Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of an underlying RBC enzymopathy

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

Genetics Test Information

See Targeted Genes Interrogated by NGENZ Next-Generation Sequencingin 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 NGENZ 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

Shipping Instructions

Peripheral blood specimens must arrive within 30 days of collection.

Necessary Information

1. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information i**s required,** see Special Instructions. 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, CBC 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 or (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. Metabolic Hematology Next-Generation Sequencing (NGS) Patient Information **is required**, see Special Instructions.

2**. 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)

Specimen Minimum Volume

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

Reject Due To

Gross hemolysis	Reject

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

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

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 panel aids in the diagnosis and genetic counseling of individuals with inherited RBC enzymopathies, possible carrier states, or compound mutations with severity modulating interactions. This panel always should be interpreted in the context of protein functional findings by enzymatic assay and complete blood count and peripheral blood findings. This complete interpretation can be provided by also ordering the EEEVP / RBC Enzyme Evaluation. Please fill out the information sheet and indicate that NGS testing was ordered. Providing CBC data and clinical notes will also allow more precise interpretation of results.

Mature erythrocytes are dependent upon glycolysis for energy production and the hexose monophosphate shunt for oxidation-reduction stability. Hereditary deficiencies in RBC enzymes within these pathways cause nonspherocytic hemolytic anemia (NSHA) with variable clinical presentations, therapeutic considerations and inheritance patterns.(1-3) Most of these deficiencies cause chronic hemolysis with little to no pathognomonic morphologic changes in the peripheral blood smear making correlation with enzyme activity critical for diagnosis. Some are associated with acute episodic anemia triggered by medications, food, or viral illness. Variable additional symptoms may be present for some deficiency types, including myopathy, neuropathy, and developmental delay. Because a subset of clinically significant RBC enzyme disorders can have indeterminate to normal enzyme activity (masking in the presence of increased reticulocytes), the proteinÂ (enzymatic activity) studies are more sensitive when performed as a panel of RBC enzymes, which allows comparison of multiple enzyme activities. This genetic panel can aid in the interpretation of equivocal protein findings and genetically confirm an enzyme deficiency. Additionally, there are genes interrogated on this panel for which an enzyme test is not clinically available for correlation.

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.(4,5) 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 positive results. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/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. Nathan and Oski's Hematology of Infancy and Childhood. Edited by SH Orkin, DG Nathan, D Ginsburg, et al. Seventh edition. Philadelphia, Saunders Elsevier, 2009, pp 360-364

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. 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;17:405-424

Performance

Method Description

This next-generation sequencing (NGS) assay is performed to test for the presence of a pathogenic mutation in targeted regions of the following 17 genes: *AK1, ALDOA, G6PD, GCLC, GPI, GSR, GSS, HBB, HBD, HK1, HMOX1, NT5C3A, PFKM, PGK1, PKLR, TPI1*, and *UGT1A1*. See Targeted Genes Interrogated by NGENZ Next-Generation

Sequencing 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.

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

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

Test ID	Test Order Name	Order LOINC Value
NGENZ	RBC Enzyme Sequencing, V	In Process

Result ID	Test Result Name	Result LOINC Value
NGENS	Specimen Type	31208-2
NGEND	Indication for Test	42349-1

Result ID	Test Result Name	Result LOINC Value
40560	Alterations Detected	82939-0
40561	Interpretation	59465-5
40562	Additional Notes	48767-8
40563	Method Summary	49549-9
40564	Disclaimer	62364-5
40566	Panel Gene List	36908-2
40567	Reviewed By	18771-6