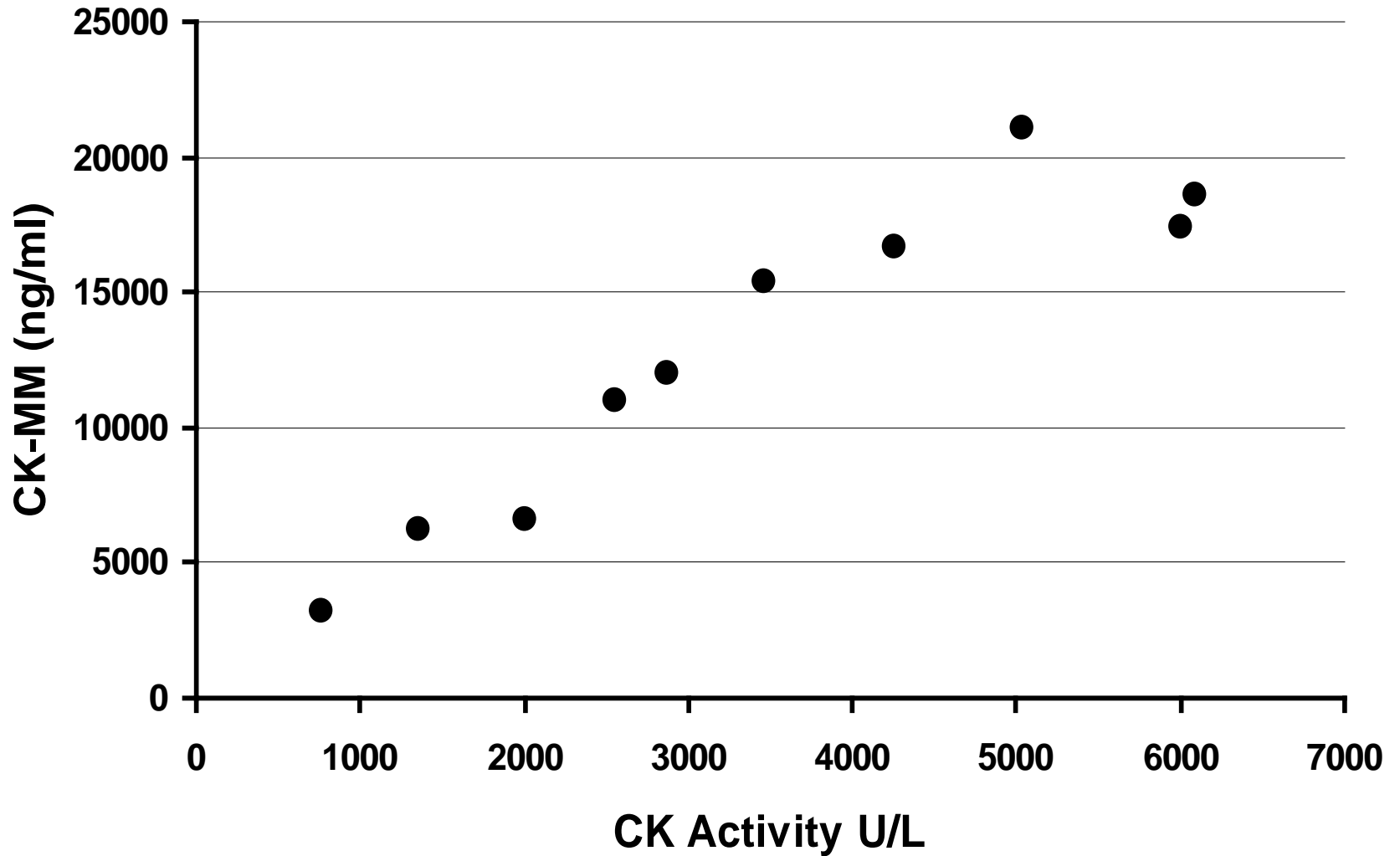
Recombinant

CK-MM



Purified Human  
CK-MM

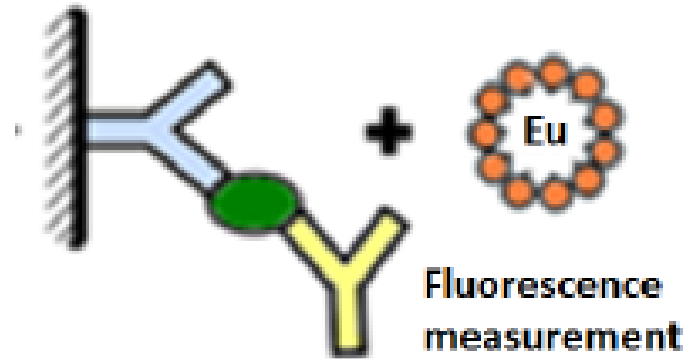
# DMD patient bloodspot