Test Definition: G6PD1

Glucose 6-Phosphate Dehydrogenase Enzyme Activity, Blood

Overview

Useful For

Evaluation of individuals with episodic or chronic Coombs-negative nonspherocytic hemolytic anemia

Rapid testing to assess glucose 6-phosphate dehydrogenase (G6PD) enzyme capacity prior to rasburicase or other therapies that may cause hemolysis or methemoglobinemia in G6PD deficient patients

May aid in the creation of a comprehensive patient profile and can ensure appropriate patient monitoring for developing anemia

Testing Algorithm

The following are available:

-Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Diagnostic Algorithm

-Glucose-6-Phosphate Dehydrogenase (G6PD) Genotyping Interpretive Algorithm

-Newborn Screen Follow-up for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

-Newborn Screening Act Sheet Glucose-6-Phosphate Dehydrogenase Deficiency

Special Instructions

- Newborn Screening Act Sheet Glucose-6-Phosphate Dehydrogenase Deficiency
- Newborn Screen Follow-up for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency
- Glucose-6-Phosphate Dehydrogenase (G6PD) Genotyping Interpretive Algorithm
- Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Diagnostic Algorithm

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)

Acceptable: Lavender top (EDTA) or yellow top (ACD solution A)

Specimen Volume: 6 mL

Collection Instructions: Send specimen in original tube. **Do not** aliquot.

Forms

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

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood ACD-B	Refrigerated	20 days	

Clinical & Interpretive

Clinical Information

Hemolytic anemia may be associated with deficiency of erythrocyte enzymes. The most common enzyme defect worldwide is a deficiency of glucose 6-phosphate dehydrogenase (G6PD).

As an enzyme in the hexose monophosphate pathway, G6PD plays a key role in the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Because red blood cells lack the citric acid cycle, this NADPH generation is critical for protection against oxidative stress. Normal conditions require approximately 2% of capacity, leaving 98% reserve for stressor events. More than 400 molecular variants of G6PD are known, and the clinical and laboratory features of G6PD deficiency vary according to the degree to which enzyme reserve is decreased. G6PD deficiency (OMIM 300908, X-linked) therefore results in various forms of anemia and is classified by World Health Organization (WHO) criteria according to enzyme activity and chronic versus acute episodic clinical course.(1-3) In 2022, the WHO Malaria Policy Advisory Group panel proposed updated guidance for the classification of *G6PD* variants.(3) This revised guidance is based on the median residual enzyme activity (per allele) and seeks to resolve problems identified with the WHO *G6PD* classification system that has been in place since 1985 (see Table in Interpretation).

The *G6PD* locus is located on the X chromosome and, therefore, G6PD deficiency is a sex-linked disorder. Most people with G6PD deficiency are asymptomatic until a stressor event occurs resulting in acute hemolytic anemia that resolves after stimulus removal. Symptoms can include neonatal jaundice (presents at 1-4 days of age) or acute hemolysis triggered by medications (antimalarials, sulfonamides, dapsone, nitrofurantoin, and naphthalene), infection (hepatitis, CMV, typhoid), or fava bean ingestion. Hemolysis and jaundice begin 24 to 72 hours after a triggering stimulus, with accompanying dark urine/hemoglobinuria. Anemia worsens for approximately 1 week and begins to recover 10 days after cessation. Splenomegaly, gallstones, and recurrent jaundice are additional clinical symptoms. Because it is X-linked, male patients are usually more severely affected, but homozygous female patients are seen due to the prevalence of

genetic variants. Heterozygous female patients (carriers) range from asymptomatic to severe anemia due to mosaicism/lyonization. Acquired G6PD may occur due to increasing X inactivation in aging women.(4) Acute episodic G6PD deficiency (WHO class B, formerly class II and III) is not expected to affect length or quality of life. Less commonly seen are genetic variants that result in chronic nonspherocytic hemolytic anemia, which manifests similarly to other enzyme deficiencies (WHO class A, formerly class I).

The major *G6PD* variants occur in specific ethnic groups. Thus, knowledge of the ethnic background of the patient is helpful. G6PD deficiency has very high frequency in persons of southeast Asian, African, southern European, and Middle Eastern descent.

