Test Definition: NENZ

Red Blood Cell Enzyme Disorders Gene Panel, Next-Generation Sequencing, Varies

Overview

Useful For

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

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 15 genes associated with inherited red blood cell enzymopathies: *AK1, ALDOA, G6PD, GCLC, GPI, GSR, GSS, HK1, HMOX1, NT5C3A, PFKM, PGK1, PGLS, PKLR,* 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 inherited red blood cell enzymopathies.

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 Red Blood Cell Enzyme Disorders Gene Panel

Highlights

This profile evaluates for hereditary (congenital) causes of red blood cell enzymopathies. Symptoms should be long-standing or familial in nature.

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.

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 panel aids in the diagnosis and genetic counseling of individuals with inherited red blood cell enzymopathies, possible carrier states, or compound variants with severity modulating interactions. This test is best interpreted in the context of protein functional findings by enzymatic assay, complete blood cell count, and peripheral blood findings. This complete interpretation can be provided by also ordering the EEEV1 / Red Blood Cell (RBC) Enzyme Evaluation, Blood or HAEV1 / Hemolytic Anemia Evaluation, 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

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.
Specimen Type: Whole blood
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.

Mature erythrocytes are dependent upon glycolysis for energy production and the hexose monophosphate shunt for oxidation-reduction stability. Hereditary deficiencies in red blood cell (RBC) enzymes within these pathways cause nonspherocytic hemolytic anemia 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 protein (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, especially if the patient has been recently transfused with red blood cells. 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

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(5) 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 the 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.

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.

Genes may be added or removed based on updated clinical relevance. 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.(5) 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; 2009:360-364
2. Iolascon A, Andolfo I, Barcellini W, et al. Recommendations for 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
4. Zanella A, Fermo E, Bianchi P, Chiarelli LR, Valentini G. Pyruvate kinase deficiency: the genotype-phenotype association. Blood Rev. 2007;21(4):217-231. doi:10.1016/j.blre.2007.01.001
5. 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

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 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 Red Blood Cell Enzyme Disorders 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: *AK1, ALDOA, G6PD, GCLC, GPI, GSR, GSS, HK1, HMOX1, NT5C3A, PFKM, PGK1, PGLS, PKLR,* and *TPI1*

PDF Report
Supplemental

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
NENZ	RBC Enzyme Sequencing, NGS	107542-3

Result ID	Test Result Name	Result LOINC® Value
619048	Test Description	62364-5
619049	Specimen	31208-2
619050	Source	31208-2
619051	Result Summary	50397-9
619052	Result	82939-0
619053	Interpretation	59465-5
619054	Additional Results	82939-0
619055	Resources	99622-3
619056	Additional Information	48767-8
619057	Method	85069-3
619058	Genes Analyzed	82939-0
619059	Disclaimer	62364-5
619060	Released By	18771-6