Overview

Useful For

Definitive evaluation of an individual with *JAK2*-negative erythrocytosis associated with lifelong sustained increased RBC mass, elevated RBC count, hemoglobin, or hematocrit

This test is **not intended for** prenatal diagnosis.

Genetics Test Information

This test is a third-order test and should be ordered when the patient meets the following criteria: diagnosis of erythrocytosis, *JAK2* V617F is negative, and p50 values are normal.

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
MINT	Molecular Interpretation	No	Yes
EPOR	EPOR Gene, Mutation Analysis, B	No	Yes
HIF2A	HIF2A Gene, Mutation Analysis, B	No	Yes
PHD2	PHD2 Gene, Mutation Analysis, B	No	Yes

Additional Tests

Test ID	Reporting Name	Available Separately	Always Performed
BPGMM	BPGM Full Gene Sequencing	Yes	Yes
VHLE	VHL Gene Erythrocytosis Mutations	No, (Order VHLZ)	Yes

Testing Algorithm

This evaluation is recommended for patients presenting with lifelong erythrocytosis, 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. Additionally, p50 testing should be performed and a normal result confirmed before ordering this test.

Additional testing for *BPGM* full gene sequencing and *VHL* gene erythrocytosis variant analysis will always be performed and charged separately when this test is ordered.

See Erythrocytosis Evaluation Testing Algorithm in Special Instructions.

Special Instructions

- Informed Consent for Genetic Testing

- Erythrocytosis Patient Information
- Erythrocytosis Evaluation Testing Algorithm
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR) Amplification/Sanger Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Advisory Information

This is a third-order test for specific variants. For a complete evaluation including p50 testing, hemoglobin electrophoresis testing, and hereditary erythrocytosis variant analysis in an algorithmic fashion, order REVE / Erythrocytosis Evaluation, Whole Blood.

This test does not provide a serum erythropoietin (EPO) level. If EPO testing is desired, see EPO / Erythropoietin (EPO), Serum.

Specimen Required

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions: Send specimen in original tube.

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 in Special Instructions:

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

2. Erythrocytosis Patient Information (T694) in Special Instructions

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

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

Other	Moderately to severely clotted

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Refrigerated	30 days	

Clinical and Interpretive

Clinical Information

Erythrocytosis (ie, increased 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 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: *HIF/EPAS1*, prolyl hydroxylase domain: *PHD2/EGLN1*, and von Hippel Lindau: *VHL*) can result in hereditary erythrocytosis (see Table). High-oxygen-affinity hemoglobin variants and *BPGM* abnormalities result in a decreased p50 result, whereas those affecting *EPOR*, *HIF*, *PHD*, and *VHL* have normal p50 results. The true prevalence of hereditary erythrocytosis-causing variants is unknown.

Genes Associated with Hereditary Erythrocytosis

Gene	Inheritance	Serum EPO	p50
JAK2 V617F	Acquired	Decreased	Normal
JAK2 exon 12	Acquired	Decreased	Normal
EPOR	Dominant	Decreased to normal level	Normal
PHD2/*EGLN1*	Dominant	Normal level	Normal
BPGM	Recessive	Normal level	Decreased
Beta Globin	Dominant	Normal level to increased	Decreased
Alpha Globin	Dominant	Normal level to increased	Decreased
HIF2A/*EPAS1*	Dominant	Normal level to increased	Normal

VHL	Recessive	Markedly Increased	Normal

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, VEGF, and GLUT1*. HIF-alpha is regulated by von Hippel-Lindau (VHL) protein-mediated ubiquitination and proteosomal degradation, which requires prolyl hydroxylation of HIF proline residues. The HIF-alpha subunit is encoded by the *HIF2A (*official name *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* (official name *EGLN1*) gene. Variations resulting in altered HIF-alpha, PHD2, and VHL proteins can lead to clinical erythrocytosis. A small subset of variants, in *PHD2* and *HIF2A*, 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, are mainly missense or small deletion and insertions resulting in stop codons, and are heterozygous. *EPOR* variants are associated with decreased to normal EPO levels and normal p50 values (see Table).

