Pompe Disease - Biochemical Investigation and Monitoring

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The location of glucosidase enzymes in the cell

- Neutral α-glucosidase (pH 7)
- Maltase-glucoamylase
- Lysosomal acid α-glucosidase (GAA)
  pH 3.8 + acarbose

Enzyme activity measured at pH 3.8
8 week cut-off

£0

Very Clear information required, including request for Pompe!
Good Quality blood spots – essential – otherwise rejection!

4-methylumbelliferyl α-D-glucoside
Fluorimetric GAA Assay: 4-methylumbelliferyl α-D-glucoside

4-MU α-D-glucoside → GAA
pH 3.8
Glucose

Maltase-glucoamylase: Interfering Enzyme

4-MU α-D-glucoside → MGA
pH 3.8
Glucose

tGAA
Fluorimetric GAA Assay: 4-methylumbelliferyl \( \alpha \)-D-glucoside

Maltase-glucoamylase: Interfering Enzyme

Inhibition with Acarbose
Fluorimetric GAA Assay

- Potential false positives due to specimen deterioration
- Correction for specimen deterioration:
  - Ratio of GAA/tGAA (+/- acarbose)
  - Measurement of other control enzymes

Neutral α-glucosidases: Control Enzyme

4-MU α-D-glucoside → NAG → 4-MU
pH 7 → Glucose
Brief protocol of the DBS Pompe assay

- Extract enzyme from blood spot with water
- Set up using the Tecan Robotic pipetting station the three assay condition in a 96 well plate
- All wells contain substrate and either:
  - pH 3.8 buffer + acarbose
  - pH 3.8 buffer
  - pH 7.0 buffer
- Add sample to test wells to start the reaction and add sample to blank wells after the reaction has terminated.
- After 20 hours incubation, stop reaction. Set up a calibration curve then read fluorescence.
DBS 4MU-GAA Assay Ratio +/- Acarbose

- Control: n = 92
- Pompe: n = 13
- Obligate Heterozygote: n = 2
- Infantile: n = 6
- Adult onset: n = 7

Ratio +/- acarbose
DBS 4MU-GAA Assay. Ratio pH7.0 / pH3.8 + acarbose

- Pompe
- Unaffected

Legend:
- Control
- Obligate heterozygote
- Infantile Pompe
- Adult Onset Pompe
Pompe Disease – Post DBS Investigations

- Pseudodeficiency - Follow up testing required

- Vacuolated lymphocytes

- Urine tetrasacharride (Glc₄)

- Mutation analysis
Vacuolated Lymphocytes

- Range of metabolic diseases lead to cytoplasmic vacuolation
- Pompe Disease
- Less frequently seen in adult form

Anderson et al., 2005
Tetrasaccharide (Glc$_4$) as a Biomarker for Pompe Disease

$\text{Glc}_4$: from glycogen

- Urine Glc$_4$ reflects clinical response to treatment?

Urine Tetrasaccharide in Pompe Disease – Response to ERT

Patient 2

Pre-treatment
Post-treatment (1 DAY)
Post-treatment (2 WKS)

Glc4 umol/mmol creatinine

0
10
20
30
40
50
60
70
CRIM Analysis

- **Cross-reacting immunologic material**

- CRIM +VE patients tend to show better clinical response to ERT

Klinge et al. 2005, Amalfitano et al. 2001, Kishani et al., 2010

Kaplan-Meier curve of ventilator-free survival of the CRIM-negative (n = 11) and CRIM positive (n=21) patients. (Kishnani et al., 2010)
CRIM Analysis

- Currently detection of CRIM is in cultured fibroblasts by Western blotting
- Only available in a few laboratories worldwide
- Long TAT: fibroblasts required (6 - 8 weeks to grow to confluence)
- In Development – CRIM analysis in white cells
Immune Modulation

Mendelsohn et al., 2009
Pompe – Diagnosis & Monitoring

Enzymology
Vacuolated Lymphocytes
Genetics
Tetrasaccharide (CRIM)
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