UDP-Galactose-4'-epimerase activity determination in Red Blood Cells by Liquid Chromatography Tandem Mass Spectrometry

Stephen J. Welna[1], Patricia L. Hall[2], Coleman T. Turgeon[1], Dimitar K. Gavrilov[1], Silvia Tortorelli[1], Devin Oglesbee[1], Piero Rinaldo[1], Kimiyo M. Raymond[1], Dietrich Matern[1]

[1]Biochemical Genetics Laboratory, Mayo Clinic, 200 First Street SW, Rochester, MN 55905

[2]Emory Genetics Laboratory, Department of Human Genetics, Emory University, 2165 North Decatur Road, Decatur, GA 30033

Introduction: Uridine diphosphate galactose-4-epimerase (GALE) deficiency is a rare differential diagnosis of galactosemia which includes two other autosomal recessive conditions, classic galactosemia due to galactose-1-phosphate uridyltransferase (GALT) deficiency and galactokinase (GALK) deficiency. GALE catalyzes the conversion of UDP-galactose to UDP-glucose in a reversible enzymatic reaction. Demonstration of reduced GALE activity in red blood cells (RBC) is a useful diagnostic test following an abnormal newborn screening result for galactosemia.

Methods: The concentration of the enzyme product of UDP-glucose is determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Washed and lysed RBC are incubated at 37°C in the presence of UDP-galactose substrate and NAD^+. LC-MS/MS is performed using a normal phase column (100 mm x 4.6 mm, 3.5 µm) to separate the isomeric enzyme product from residual substrate. The MS/MS is operated in the multiple reaction monitoring (MRM) negative mode with a total analysis time of 10 minutes per sample. The concentration of UDP-glucose is established by comparison of its ion intensity to that of an internal standard. The final enzyme activity is normalized to incubation time and hemoglobin (Hb) concentration.

Preliminary Data: Intra and inter-assay precision was assessed using RBC samples (N=4) of varying enzyme activities (intra-assay mean: 14.0, 13.1, 8.3 and 2.0 nmol/h/mg Hb; inter-assay mean: 15.1, 14.4, 12.3, and 2.2 nmol/h/mg Hb). Intra-assay precision CVs were 5.3, 4.6, 4.3, and 5.7% respectively (N=20). Inter-assay precision CVs were 6.0, 7.4, 7.2, 9.8% respectively (N=20). Accuracy was assessed as clinical concordance by an ongoing blinded sample exchange with Emory University. Six abnormal results were identified out of the ten specimens, 4 true

positive and 2 suspected carriers. Clinical interpretation was in agreement with findings from Emory University. A reference range evaluation has yielded a mean of 16.7 nmol/h/mg Hb (min-max = 7.1-36.8, stdev = 4.7, N=181).

Novel Aspect: The use of a rapid LC separation of UDP-glucose and UDP-galactose allows direct quantification of enzymatic products and is an improvement upon recently published enzyme assays using LC-MS/MS and either fragment intensity ratios or a linked enzyme process to calculate enzymatic product concentrations and thus enzyme activity (1, 2).

1. Li Y et al. Liquid chromatography-tandem mass spectrometry enzyme assay for UDP-galactose 4'-epimerase: use of fragment intensity ratio in differentiation of structural isomers. Clin Chem. 2014; 60: 783-90
2. Chen J et al. An interference-free two-step enzyme assay with UPLC-tandem mass spectrometric product measurement for the clinical diagnosis of uridine diphosphate galactose-4-epimerase deficiency. J Chromatogr B Analyt Technol Biomed Life Sci. 2014; 959: 5-9.
3. McHugh et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet Med. 2011 Mar; 13(3):230-54
4. Marquart et al. Enhanced Interpretation of Newborn Screening Results without Analyte Cutoff Values. Genet Med. 2012 Jul; 14(7):648-55