samples





# Cardiff – PerkinElmer Collaboration

**GSP® CK-MM**



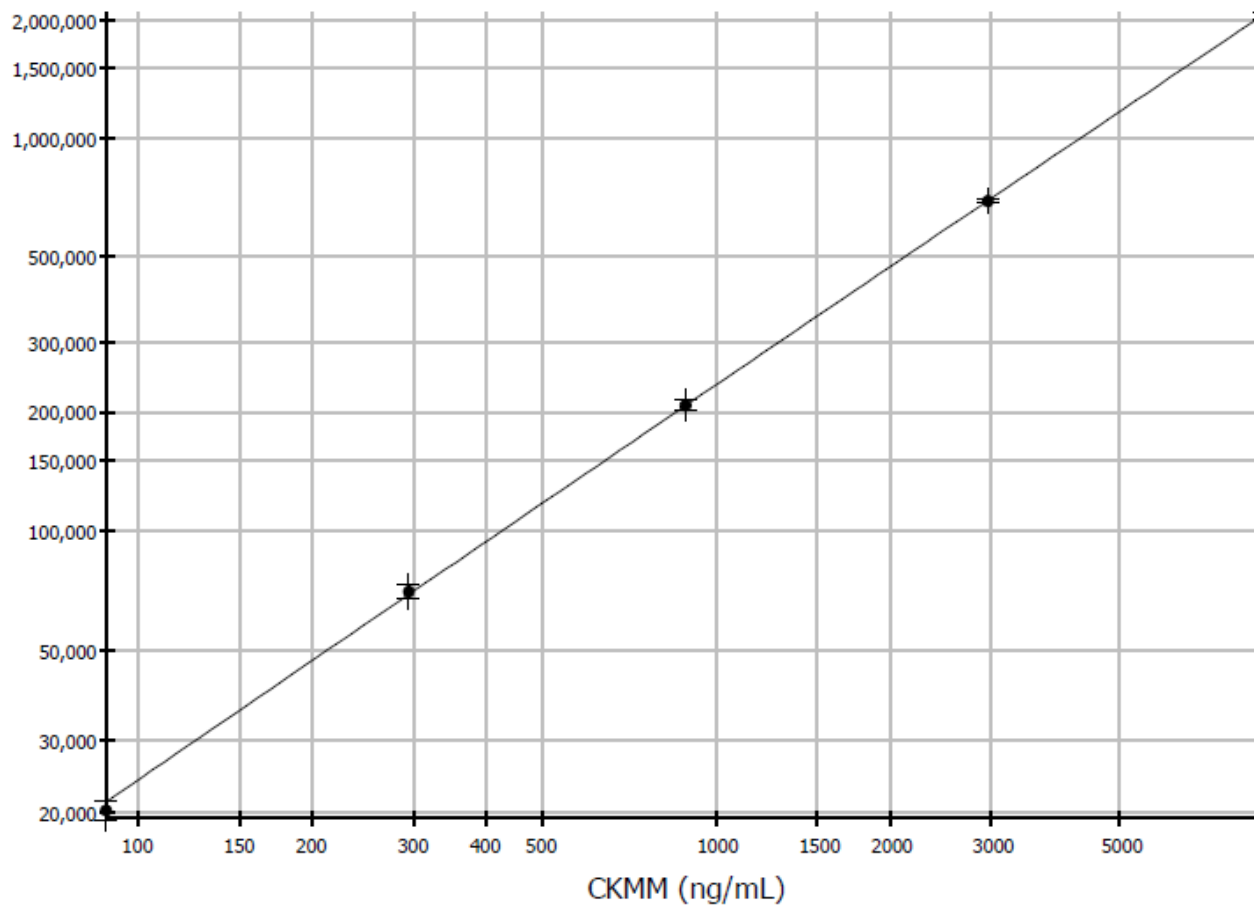
**Assay cross reactivity to CK isoenzymes**

