

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of hereditary hemolytic anemias, including red blood cell (RBC) membrane/hydration disorders, RBC enzymopathies and congenital dyserythropoietic anemia

Comprehensive testing for patients in whom previous targeted gene variant analyses were negative for a specific hereditary hemolytic anemia

Establishing a diagnosis of a hereditary hemolytic anemia or related disorder, allowing for appropriate management and surveillance of disease features based on the gene involved, especially if splenectomy is a consideration(2)

Identifying variants within genes associated with phenotypic severity, allowing for predictive testing and further genetic counseling

Genetics Test Information

For a list of the genes and exons targeted by this test, see [Targeted Genes Interrogated by NGHHA Next-Generation Sequencing](#) .

Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Targeted Genes Interrogated by NGHHA Next-Generation Sequencing](#)
- [Metabolic Hematology Next-Generation Sequencing \(NGS\) Patient Information](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)
- [NGHHA and Subpanel Comparison Gene List](#)

Method Name

Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Multiple hematology gene panels are available. For more information, see [NGHHA and Subpanel Comparison Gene List](#).

Additional Testing Requirements

This test is best interpreted in the context of protein studies and peripheral blood findings. This can be provided by ordering the HAEV1 / Hemolytic Anemia Evaluation Profile test. Fill out the information sheet and indicate that a next-generation sequencing test was also ordered. Providing complete blood cell count data and clinical notes will also allow more precise interpretation of results.

Shipping Instructions

Peripheral blood specimens must arrive within 30 days of collection.

Necessary Information

1. [Metabolic Hematology Next-Generation Sequencing \(NGS\) Patient Information](#) is required,. Testing may proceed without the patient information, however, the information aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to fill out the form and send with the specimen.
2. If form not provided, include the following information with the test request: clinical diagnosis, pertinent clinical history (ie, complete blood cell count results and relevant clinical notes) and differentials based on clinical or morphologic presentation.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Peripheral blood (preferred)

Container/Tube:

Preferred: Lavender top (EDTA) or yellow top (ACD)

Acceptable: Green top (heparin)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot**

3. Label specimen as blood.

Specimen Stability: Refrigerated < or =30 days

Specimen Type: Extracted DNA

Container/Tube: 1.5- to 2-mL tube

Specimen Volume: Entire specimen

Collection Instructions:

1. Indicate volume and concentration of the DNA.
2. Label specimen as extracted DNA and source of specimen.

Specimen Stability: Frozen/Refrigerated/Ambient < or =30 days

Forms

1. New York Clients-Informed consent is required. Document on the request form or electronic order that a copy is on file.:

[-Informed Consent for Genetic Testing \(T576\)](#)

[-Informed Consent for Genetic Testing-Spanish \(T826\)](#)

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

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Bone marrow biopsies	Reject
Slides	
Paraffin shavings	
Frozen tissues	
Paraffin-embedded tissues	
Paraffin-embedded bone marrow aspirates	

Specimen Minimum Volume

Blood: 1 mL

Extracted DNA: 100 mL at 20 ng/mL concentration

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies (preferred)		

Clinical & Interpretive**Clinical Information**

Next-generation sequencing (NGS) is a methodology that can interrogate large regions of genomic DNA in a single assay. The presence and pattern of gene variants can provide critical diagnostic, prognostic, and therapeutic information for managing physicians.

Hereditary hemolytic anemias are caused by defects in one or more of the genes that control red blood cell (RBC) production, metabolism, or structure, resulting in faulty erythropoiesis, cell membranes, or enzymes required for normal RBC function.

This panel aids in the diagnosis and treatment for hereditary (congenital) hemolytic anemia.(1,2) The panel includes genes known to cause hereditary anemia including those implicated in RBC enzyme,(3) RBC membrane/RBC hydration,(4) and congenital dyserythropoietic anemia(5) disorders. This panel can aid in the differential diagnosis of early onset and lifelong myopathic or neurologic syndromes, especially if associated with hemolysis. Specifically, this panel assays genes associated with hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, Southeast Asian ovalocytosis, hereditary stomatocytosis (both overhydrated and dehydrated/hereditary xerocytosis subtypes), and cryohydrocytosis. Hereditary stomatocytosis is an RBC membrane permeability disorder that can manifest as the more common dehydrated hereditary stomatocytosis, also known as hereditary xerocytosis and the rarer overhydrated hereditary stomatocytosis subtypes. These disorders are important to confirm or exclude as splenectomy has been associated with an increased risk for serious venous thrombosis and thromboembolism events and is contraindicated in published guidelines.(7) It also includes genes associated with RBC enzymopathies, ranging from the common glucose 6-phosphate dehydrogenase and pyruvate kinase deficiencies, to the rarer disorders of adenylate kinase , hexokinase , phosphofructokinase , phosphoglycerate kinase , pyruvate kinase , glutathione pathway, and triosephosphate isomerase .

