Overview

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

Comprehensive testing for patients in whom previous targeted gene mutation 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

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

Genetics Test Information
See Targeted Genes Interrogated by NGHHA Next-Generation Sequencing in Special Instructions for a list of the genes and exons targeted by this test.

Testing Algorithm
See NGHHA and Subpanel Comparison Gene List in Special Instructions.

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
Hereditary Mutation Detection by Next-Generation Sequencing (NGS)

NY State Available
Yes

Specimen

Specimen Type
Varies

Shipping Instructions
Peripheral blood specimens must arrive within 30 days of collection.

Necessary Information
The following information is required on patient information or test request form:

1. Clinical diagnosis
2. Pertinent clinical history (submit CBC results and relevant clinical notes)

3. Differentials based on clinical or morphologic presentation

4. Date of collection

5. Specimen type, whole blood or extracted DNA

**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 specimen in original tube.
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. The following documents are available in Special Instructions:

   - [Informed Consent for Genetic Testing](#) (T576)
Informed Consent for Genetic Testing-Spanish (T826)

2. Metabolic Hematology Next-Generation Sequencing (NGS) Information in Special Instructions.

**Specimen Minimum Volume**

Blood: 1 mL  
Extracted DNA: 100 mcL at 20 ng/mcL concentration

**Reject Due To**

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**Specimen Stability Information**

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**Clinical and 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 mutations can provide critical diagnostic, prognostic, and therapeutic information for managing physicians.

This test is best interpreted in the context of protein studies and peripheral blood findings. This can be provided by ordering the HAEP / Hemolytic Anemia Evaluation Profile test. Please fill out the information sheet and indicate that NGS testing was also ordered. Providing CBC data and clinical notes will also allow more precise interpretation of results.

Hereditary hemolytic anemias are caused by defects in one or more of the genes that control 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 (HS), hereditary elliptocytosis (HE), hereditary pyropoikilocytosis (HPP), Southeast Asian ovalocytosis, hereditary stomatocytosis (both overhydrated and dehydrated/hereditary xerocytosis subtypes), and cryohydropoikilocytosis. Hereditary stomatocytosis is a RBC membrane permeability disorder that can manifest as the more common dehydrated hereditary stomatocytosis (DHSt), also known as hereditary xerocytosis (HX) and the rarer overhydrated hereditary stomatocytosis (OHSt) 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 (G6PD) and pyruvate kinase (PK) deficiencies, to the rarer disorders of adenylate kinase (AK1), hexokinase (HK1), phosphofructokinase (PFKM), phosphoglycerate kinase (PGK1), pyruvate kinase (PKLR), glutathione pathway, and triosephosphate isomerase (TPI1).

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.(6,7) 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 mutation that is not identified by the methods performed. The absence of a mutation, 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 (VAF) 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 pathogenic 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 insertion/deletion (indel) 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 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**


**Performance**

**Method Description**

This next-generation sequencing assay is performed to test for the presence of a mutation in targeted regions of the following 39 genes: AK1, ALDOA, ANK1, C15ORF41, CD59, CDAN1, EPB41, EPB42, FANCA, FANCC, FANCG, G6PD, GATA1, GCLC, GPI, GSR, GSS, GYPB, HBB, HBD, HK1, HMOX1, KIF23, KLF1, NT5C3A, PFKM, PGK1, PIEZO1, PKLR, RHAG, RPS19, SEC23B, SLC2A1, SLC4A1, SPTA1, SPTB, STOM, TPI1, and UGT1A1. See Targeted Genes Interrogated by NGHHA Next-Generation Sequencing in Genetics Information in Special Instructions for details regarding the targeted gene regions identified by this test. This is a laboratory-developed test using Research Use Only reagents.

Next-generation sequencing (NGS) is performed using the Illumina MiSeq 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)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday
Test Definition: NGHHA
Hereditary Hemolytic Anemia Seq, V

Analytic Time
8 weeks

Maximum Laboratory Time
10 weeks

Specimen Retention Time
DNA 3 months

Performing Laboratory Location
Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
81443

LOINC® Information

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