
Overview

Useful For

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

Second-tier testing for patients in whom previous targeted gene mutation analyses were negative for a specific RBC membrane disorder

Establishing a diagnosis of a hereditary RBC membrane disorder, allowing for appropriate management and surveillance of disease features based on the gene involved, especially if splenectomy is a consideration (4)

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

Genetics Test Information

See [Targeted Gene Interrogated by NGMEM 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 NGMEM Next-Generation Sequencing](#)
- [Metabolic Hematology Next-Generation Sequencing \(NGS\) Patient Information](#)
- [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](#) is 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 (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. [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, slides, paraffin shavings	Reject
Frozen tissues	OK
Paraffin-embedded tissues	OK
Paraffin-embedded bone marrow aspirates	Reject

Specimen Minimum Volume

Blood: 1 mL

Extracted DNA: 100 mcL at 20 ng/mcL 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 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 also

ordering the RBCME / Red Blood Cell Membrane Evaluation test. 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.

This panel aids in the diagnosis and genetic counseling of individuals with RBC membrane disorders including hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, Southeast Asian ovalocytosis, hereditary stomatocytosis (both overhydrated and dehydrated/hereditary xerocytosis subtypes), and cryohydrocytosis(1-3).

The functional red cell membrane is composed of a cholesterol and phospholipid bilayer anchored by integral proteins to an elastic cytoskeletal network. These interactions form the shape, deformability, and proper ion balance of the cell. Abnormalities in these moieties result in red blood cell membrane disorders. Hereditary spherocytosis (HS) is a common membrane disorder that can be present in all ethnic groups. It is usually associated with visible spherocytes on the peripheral blood smear and can be associated with variable clinical features of hemolysis ranging from mild to severe. Paradoxically, erythrocytosis can occur after splenectomy. Hereditary elliptocytosis (HE) is another fairly common and clinically variable disorder that can range from normal RBC indices in the large majority of cases to a minor subset of patients with moderate to severe anemia. Common hereditary elliptocytosis (CHE) is characterized by the presence of elliptocytes on the peripheral blood smear and the absence of anemia. Mutations associated with HE have been reported in widely variable ethnicities with greater prevalence in populations overlapping the malaria belt. Hereditary pyropoikilocytosis (HPP) is now best classified as a severe form of hereditary elliptocytosis. It is uncommon and presents in early childhood as a severe hemolytic anemia. These disorders are associated with marked poikilocytosis on the peripheral blood smear.(1,2) Hereditary stomatocytosis is an 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.(4) DHSt/HX manifests variably as mild to compensated anemia to some cases with increased hemoglobin levels. Some patients are asymptomatic, others show hemolysis after even nontraumatic exercise sessions. Others display perinatal edema and susceptibility to iron overload. DHSt/HX is associated with pseudohyperkalemia, increased MCHC, and decreased osmotic fragility due to relative dehydration of the red blood cell. OHSt is similarly associated with anemia of variably severity, but is associated with increased osmotic fragility due to a relatively overhydrated steady state.

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.(5,6) 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 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/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. Gallagher PG: Abnormalities of the erythrocyte membrane. *Pediatr Clin North Am* 2013 Dec;60(6):1349-1362
2. Barcellini W, Bianchi P, Fermo E, et al: Hereditary red cell membrane defects: diagnostic and clinical aspects. *Blood Transfus* 2011 Jul;9(3):274-277
3. Zarychanski R, Schulz VP, Houston BL, et al: Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis. *Blood* 2012 Aug 30;120(9):1908-1915

4. 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

5. 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 assay is performed to test for the presence of a mutation in targeted regions of the following 15 genes: *ANK1*, *EPB41*, *EPB42*, *GYPC*, *HBB*, *HBD*, *PIEZO1*, *RHAG*, *SLC2A1*, *SLC4A1*, *SPTA1*, *SPTB*, *STOM*, and *UGT1A1*. See [Targeted Genes Interrogated by NGMEM Next-Generation Sequencing](#) in Genetics Information 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

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

81364

81405

81479

81404

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
NGMEM	RBC Membrane Sequencing, V	In Process

Result ID	Reporting Name	LOINC®
NGMES	Specimen Type	31208-2
NGMED	Indication for Test	42349-1
40568	Alterations Detected	82939-0
40569	Interpretation	59465-5
40570	Additional Notes	48767-8
40571	Method Summary	85069-3
40572	Disclaimer	62364-5
40574	Panel Gene List	36908-2
40575	Reviewed By	18771-6