This panel also includes multiple genes associated with congenital dyserythropoietic anemia (CDA), types 1a, 1b, 2, 3, and 4. CDA is a disorder of ineffective erythropoiesis associated with distinctive bone marrow morphologic changes. A limited number of the most common genes associated with Fanconi anemia (FA) and Diamond-Blackfan anemia (DBA) are also analyzed by this panel; however, this panel is not intended as a thorough investigation of FA or DBA.

Reference Values

An interpretive report will be provided.

Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics recommendations as a guideline.⁽⁶⁾ Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Cautions**Clinical:**

Some individuals may have a variant that is not identified by the methods performed. The absence of a variant, therefore, does not eliminate the possibility of hereditary hemolytic anemia or a related disorder. This assay does not distinguish between germline and somatic alterations, particularly with variant allele frequencies significantly lower than 50%. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If there is a family history of hereditary hemolytic anemia or a related disorder, it is often useful to test first-degree family members to help establish the clinical significance of variants of unknown significance.

At this time, it is not standard practice for the laboratory to systematically review likely disease-associated variants or variants of uncertain significance that have been previously detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

Technical:

Some genetic or genomic alterations, such as large deletion/insertion (delin) events, copy number alterations, and gene translocation events are not detected by this assay. The depth of coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria or for failed regions will be noted. Additionally, rare variants (ie, polymorphisms) may be present that could lead to false-negative or false-positive results. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion and duplication analysis. If the patient has had an allogenic blood transfusion, these results may be inaccurate due to the presence of donor DNA.

Clinical Reference

1. Orkin SH, Nathan DG, Ginsburg D, et al, eds. Nathan and Oski's Hematology of Infancy and Childhood. 7th ed. Saunders Elsevier; 2009: 455-1108
2. Iolascon A, Andolfo I, Barcellini W, et al: Recommendations for splenectomy in hereditary hemolytic anemias. Haematologica. 2017 May 26. PMID: 28550188. doi: 10.3324/haematol.2016.161166.
3. 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 Jun;36(3):388-397
4. King MJ, [Garcon L](#), Hoyer JD, et al: International Council for Standardization in Haematology. ICSH guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol. 2015 Jun;37(3):304-325
5. Gambale A, Iolascon A, Andolfo I, Russo R: Diagnosis and management of congenital dyserythropoietic anemias. Expert Rev Hematol. 2016 Mar;9(3):283-296
6. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-424

Performance**Method Description**

This next-generation sequencing (NGS) assay is performed to test for the presence of a variant in targeted regions of 39 genes. See [Targeted Genes Interrogated by NGHHA Next-Generation Sequencing in Genetics Information](#) for details regarding the targeted gene regions identified by this test. This is a laboratory-developed test.

NGS is performed using an Illumina instrument with paired-end, 151-base pair (bp) reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with the CLC Genomics Server Program. Supplemental or confirmatory Sanger sequencing is performed when necessary. (Unpublished Mayo method)

Genes analyzed: *AK1, ALDOA, ANK1, C15ORF41, CD59, CDAN1, EPB41, EPB42, FANCA, FANCC, FANCG, G6PD, GATA1, GCLC, GPI, GSR, GSS, GYPC, HBB, HBD, HK1, HMOX1, KIF23, KLF1, NT5C3A, PFKM, PGK1, PIEZO1, PKLR, RHAG, RPS19, SEC23B, SLC2A1, SLC4A1, SPTA1, SPTB, STOM, TPI1, and UGT1A1.*

PDF Report

No

Specimen Retention Time

DNA: 3 months

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

81443

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
NGHHA	Hereditary Hemolytic Anemia Seq, V	In Process

Result ID	Reporting Name	LOINC®
NGHHS	Specimen Type	31208-2
NGHHD	Indication for Test	42349-1
40552	Alterations Detected	82939-0
40553	Interpretation	59465-5
40554	Additional Notes	48767-8
40555	Method Summary	85069-3
40556	Disclaimer	62364-5
40558	Panel Gene List	36908-2
40559	Reviewed By	18771-6