MAYO CLINIC LABORATORIES

Test Definition: NHEP

Hereditary Erythrocytosis Gene Panel, Next-Generation Sequencing, Varies

Overview

Useful For

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

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

Comprehensive testing for patients in whom previous targeted gene variant analyses were negative for a specific 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 24 genes associated with hereditary erythrocytosis and polycythemia: *ACO1, ANKRD26, BHLHE41, BPGM, CYB5A, CYB5R3, EGLN1, EGLN2, EGLN3, EPAS1, EPO, EPOR, GFI1B, HIF1A, HIF1AN, HIF3A, JAK2, KDM6A, PFKM, PIEZO1, PKLR, SH2B3, SOCS3,* 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

For skin biopsy or cultured fibroblast specimens, 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 Gene Panel
- Hereditary Erythrocytosis Gene Panel and Subpanel Comparison

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 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 testing 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.

See Erythrocytosis Genotyping Comparison Chart for a comparison of erythrocytosis testing options. If this test is ordered in the setting of erythrocytosis and suspicion of polycythemia vera, interpretation requires correlation with a concurrent or recent bone marrow evaluation.

Recommended Testing for *JAK2* negative lifelong or familial elevated hemoglobin levels:
Tier 1: REVE2 / Erythrocytosis Evaluation, Blood
Tier 2: NHEP / Hereditary Erythrocytosis Gene Panel, Next-Generation Sequencing, Varies

This comprehensive panel (NHEP) assesses 24 genes and includes deletion/duplication analysis. It is recommended for most patients with idiopathic erythrocytosis because hereditary erythrocytosis (HE) can be associated with novel variants with low incidence.

For an evaluation including hemoglobin electrophoresis testing and a Sanger sequencing panel of hereditary erythrocytosis variant analysis of the most common gene regions associated with hereditary erythrocytosis in an algorithmic fashion, order REVE2.

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.

Upon request and after initial testing is complete, WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies may be added to this test. To obtain more information about this option or add WESPR testing, call 800-533-1710.

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.

Necessary Information

1. Erythrocytosis 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 is 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

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. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.
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.

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) < 24 hours/Ambient < 24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are 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) < 24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

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. Erythrocytosis Patient Information (T694)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

Specimen Minimum Volume
Whole blood: 1 mL; Skin biopsy, Cultured fibroblast: See Specimen Required

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, kidney 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 are associated with neoplasia (eg,

pheochromocytoma 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, the hemoglobin genes, *HBA1/HBA2* and *HBB*, are not interrogated in this panel.

The oxygen-sensing pathway functions through an enzyme, hypoxia-inducible factor (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*, has 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.

However, the cause of erythrocytosis remains unknown in greater than 70% of cases after testing genes most associated with hereditary erythrocytosis. Therefore, additional genes associated with erythropoiesis are also interrogated in this assay. These include additional oxygen sensing erythrocytosis pathway genes (*EGLN2, EGLN3, HIF1A, HIF1AN, HIF3A*), some genes associated with myeloid proliferation (*JAK2, SH2B3, SOCS3*), and those associated with other conditions that also variably present with erythrocytosis (*ANKRD26, PFKM, PIEZO1, PKLR*), or methemoglobinemia (*CYB5A, CYB5R3*). In addition, genes identified by whole genome studies (*ACO1*, *GFI1B, KDM6A,* and *BHLHE41*) are included.(1)

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(2) 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 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.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

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 heterologous 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.(2) 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. 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
2. 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(5):405-424
3. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. Leukemia. 2009;23(5):834-844. doi:10.1038/leu.2009.54
4. Prchal JF, Prchal JT. Polycythemia vera. In: Kaushansky K, Lichtman MA, Prchal JT, eds. Williams Hematology. 9th ed. McGraw Hill; 2015
5. Oliveira JL, Coon LM, Frederick LA, et al. Genotype-phenotype correlation of hereditary erythrocytosis mutations, a single center experience. Am J Hematol. Published online May 23, 2018. doi:10.1002/ajh.25150
6. 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
7. Tarade D, Robinson CM, Lee JE, Ohh M. HIF-2 alpha-pVHL complex reveals broad genotype-phenotype correlations in HIF-2 alpha-driven disease. Nat Commun. 2018;9(1):3359. doi:10.1038/s41467-018-05554-1

8. 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
9. 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
10. Iolascon A, Bianchi P, Andolfo I, et al. Recommendations for diagnosis and treatment of methemoglobinemia. Am J Hematol. 2021;96(12):1666-1678. doi:10.1002/ajh.26340

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 content, and repetitive sequences.(Unpublished Mayo method)

See Targeted Genes and Methodology Details for Hereditary Erythrocytosis 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: *ACO1, ANKRD26, BHLHE41, BPGM, CYB5A, CYB5R3, EGLN1, EGLN2, EGLN3, EPAS1, EPO, EPOR, GFI1B, HIF1A, HIF1AN, HIF3A, JAK2, KDM6A, PFKM, PIEZO1, PKLR, SH2B3, SOCS3,* and *VHL*

PDF Report

No

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
81405
81479
81479 (if appropriate for government payers)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NHEP	Erythrocytosis Full Panel, NGS	103735-7

Result ID	Test Result Name	Result LOINC® Value
619020	Test Description	62364-5
619021	Specimen	31208-2
619022	Source	31208-2
619023	Result Summary	50397-9
619024	Result	82939-0
619025	Interpretation	59465-5
619026	Additional Results	82939-0
619027	Resources	99622-3
619028	Additional Information	48767-8
619029	Method	85069-3
619030	Genes Analyzed	82939-0
619031	Disclaimer	62364-5
619032	Released By	18771-6