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<sup>®</sup> CK-MM analytical performance



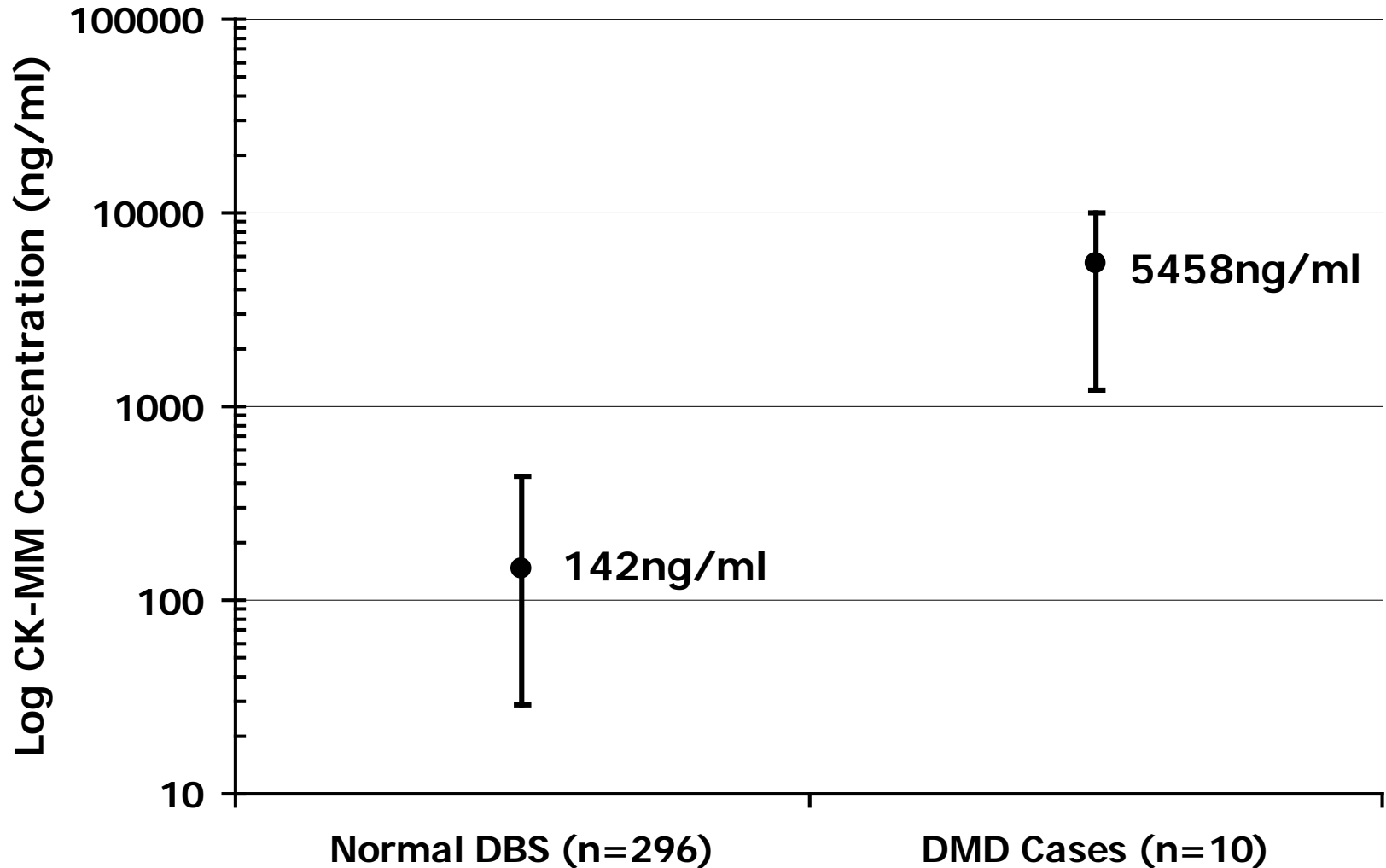
Calibrators	
BLANK	
C2	87.7
C3	292
C4	877
C5	2920
C6	8770
Fitting	
Algorithm	Spline
Slope at 50%	0.4355
ED20	210.3
ED50	841.9
ED80	3404
Iterations	2
Metrics	
X-axis	Logarithmic
Y-axis	LogB

