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

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Abstract

Intended use: The intended use of this assay is to support the biochemical diagnosis of UDP-galactose-4’-epimerase deficiency (OMIM #230350), an autosomal recessive inborn error of galactose metabolism. Clinically two variants of GALE deficiency are known, a benign “peripheral” deficiency and a systemic deficiency. The latter may present similar to classic galactosemia due to GALT deficiency (hypotonia, hepatomegaly, liver dysfunction, aminoaciduria, and cataracts). Both variants of GALE deficiency are characterized by reduced enzyme activity in RBC.

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 mmol/h/mg Hb; inter-assay mean: 15.1, 14.4, 12.3, and 2.2 mmol/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, and 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 facilitates 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).

Discussion

Specimen and Extract Stability: Less than 20% change in calculated enzyme activity was observed for the time frames listed.

Clinical Specificity: Five clinical waste RBC specimens deficient in Thiopurine methyltransferase activity were assayed for GALE and found to be within the reference range.

Clinical Sensitivity: Four true positive GALE deficient patients and two suspected carriers were identified in a blinded sample exchange with Emory University.

References