MAYO CLINIC LABORATORIES

Test Definition: NHEM

Hereditary Erythrocytosis Focused Gene Panel, Next-Generation Sequencing, Varies

Overview

Useful For

Focused evaluation of an individual with *JAK2*-V617F negative erythrocytosis associated with lifelong sustained increased red blood cell (RBC) mass, hemoglobin, or hematocrit

Providing a focused genetic evaluation for patients with a personal or family history suggestive of hereditary erythrocytosis

Establishing a diagnosis of a hereditary erythrocytosis or related disorder, allowing for appropriate management and surveillance of disease features based on the gene involved

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 5 genes associated with hereditary erythrocytosis: *BPGM, EGLN1, EPAS1, EPOR,* and *VHL.* 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 erythrocytosis.

Testing Algorithm

This evaluation is recommended for patients presenting with lifelong elevation in hemoglobin or hematocrit, usually with a positive family history of similar symptoms. Polycythemia vera should be excluded prior to testing as it is much more common than hereditary erythrocytosis and can be present even in young patients. A *JAK2* V617F or *JAK2* exon 12 variant should not be present. More sensitive variant-specific test for *JAK2* V617F is highly recommended prior to ordering this test. Additionally, alpha and beta chain high-oxygen affinity hemoglobin variants should be excluded prior to ordering this test panel.

If skin biopsy (fresh) is received, fibroblast culture will be added at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Informed Consent for Genetic Testing
- Erythrocytosis Patient Information
- Erythrocytosis Evaluation Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)
- Erythrocytosis Genotypic Comparison Chart
- Targeted Genes and Methodology Details for Hereditary Erythrocytosis Focused Gene Panel

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
Polycythemia vera and acquired causes of erythrocytosis should be excluded before ordering this evaluation.

For a complete evaluation including hemoglobin electrophoresis testing and hereditary erythrocytosis variant analysis of the most common gene regions associated with hereditary erythrocytosis in an algorithmic fashion, order REVE2 / Erythrocytosis Evaluation, Blood. See Erythrocytosis Genotyping Comparison Chart for a comparison of erythrocytosis testing options.

The hemoglobin genes, *HBA1*/*HBA2* and *HBB*, are not interrogated in this assay.

Multiple gene panels are available. For more information see Hereditary Erythrocytosis Gene Panel and Subpanel Comparison.

Customization of this panel and single gene analysis for any gene present on this panel are available. For more information see CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies.

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.

Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

Necessary Information
1. **Erythrocytosis Patient Information** **is required.** 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, complete blood cell count results and relevant clinical notes), and differentials based on clinical presentation

and/or laboratory findings.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with whole blood testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.
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

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblast
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
Specimen Stability Information: Ambient (preferred)/Refrigerated (<24 hours)
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Forms

1. **Erythrocytosis Patient Information (T694) is required.**
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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
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.

Erythrocytosis (ie, increased red blood cell [RBC] mass or polycythemia) may be primary, due to an intrinsic defect of bone marrow stem cells (ie, polycythemia vera: PV), or secondary, in response to increased serum erythropoietin (EPO) levels. Secondary erythrocytosis is associated with a number of disorders including chronic lung disease, chronic increase in carbon monoxide (due to smoking), cyanotic heart disease, high-altitude living, renal cysts and tumors, hepatoma, and other EPO-secreting tumors. When these common causes of secondary erythrocytosis are excluded, a heritable cause involving hemoglobin or erythrocyte regulatory mechanisms may be suspected.

Unlike polycythemia vera, hereditary erythrocytosis is not associated with the risk of clonal evolution and should present with isolated erythrocytosis that has been present since birth. A small subset of cases is associated with neoplasia (ie, pheochromocytoma and/or paraganglioma formation). It is caused by variations in several genes and may be inherited in either an autosomal dominant or autosomal recessive manner. A family history of erythrocytosis would be expected in these cases, although it is possible for new variants to arise in an individual.

The genes coding for hemoglobin, beta globin and alpha globin (high-oxygen-affinity hemoglobin variants), hemoglobin-stabilization proteins (2,3 bisphosphoglycerate mutase: *BPGM*), and the erythropoietin receptor (*EPOR*) and oxygen-sensing pathway enzymes (hypoxia-inducible factor [*HIF2A*/*EPAS1*], prolyl hydroxylase domain 2 [*PHD2*/*EGLN1*], and von Hippel Lindau [*VHL*]) can result in hereditary erythrocytosis. High-oxygen-affinity hemoglobin variants and *BPGM* abnormalities result in a decreased p50 result, whereas those affecting *EPOR*, *HIF2A*, *PHD2*, and *VHL* have normal p50 results. The true prevalence of hereditary erythrocytosis-causing variants is unknown. Due to high homology, hemoglobin genes, *HBA1/HBA2* and *HBB*, are not interrogated in this panel.