# PerkinElmer GSP CK-MM

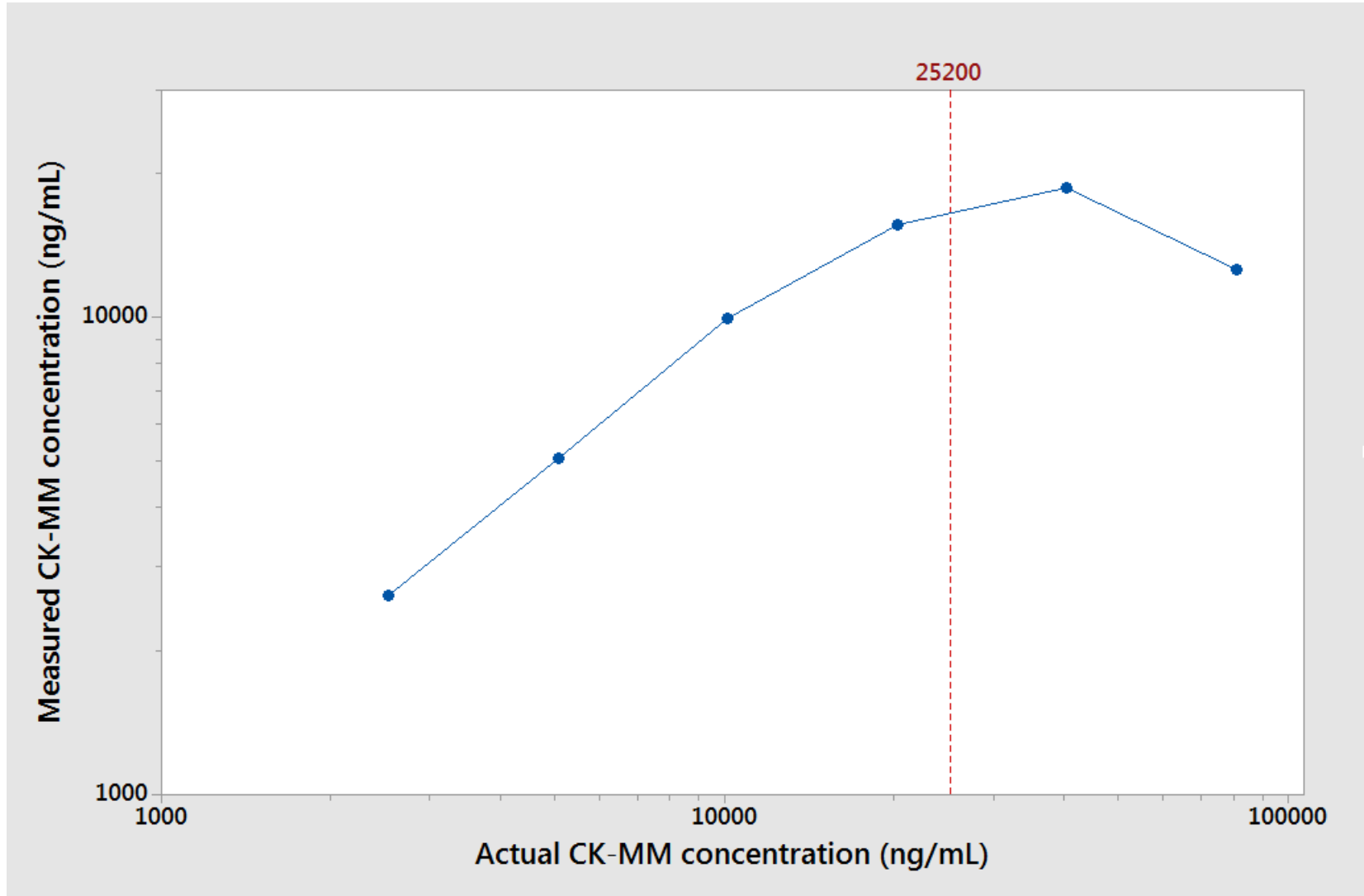
## Precision studies – Inter-assay (n=40)