Rasburicase therapy is contraindicated in patients with G6PD deficiency. US Food and Drug Administration (FDA) guidelines state to screen patients at higher risk for G6PD deficiency (eg, patients of African or Mediterranean ancestry) prior to starting therapy.(5)

Deficiency can be assessed by enzymatic and/or genetic assays. Due to limitations of genetic testing, in most cases it is preferential to perform G6PD enzyme testing to assign G6PD status. However, enzyme activity can be affected by recent red blood cell transfusion, marked reticulocytosis, and very high white blood cell count. In these settings, genotyping may be useful for correlation with the red blood cell enzyme level.(6,7)

Due to historic issues with other similar antimalarial medications, questions arise if hydroxychloroquine (HCQ) or chloroquine (CQ) therapy may trigger acute hemolytic episodes in some G6PD subtypes. Data is limited in this regard. Available published data did not find hemolytic episodes associated with HCQ therapy in G6PD deficient African American (8) or CQ therapy in G6PD deficient African (9) patients. Both studied populations were assumed to have mild forms of the disorder. Data regarding these medications in populations with more severe G6PD phenotypes is lacking. While patients receiving HCQ do not routinely need G6PD levels checked before initiating therapy, testing may be considered in patients who are from ethnic backgrounds with high G6PD variant rates, such as those from Mediterranean, African, or Asian descent. Although specific details are not described, hemolysis has been reported in at least one individual with G6PD deficiency during the post-approval use of HCQ sulfate tablets, United States Pharmacopeia (USP) per FDA label information.(10)

Reference Values

> or =12 months of age: 8.0-11.9 U/g Hb

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

Interpretation

The World Health Organization (WHO) classification of glucose 6-phosphate dehydrogenase (G6PD) deficiency is historically based on enzyme activity level, and, in most cases, enzyme activity level is sufficient. Accurate classification requires correlation with clinical, and in certain cases, genetic data. The revised WHO classification (2022) has updated classification subtypes from classes I, II, III, IV and V to class A, B, C and U.

The Advisory Group panel concluded:(3)
"In future, *G6PD* variants should be classified based on the median residual enzyme activity expressed as a percentage of normal activity. It should be emphasized that this system is for classifying genetic variants of *G6PD* and should not be

used to classify individual patients with G6PD deficiency. Currently, no variants have been identified in homozygous deficient females or hemizygous deficient males that have median G6PD enzyme activity falling between 45% and 60%. Therefore, a gap has been left between Classes B and C. If new variants are found with median G6PD enzyme activity in this range, these should be included in the "U" class and studied until solid evidence is found that they induce acute haemolytic anaemia (= Class B) or do not pose a haemolytic risk (= Class C). Based on new evidence, the thresholds may then need to be revisited."

Table. **Updated (2022) and Legacy *G6PD* Variant WHO Classification and Associated G6PD Deficiency Phenotype**

2022 WHO class	Median* G6PD activity	Hemolysis	Legacy** WHO class	Level of residual enzyme activity (% of normal)
A	<20%	Chronic (CNSHA)	I	<10%
B	<45%	Acute, triggered	II	<10%
			III	10%-60%
C	60-150%	No hemolysis	IV	Normal
U	Any	Uncertain clinical significance		

*The activity is per variant (ie, per allele) and most straightforward to assess in hemizygous male patients and homozygous female patients. Compound heterozygous female patients are more complex and rely on clinical and familial correlation.
**Legacy WHO Class V: increased activity (enzyme activity >150%) has been discontinued in the 2022 recommendations. It was originally created due to a single variant that has not been corroborated and is not deemed clinically relevant.

Although G6PD deficiency is an X-linked recessive disorder and most often seen in hemizygous male patients, some female patients are affected. In addition, older women who are heterozygous can develop deficiency due to differential X-skewing with age.(4) It is important to note that clinically significant G6PD deficiency can be masked in the setting of significant reticulocytosis, markedly elevated white blood cell count, or recent red blood cell transfusion. If any of these are present in the setting of a history of neonatal, chronic, or episodic jaundice or anemia, genotyping for *G6PD* genetic alterations is recommended. If desired, order G6PDZ / Glucose-6-Phosphate Dehydrogenase (*G6PD*) Full Gene Sequencing, Varies.