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided and will include specimen information, assay information, and whether the specimen was positive for any variations in the gene. If positive, the variant will be correlated with clinical significance, if known.

Cautions

Polycythemia vera and acquired causes of erythrocytosis should be excluded before ordering this evaluation. The p50 value should be normal.

This test will not detect somatic or gonadal mosaicism.

Certain sequence alterations have no clinical manifestations and, in essence, are clinically benign. Correlation with all relevant clinical information is necessary to provide appropriate patient care.

Clinical Reference

1. Patnaik MM, Tefferi A: The complete evaluation of erythrocytosis: congenital and acquired. Leukemia 2009 May;23(5):834-844

2. McMullin MF: The classification and diagnosis of erythrocytosis. Int J Lab Hematol 2008;30:447-459

3. Percy MJ, Lee FS: Familial erythrocytosis: molecular links to red blood cell control. Haematologica 2008 Jul;93(7):963-967

4. Huang LJ, Shen YM, Bulut GB: Advances in understanding the pathogenesis of primary familial and congenital polycythaemia. Br J Haematol 2010 Mar;148(6):844-852

5. Maran J, Prchal J: Polycythemia and oxygen sensing. Pathologie Biologie 2004;52:280-284

6. Lee F: Genetic causes of erythrocytosis and the oxygen-sensing pathway. Blood Rev 2008;22:321-332

7. Merchant SH, Oliveira JL, Hoyer JD, Viswanatha DS: Erythrocytosis. In Hematopathology. Second edition. Edited by ED His. Philadelphia, Elsevier Saunders, 2012, pp 22-723

8. Zhuang Z, Yang C, Lorenzo F, et al: Somatic *HIF2A* gain-of-function mutations in paraganglioma with polycythemia. N Engl J Med 2012 Sep 6;367(10):922-930

9. Ladroue C, Carcenac R, Leporrier M, et al: *PHD2* mutation and congenital erythrocytosis with paraganglioma. N Engl J Med 2008 Dec 18;359(25):2685-2692

10. Lorenzo FR, Yang C, Ng Tang Fui M, et al: A novel *EPAS1*/*HIF2A* germline mutation in congenital polycythemia with paraganglioma. J Mol Med 2013 Apr;91(4):507-512

Performance

Method Description

DNA is extracted from whole peripheral blood and amplified in 7 separate PCR reactions to cover *EPOR* exon 8, *HIF2A* exons 9 and 12, and *PHD2* exons 1 through 5. PCR products are then sequenced by the Sanger sequencing method and analyzed with sequencing software. Patient sequence results are compared with the genomic reference sequences and the single nucleotide variants known to occur in the genes. If a variant is detected, the messenger RNA reference sequence will be used to determine the amino acid number and resulting amino acid change if there is one.(Percy MJ, McMullin MF, Roques AW, et al: Erythrocytosis due to a mutation in the erythropoietin receptor gene. Br J Haematol 1998;100:407-410; Martini M, Teofili L, Cenci T, et al: A novel heterozygous HIF2a[M535I] mutation reinforces the role of oxygen sensing pathway disturbances in the pathogenesis of familial erythrocytosis. Haematologica 2008;93[7]:1068-1071; Percy MJ, Zhao Q, Flores A, et al: A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. PNAS 2006;103[3]:654-659; Oliveira JL, Coon LM, Frederick LA, et al: Genotype-Phenotype Correlation of Hereditary Erythrocytosis Mutations, a single center experience. Am J Hematol 2018 May 23. doi: 10.1002/ajh.2515)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Analytic Time

10 days

Maximum Laboratory Time

25 days

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

81479-Unlisted molecular pathology procedure

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HEMP	Hereditary Erythrocytosis Mut, B	In Process

Result ID	Test Result Name	Result LOINC Value
34645	EPOR Gene Sequencing Result	82939-0
34646	PHD2 Gene Sequencing Result	82939-0
34647	HIF2A Gene Sequencing Result	82939-0
34648	Molecular Interpretation	69047-9
35000	Reviewed By	18771-6