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

# PerkinElmer GSP CK-MM



# 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 immunoassay 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**

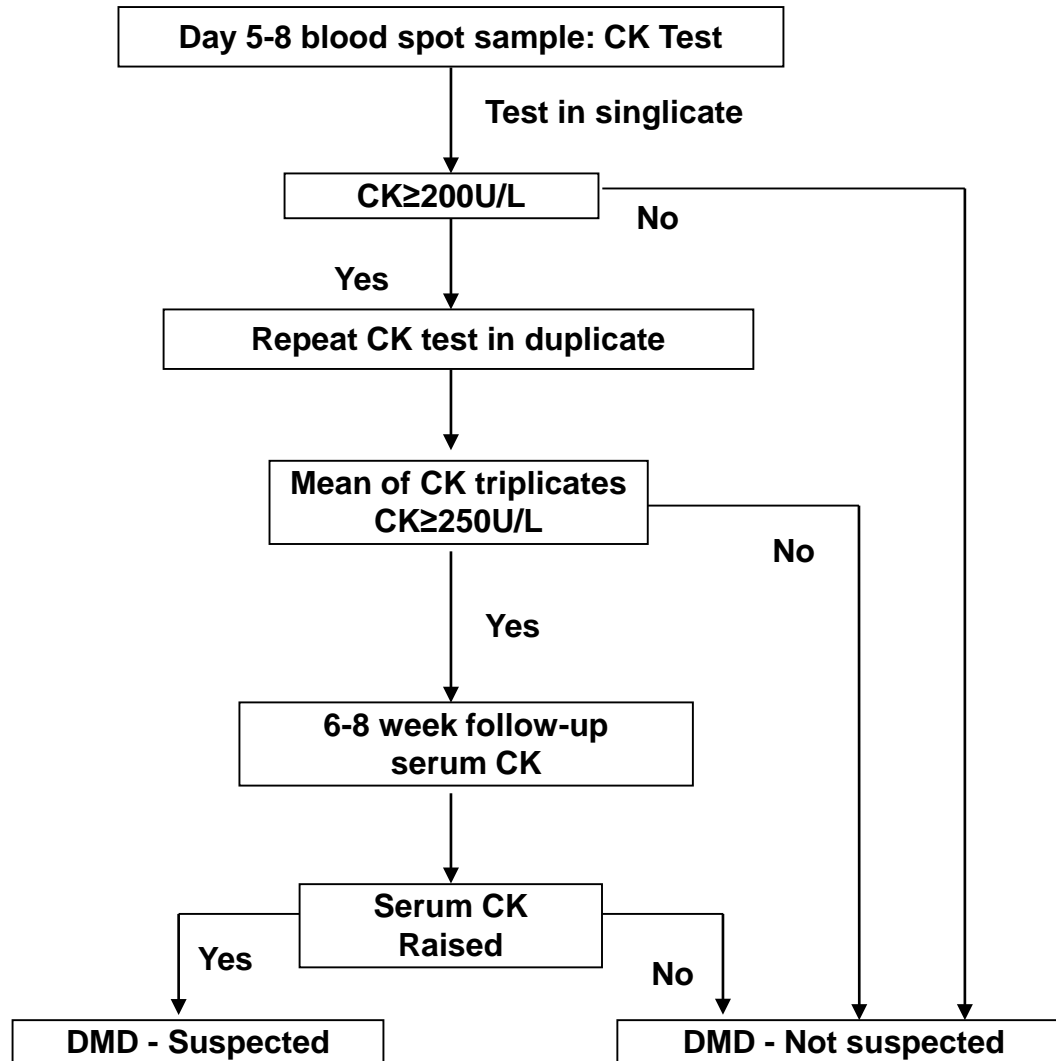
Petra Furu, Harri Hakala, Hanna Polari, Paulina Mäkinen, Liisa Meriö & Sari Aireenne.

