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

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Abstract

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).

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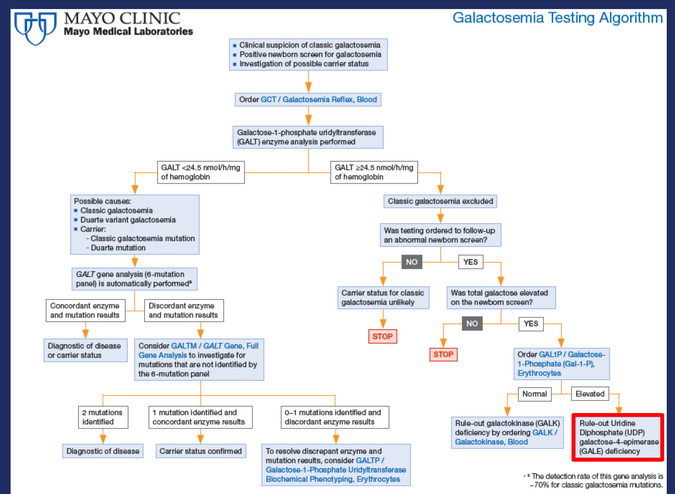
Objectives

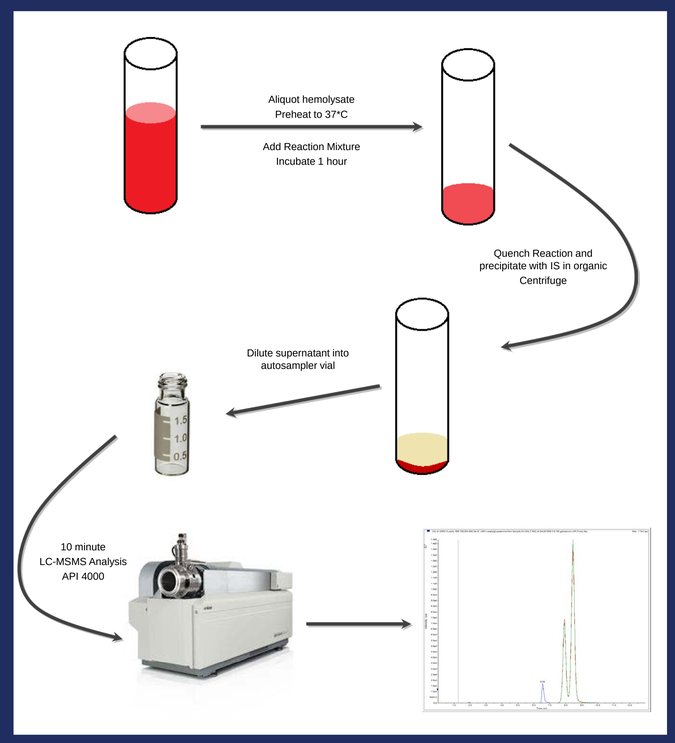
Intended use: The intended use of this assay is to support the biochemical diagnosis of UDP-galactose-4’-epimerase deficiency (OMIM #230350), an autosomal recessive inborn error of galactose metabolism. Clinically two variants of GALE deficiency are known, a benign “peripheral” deficiency and a systemic 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.

GALE Test Algorithm

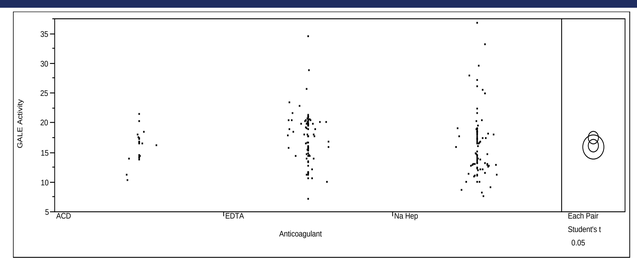


Order of testing: This chart shows the appropriate procedure to follow when ordering tests for a patient suspected of galactosemia. GALE is indicated following a normal GALT enzyme activity and elevated RBC GAL1P.

Methods



Acceptable Anticoagulants for Testing

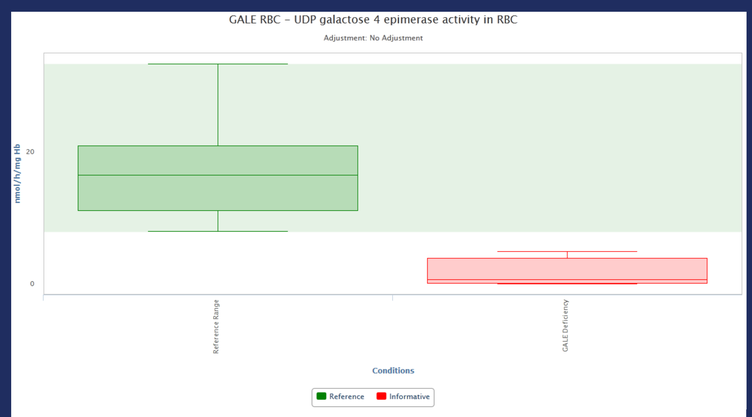


Conclusion: Pools of reference specimens from Acid Citrate Dextrose (ACD), EDTA, and Sodium Heparin (Na Hep) showed no statistical difference at the 95% confidence interval.

Assay Performance

Analytical Parameter	Assay Performance
Limit of Detection	70 nM
Intra-assay Precision	<6%
Inter-assay Precision	<10%
Measurable Range	70nM - 87µM
Carryover	0%
Ion Suppression	None observed
Specimen Stability (25ºC)	6 days
Specimen Stability (4ºC)	14 days
Extract Stability (10ºC)	5 days
Clinical Specificity	100%

Reference Range



	True Positive Cases	Reference Range
Mean	1.7	16.7
SD	2.3	4.7
N	4	181
Range Min	0.1	7.1
Range Max	5.1	36.8

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.

Conclusions

When to consider GALE: Testing for GALE deficiency should be considered after a positive Newborn Screening and/or elevated RBC galactose-1-phosphate with normal GALT activity.

Clinical Utility: RBC GALE testing is currently of limited availability. This method provides a useful tool for the diagnosis of a rare form of Galactosemia with the potential for rapid turn around time.

References

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4. Marquart et al. Enhanced Interpretation of Newborn Screening Results without Analyte Cutoff Values.Genet Med. 2012 Jul; 14(7):648-55