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

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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 uridylyltransferase (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).