Test Definition: NHHA

Hereditary Hemolytic Anemia Gene Panel, Next-Generation Sequencing, Varies

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

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 37 genes associated with hereditary hemolytic anemia: *ABCB6, AHSP, AK1, ALDOA, ANK1, BCL11A, CDIN1 (C15orf41), CD59, CDAN1, EPB41, EPB42, G6PD, GATA1, GCLC, GPI, GSR, GSS, GYPC, HK1, HMOX1, KCNN4, KIF23, KLF1, NT5C3A, PFKM, PGK1, PGLS, PIEZO1, PKLR, RHAG, SEC23B, SLC2A1, SLC4A1, SPTA1, SPTB, TMPRSS6,* and *TPI1*. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for hereditary hemolytic anemia.

Special Instructions

- Informed Consent for Genetic Testing
- Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information
- Informed Consent for Genetic Testing (Spanish)
- Hereditary Hemolytic Anemia Gene Panel and Subpanel Comparison
- Targeted Genes and Methodology Details for Hereditary Hemolytic Anemia Gene Panel
- Hereditary Hemolytic Anemia Evaluation Testing Algorithm

Highlights

This profile evaluates for hereditary (congenital) causes of hemolytic anemia. Symptoms should be long-standing or familial in nature. See Hereditary Hemolytic Anemia Evaluation Testing Algorithm.

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type
Varies

Ordering Guidance
Multiple hematology gene panels are available. For more information see Hereditary Hemolytic Anemia Gene Panel and Subpanel Comparison.

Upon request and after initial testing is complete, WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies may be added to this test. To obtain more information about this option or add WESPR testing, call 800-533-1710.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements
This test is best interpreted in the context of protein studies and peripheral blood findings. Prior to sending this test, Coombs testing should be negative, laboratory testing should indicate a hemolytic process and consider evaluating a peripheral blood smear. In addition, protein analysis for hereditary causes of hemolytic anemia can be provided by ordering HAEV1 / Hemolytic Anemia Evaluation Profile, Blood. Fill out the information sheet and indicate that a next-generation sequencing test was also ordered. Additionally, providing complete blood cell count data and clinical notes will allow a more precise interpretation of results.

Necessary Information
1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information is strongly recommended but not required. Testing may proceed without the patient information; however, it aids in providing a more thorough interpretation. Ordering healthcare professionals are strongly encouraged to complete the form and send it 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 any previous bone marrow studies, clinical or morphologic presentation.

Specimen Required
Specimen Type: Whole blood
Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)

Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days
Additional Information: To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

Forms

1. New York Clients-Informed consent is required. Document on the request form or electronic order that a copy is on file. The following documents are available.
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information (T816)
3. 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

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Next-generation sequencing 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,(4,5) and congenital dyserythropoietic anemia(6) 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 analyzes 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.(2) 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.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(7) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions

(delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(7) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

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 periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

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 regarding splenectomy in hereditary hemolytic anemias. Haematologica. 2017;102(8):1304-1313. 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;36(3):388-397. doi:10.1111/ijlh.12223
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;37(3):304-325. doi:10.1111/ijlh.12335
5. Andolfo I, Russo R, Gambale A, Iolascon A. Hereditary stomatocytosis: an underdiagnosed condition. Am J Hematol. 2018;93(1):107-121. doi:10.1002/ajh.24929
6. Gambale A, Iolascon A, Andolfo I, Russo R. Diagnosis and management of congenital dyserythropoietic anemias. Expert Rev Hematol. 2016;9(3):283-296. doi:10.1586/17474086.2016.1131608
7. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee: 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;17(5):405-424. doi:10.1038/gim.2015.30

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

See Targeted Genes and Methodology Details for Hereditary Hemolytic Anemia Gene Panel for details regarding the targeted genes analyzed for each test and specific gene regions not routinely covered.

Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report

for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

Genes analyzed: *ABCB6, AHSP, AK1, ALDOA, ANK1, BCL11A, CDIN1 (C15orf41), CD59, CDAN1, EPB41, EPB42, G6PD, GATA1, GCLC, GPI, GSR, GSS, GYPC, HK1, HMOX1, KCNN4, KIF23, KLF1, NT5C3A, PFKM, PGK1, PGLS, PIEZO1, PKLR, RHAG, SEC23B, SLC2A1, SLC4A1, SPTA1, SPTB, TMPRSS6*, and *TPI*

PDF Report
No

Day(s) Performed
Varies

Report Available
28 to 42 days

Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months

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
81443

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NHHA	Hereditary Hemolytic Anemia, NGS	107541-5

Result ID	Test Result Name	Result LOINC® Value
619034	Test Description	62364-5

619035	Specimen	31208-2
619036	Source	31208-2
619037	Result Summary	50397-9
619038	Result	82939-0
619039	Interpretation	59465-5
619040	Additional Results	82939-0
619041	Resources	99622-3
619042	Additional Information	48767-8
619043	Method	85069-3
619044	Genes Analyzed	82939-0
619045	Disclaimer	62364-5
619046	Released By	18771-6