Overview

Useful For
Evaluation of neonatal hyperbilirubinemia, favism or chronic or episodic hemolysis or jaundice
Evaluation for gamma-glutamylcysteine synthetase deficiency (OMIM 230450)
Evaluation for glutathione synthetase deficiency causing hemolytic anemia (OMIM 231900)
Evaluation for generalized glutathione synthetase deficiency with 5-oxoprolinuria (OMIM 266130)

Method Name
Kinetic Spectrophotometry

NY State Available
Yes

Specimen

Specimen Type
Whole Blood ACD-B

Specimen Required
Collection Container/Tube:
Preferred: Yellow top (ACD solution B)
Specimen Volume: 6 mL

Collection Instructions: Send specimen in original tube. Do not transfer blood to other containers.

Forms
If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

Reject Due To
Gross hemolysis  Reject

Specimen Minimum Volume
1 mL

Specimen Stability Information

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<th>Temperature</th>
<th>Time</th>
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Clinical & Interpretive

Clinical Information
Hemolytic anemia may be associated with deficiency of erythrocyte enzymes. Red blood cell (RBC) enzymes linked to hemolysis are those important in the energy generation of glycolysis or protection from oxidative stress such as the hexose monophosphate shunt.

The hexose monophosphate pathway depends primarily upon the glucose 6-phosphate dehydrogenase (G6PD) enzyme for the generation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) with 6-phosphogluconate dehydrogenase (6PGD) providing an additive effect. Both reactions require adequate levels of reduced glutathione (GSH). Because RBCs lack the citric acid cycle, this is an important source of NADPH, and a deficiency of G6PD or GSH results in the inability to neutralize oxidative insults. GSH is synthesized from amino acids by two enzymatic steps and is present in liver, kidney, brain, muscle, and RBCs. It plays widely versatile and important roles in the synthesis of proteins and DNA, the processing of medications and toxins, and other redox reactions.

Similar to G6PD deficiency, glutathione deficiency can have an episodic acute time course of hemolysis or jaundice, be triggered by fava beans, and cause neonatal hyperbilirubinemia. Five enzymes impact GSH availability and therefore are potential candidates for abnormalities leading to glutathione deficiency:

- Two enzymes, gamma-glutamylcysteine synthetase (GCLC) and glutathione synthetase (GSS), are required for GSH synthesis
- Two enzymes, glutathione reductase (GSR) and glutathione peroxidase (GPX1), are required for reduction-oxidation cycling of oxidized glutathione (GSSG) to reduced glutathione (GSH)
- A family of enzymes, glutathione S-transferases (GSTs), utilizes GSH in the detoxification and preparation of substances for excretion into the bile or urine

Enzyme deficiencies have been reported in all of these enzymes, albeit very rarely. The best characterized are GSS and GCLC deficiencies. GSS deficiency is associated with two clinical presentations; a mild form causing isolated chronic hemolytic anemia (OMIM 231900), and a more severe form marked by urinary excretion of 5-oxoproline, metabolic acidosis, hemolytic anemia, and central nervous system disorders (5-oxoprolinuria, OMIM 266130). GCLC deficiency is associated with moderate to severe chronic hemolytic anemia present from neonatal or early childhood, or compensated hemolysis with sporadic but recurrent anemia or jaundice. Some cases have shown learning disabilities, severe and progressive ataxia with myopathy and spinocerebellar degeneration. GSR deficiency has been confirmed in three siblings with favism (episodic hemolysis after fava bean ingestion) and cataracts in early adulthood, and an unrelated infant with marked neonatal hyperbilirubinemia. GSR activity can be decreased in riboflavin deficiency, but whether this results in hemolysis is not clear. Although patients have been reported with anemia in the context of decreased GPX1 activity (OMIM 614164) and decreased GST activity was found in a person with hemolytic anemia, splenomegaly, hyperbilirubinemia, and cholelithiasis, neither have been characterized sufficiently as the definitive cause of hemolysis. All described cases have shown autosomal recessive inheritance pattern.

A deficiency of either of the synthetic enzymes, GCLC or GSS, results in GSH levels less than 25%, but many show a virtual absence of measurable GSH. Heterozygotes usually show normal GSH levels. Elevated concentrations of GSH are found in patients with myelofibrosis and in those with pyrimidine-5'-nucleotidase deficiency.

**Reference Values**

> or =12 months: 46.9-90.1 mg/dL RBC

Reference values have not been established for patients who are <12 months of age.

**Interpretation**

Measurement of reduced glutathione (GSH) is used as a surrogate for the activity of the enzymes that contribute to normal levels of GSH within the red blood cell. GSH is associated with less than 25% of mean normal in individuals with deficiencies of gamma-glutamyl cysteine synthetase or glutathione synthetase.

Elevated concentrations of GSH are of uncertain significance. This finding can be nonspecific and is seen in normal neonates, pyrimidine-5’-nucleotidase deficiency, lead poisoning, dyserythropoietic disorders (inherited and acquired),
myelofibrosis (possibly due to chromosome 8 duplication), or riboflavin supplementation. Consistently elevated glutathione levels have been reported in a family with mild hemolytic anemia of uncertain cause (1); however, whether this was causative or incidental was not determined.

Cautions
Samples with white blood cell counts greater than 20x10⁹/L have been shown to falsely increase the glutathione level by as much as 25%. Results in the normal or elevated range should be interpreted with caution if high white blood cell count is noted.
Recent transfusion may mask the patient’s intrinsic enzyme activity and cause unreliable results.

Clinical Reference

Performance

Method Description
Virtually all of the non-protein sulfhydryl of red cells is in the form of reduced glutathione (GSH). 5,5'-dithiobis (2-nitrobenzoic acid) is a disulfide compound, which is readily reduced by sulfhydryl compounds, forming a highly colored yellow anion. The absorbance of this resultant yellow substance is measured by 412 nm and compared to that of a known standard.(Beutler E: Red cell metabolism. In: A Manual of Biochemical Methods. 2nd ed. Grune and Stratton, 1984; Alisik M, Neselioglu S, Erel O: A colorimetric method to measure oxidized, reduced and total glutathione levels in erythrocytes, J Lab Med. 2019;43(5), 269-277. doi: 10.1515/labmed-2019-0098)

PDF Report
No
Test Definition: GSH
Glutathione, B

Specimen Retention Time
28 days

Performing Laboratory Location
Rochester

Fees & Codes

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
82978

LOINC® Information

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