Cautions

During hemolytic events normal glucose 6-phosphate dehydrogenase (G6PD) activity values may be measured for several weeks following hemolysis.

Reticulocytosis from any cause can mask some G6PD deficiency cases by raising the activity level. Comparison to other red blood cell enzyme activity levels may be useful.

Recent transfusion may mask the patient's intrinsic enzyme activity and cause unreliable results.

A very high white blood cell count can cause interference and falsely raise the G6PD activity, thereby masking a deficiency, although this may be dependent on the type of white blood cell.

Clinical Reference

1. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371(9606):64-74
2. Glucose-6-phosphate dehydrogenase deficiency. WHO Working Group. Bull World Health Organ. 1989;67(6):601-611
3. Global Malaria Programme, Malaria Policy Advisory Group. Meeting report of the technical consultation to review the classification of glucose-6-phosphate dehydrogenase (G6PD). World Health Organization; 2022. Accessed October 10, 2023. Available at www.who.int/publications/m/item/WHO-UCN-GMP-MPAG-2022.01
4. Au WY, Ma ES, Lam VW, et al. 6-phosphate dehydrogenase (G6PD) deficiency in elderly Chinese women heterozygous for G6PD variants. Am J Med Genet A. 2004;129A(2):208-211
5. ELITEK (rasburicase). Package insert: Sanofi-aventis; Updated December 2019. Accessed October 22, 2020; Available at products.sanofi.us/elitek/Elitek.html
6. Relling MV, McDonagh EM, Chang T, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. Clin Pharmacol Ther. 2014;96(2):169-174
7. Robinson KM, Yang W, Haider CE, et al. Concordance between glucose-6-phosphate dehydrogenase (G6PD) genotype and phenotype and rasburicase use in patients with hematologic malignancies. Pharmacogenomics J. 2019;19(3):305-314. doi:10.1038/s41397-018-0043-3
8. Mohammad S, Clowse MEB, Eudy AM, Criscione-Schreiber LG. Examination of hydroxychloroquine use and hemolytic anemia in G6PDH-deficient patients. Arthritis Care Res (Hoboken). 2018;70(3):481-485. doi:10.1002/acr.23296
9. Mandi G, Witte S, Meissner P, et al. Safety of the combination of chloroquine and methylene blue in healthy adult men with G6PD deficiency from rural Burkina Faso. Trop Med Int Health. 2005;10(1):32-38
10. PLAQUENIL Hydroxychloroquine Sulfate Tablets, USP. Package insert: Concordia Pharmaceuticals Inc; 2015 Updated January 2017. Accessed October 20, 2023. Available at www.accessdata.fda.gov/drugsatfda_docs/label/2017/009768s037s045s047lbl.pdf
11. Minucci A, Moradkhani K, Hwang MJ, et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. Blood Cells Mol Dis. 2012;48(3):154-165
12. Beutler E. Glucose-6-phosphate dehydrogenase deficiency. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ. Hematology. 5th ed. McGraw-Hill Book Company; 1995:564-586
13. Mehta A, Mason PJ, Vulliamy TJ. Glucose-6-phosphate dehydrogenase deficiency. Baillieres Best Pract Res Clin Haematol. 2000;13(1):21-38
14. Koralkova P, van Solinge WW, van Wijk R. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia-pathophysiology, clinical aspects and laboratory diagnosis. Int J Lab Hematol. 2014;36:388-397
15. Luzzatto L, Ally M, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. Blood. 2020;136(11):1225-1240. doi:10.1182/blood.2019000944

Performance

Method Description

Glucose 6-phosphate dehydrogenase in a hemolysate catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconate. Concomitantly, nicotinamide adenine dinucleotide phosphate (NADP[+]) is changed to its reduced form, NADPH, and the reaction is measured spectrophotometrically on an automated chemistry analyzer.(Beutler E. Red cell metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and

Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
1 to 4 days

Specimen Retention Time
7 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

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

CPT Code Information
82955

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
G6PD1	G6PD Enzyme Activity, B	32546-4

Result ID	Test Result Name	Result LOINC® Value
G6PCL	G6PD Enzyme Activity, B	32546-4