- **Wales Newborn Screening Laboratory**
- **School of Medicine, Cardiff University**

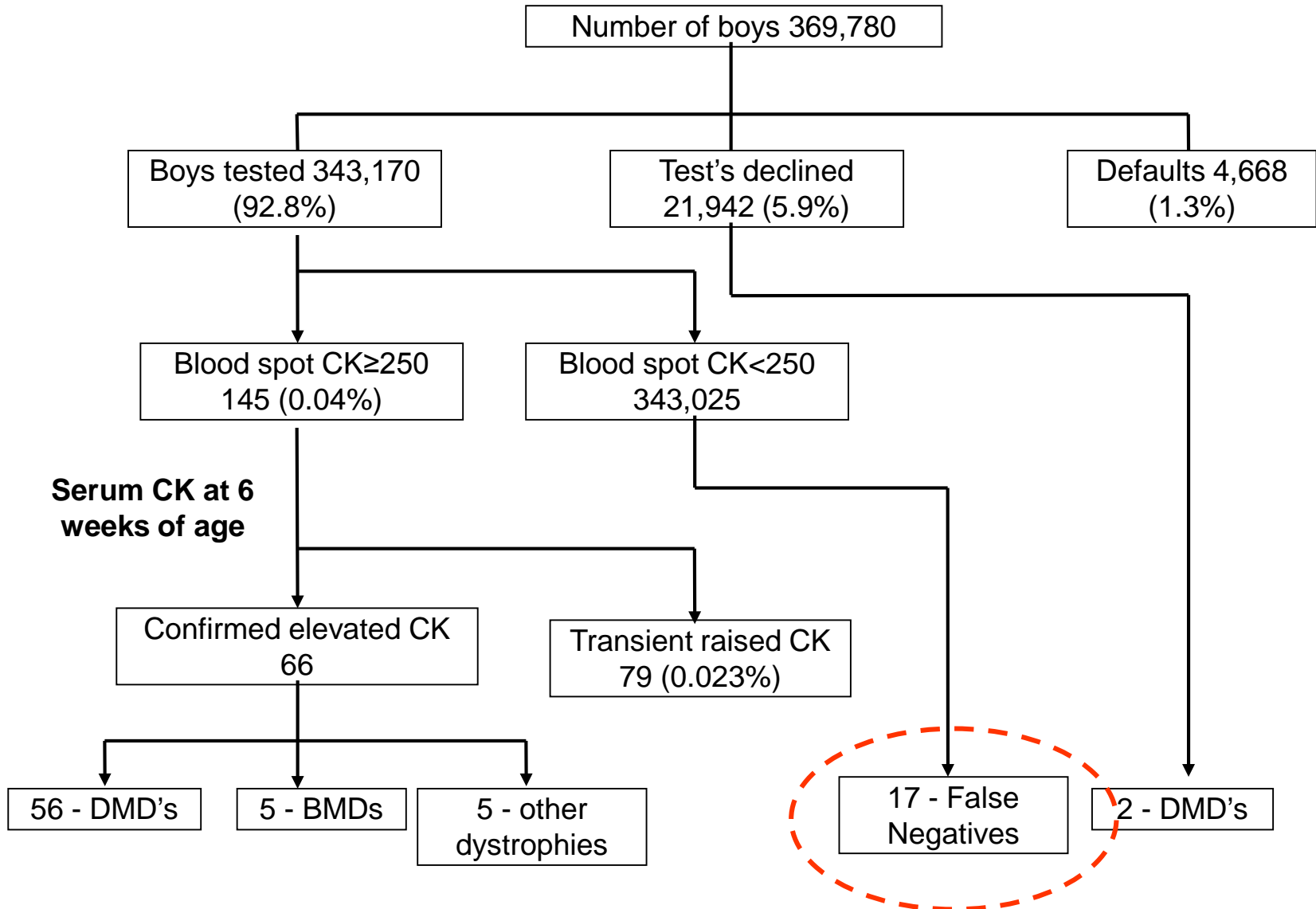


# Wales DMD Screening Protocol

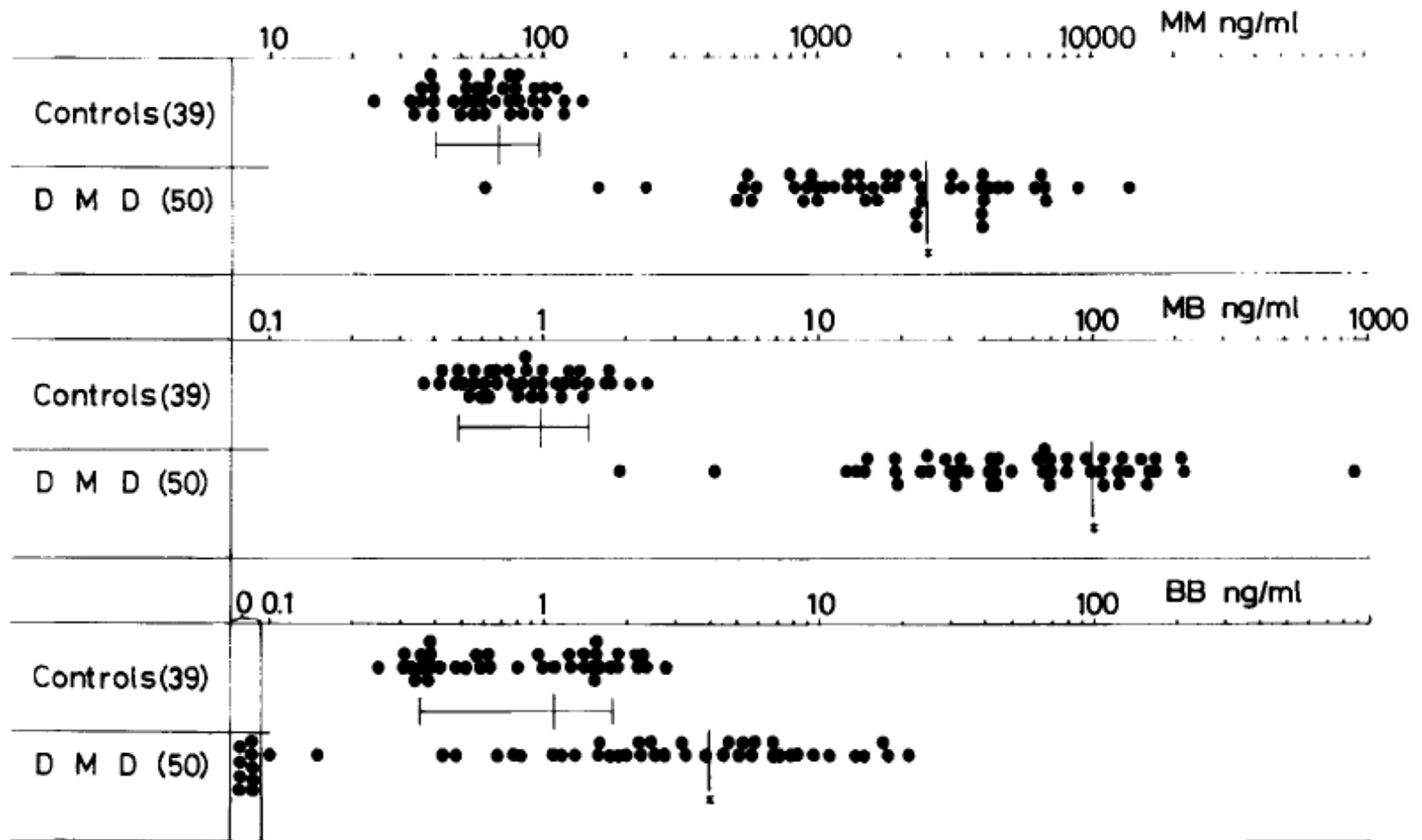
## 'Opt in test'



# Overview of results of the DMD NBS program in Wales July 1990 – November 2011



# Distribution of serum CK iso-enzymes in DMD patients and normal controls



\* P < 0.001

# Relationship between serum CKMM and age of DMD patient

