Galactose-1-phosphate uridyltransferase quantitation by HPLC-MS/MS

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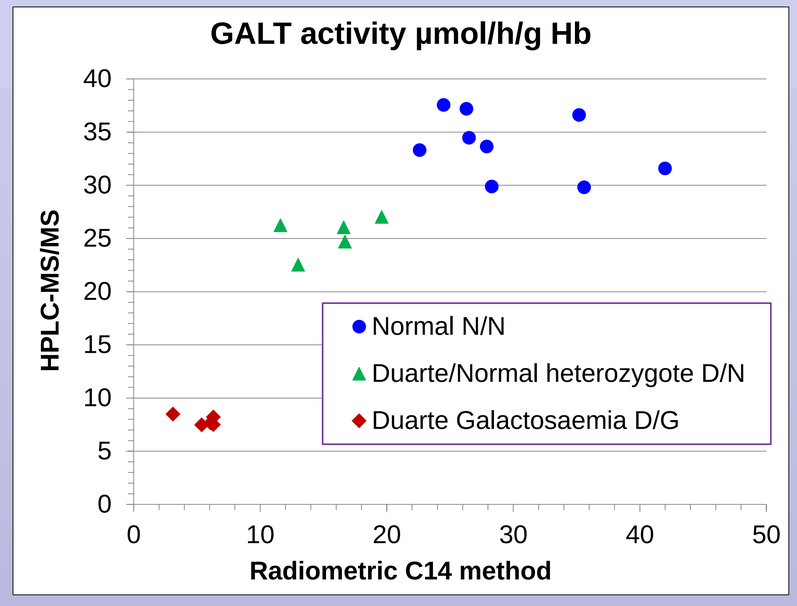
Introduction

Galactose-1-phosphate uridyltransferase (GALT) quantitation is required in the diagnosis of Classical Galactosaemia. This enzyme has been quantitated using a C14 radiometric assay in our lab for many decades; however it was necessary to switch to an HPLC-MS/MS method to eliminate the need for radioactive reagents (hazardous) and DEAE cellulose paper (withdrawn from manufacturing). Additional benefits include a smaller sample volume, larger batch sizes and improved precision.

Methods

An HPLC-MS/MS method was set up, optimised and validated to quantitate GALT activity in red blood cells:
UDP-glucose + 2-^{13}C galactose-1-phosphate → UDP 2-^{13}C galactose + glucose-1-phosphate
Thawed washed red cells (50µL) were lysed by the addition of 100µL water. Hemocue Hb201+ was used to measure the lysate haemoglobin level (g/L).
70 µL reaction mixture containing 2.5 mmol/L [2^{13}C] Gal-1-P, 2.5 mmol/L UDP glucose, 0.5 mol/L glycine NaOH (pH 8.7) and 0.04 mol/L cysteine-HCl (pH 8.7) was added to 30µL lysate, mixed, then incubated at 37°C for 30 minutes. The enzyme reaction was stopped by heating to 95°C for 5 minutes, then centrifuged at 15000g for 15 minutes. 30µL supernatant was combined with 270µL internal standard (0.06 mmol/L UDP N-acetylglucosamine in mobile phase), and 5µL injected onto a Waters T3 Atlantis column to separate the substrate, product and internal standard. Mobile phase (20 mmol/L triethylamine acetate, pH 5.10) ran isocratically at 0.25 ml/min and the column washed with water and equilibrated with mobile phase between injections (total 16 minutes). Standard curve: 0 to 2.0 mmol/L UDP-galactose. Quantitation was achieved by monitoring MRMs specific to each compound in negative ion mode.
Method adapted from Ko *et al* (2010).



Low Activity Variants

It is important to be able to distinguish patients with low activity (10-25% normal), from those with deficiency, (<10% activity, e.g. Classical Galactosaemia homozygotes) as there are implications for treatment (Welling *et al* 2017). This assay showed clear separation between the patient groups as shown in the following table

Galactosaemia type	Analyte area	Signal/ Noise	GALT activity µmol/hr/gHb
Classical (n=10)	2.8 - 43.6	2.7 - 120	<0.5
Duarte (n=6)	1802 - 2624	377 - 3322	6.6 - 9.5

Performance Characteristics

Intra batch precision n=10 CV= 3.2%
Inter batch precision n=15 CV= 6.4%
LOQ = 0.02 mmol/L.
No evidence of Ion Suppression.
No evidence of Carryover.
Linearity >0.99
All blanks [analyte] <0.001 mmol/L

Limitations

No samples from heterozygotes (N/G) were available for comparison between assays. Expected GALT activity is 50%. There are no EQA schemes available so a regular inter-laboratory comparison, is performed between ourselves and GOSH Enzyme Laboratory, London.

Patient Sample Comparison

29 samples were measured by both methods. 10 patients had Classical Galactosaemia and enzyme activity was <0.5 by both methods. The remaining 19 patient results are shown graphically. HPLC-MS/MS results show a positive bias compared with the radiometric C14 method, most likely reflecting lower background signal. All 29 results showed agreement in patient category, & hence interpretation and advice. Genetics results were available for 6 patients giving further confirmation.

New Reference Range

GALT activity 28.4– 39.4 µmol/hr/gHb
n = 27 (anonymised surplus samples)

Conclusions

This assay for GALT quantitation can:

- Confirm a diagnosis of Classical Galactosaemia, often suspected due to positive Beutler Galactosaemia screen.
- Determine variant Galactosaemia (e.g. Duarte Galactosaemia compound heterozygotes who have approximately 15-25% normal GALT activity)
- Determine carrier status for Classical Galactosaemia in family studies or in parents as part of the investigations for a child with suspected Galactosaemia (e.g. baby who has had a blood transfusion).

Sample Requirements

0.5mL lithium heparin whole blood to arrive at Southmead within 48 hours of collection. No RBC transfusion in previous 4 months. See ‘Test information’, www.severn-pathology.com.

References

- Ko DH, et al Clin Chem 2010; 56(5): 764-71
- Pasquali, M. et al ACMG Guidelines. Genet Med 20, 3–11 (2018).
- Welling L et al. International Clinical Guideline JIMD. 2017 Mar;40(2):171-176