The oxygen-sensing pathway functions through an enzyme, HIF, which regulates RBC mass. A heterodimer protein comprised of alpha and beta subunits, HIF functions as a marker of depleted oxygen concentration. When present, oxygen becomes a substrate mediating HIF-alpha subunit degradation. In the absence of oxygen, degradation does not take place, and the alpha protein component is available to dimerize with a HIF-beta subunit. The heterodimer then induces transcription of many hypoxia response genes including *EPO*. HIF-alpha is regulated by VHL protein-mediated ubiquitination and proteasomal degradation, which requires prolyl hydroxylation of HIF proline residues. The HIF-alpha

subunit is encoded by the *HIF2A* (*EPAS1*) gene. Enzymes important in the hydroxylation of HIF-alpha are the prolyl hydroxylase domain proteins, of which the most significant isoform is PHD2, which is encoded by the *PHD2* (*EGLN1*) gene. Variations resulting in altered HIF-alpha, PHD2, and VHL proteins can lead to clinical erythrocytosis. A small subset of variants, in *PHD2*/*EGLN1* and *HIF2A*/*EPAS1*, have also been detected in erythrocytic patients presenting with paragangliomas or pheochromocytomas. Truncating variants in the *EPOR* gene coding for the erythropoietin receptor can result in erythrocytosis through loss of the negative regulatory cytoplasmic SHP-1 binding domain leading to EPO hypersensitivity. All currently known variants have been localized to exon 8 and are heterozygous truncating variants. *EPOR* variants are associated with decreased EPO levels and normal p50 values. Gain of function variants in *EPO* have also been associated with hereditary erythrocytosis.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(1) 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 health care providers 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.(1) 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 judgement.

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. 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
2. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. Leukemia. 2009;23(5):834-844. doi:10.1038/leu.2009.54

3. Prchal JF, Prchal JT: Polycythemia Vera. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, Caligiuri M. eds. *Williams Hematology, 9e*. McGraw Hill; 2015
4. Oliveira JL, Coon LM, Frederick LA, et al. Genotype-Phenotype Correlation of Hereditary Erythrocytosis Mutations, a single center experience. Am J Hematol. 2018;10.1002/ajh.25150. doi:10.1002/ajh.25150
5. Zhuang Z, Yang C, Lorenzo F, et al. Somatic HIF2A gain-of-function mutations in paraganglioma with polycythemia. N Engl J Med. 2012;367(10):922-930. doi:10.1056/NEJMoa1205119
6. Tarade D, Robinson CM, Lee JE, Ohh M. HIF-2a-pVHL complex reveals broad genotype-phenotype correlations in HIF-2a-driven disease. Nat Commun. 2018;9(1):3359. doi:10.1038/s41467-018-05554-1
7. Oliveira JL. Algorithmic evaluation of hereditary erythrocytosis: Pathways and caveats. Int J Lab Hematol. 2019;41 Suppl 1:89-94. doi:10.1111/ijlh.13019
8. Gangat N, Oliveira JL, Porter TR, et al. Erythrocytosis associated with EPAS1(HIF2A), EGLN1(PHD2), VHL, EPOR or BPGM mutations: The Mayo Clinic experience. Haematologica. 2022;107(5):1201-1204. doi:10.3324/haematol.2021.280516
9. Camps C, Petousi N, Bento C, et al. Gene panel sequencing improves the diagnostic work-up of patients with idiopathic erythrocytosis and identifies new mutations. Haematologica. 2016;101(11):1306-1318. doi:10.3324/haematol.2016.144063

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 Erythrocytosis Focused 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: *BPGM, EGLN1, EPAS1, EPOR,* and *VHL*

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
81404
81479
81479 (if appropriate for government payers)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NHEM	Erythrocytosis Focused Panel, NGS	103736-5

Result ID	Test Result Name	Result LOINC® Value
618992	Test Description	62364-5
618993	Specimen	31208-2
618994	Source	31208-2
618995	Result Summary	50397-9
618996	Result	82939-0
618997	Interpretation	59465-5
618998	Additional Results	82939-0

618999	Resources	99622-3
619000	Additional Information	48767-8
619001	Method	85069-3
619002	Genes Analyzed	82939-0
619003	Disclaimer	62364-5
619004	Released By	18771-6