MAYO CLINIC LABORATORIES

Test Definition: PHD2

Prolyl Hydroxylase Domain-2 (PHD2/EGLN1)
Gene Sequencing, Whole Blood

Overview

Useful For

Assessing *PHD2*/*EGLN1* in the evaluation of an individual with *JAK2*-negative erythrocytosis associated with lifelong sustained increased red blood cell (RBC) mass, elevated RBC count, hemoglobin, or hematocrit

Method Name

Only orderable as part of a profile. For more information see HEMP / Hereditary Erythrocytosis Mutations, Whole Blood.

Polymerase Chain Reaction (PCR)/Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Specimen Required

Only orderable as part of a profile. For more information see HEMP / Hereditary Erythrocytosis Mutations, Whole Blood.

Container/Tube: Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Refrigerated (preferred)	30 days	
	Ambient	14 days	

Clinical & Interpretive

Clinical Information

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 pheochromocytoma or paraganglioma formation. Hereditary erythrocytosis is caused by variants 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 alterations 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). The true prevalence of hereditary erythrocytosis-causing alterations is unknown. The hemoglobin genes, *HBA1/HBA2* and *HBB,* are not assayed in this profile.

Table. **Genes Associated with Hereditary Erythrocytosis**

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

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 (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. Genetic variants 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 to normal EPO levels values (see Table).

Reference Values

Only orderable as part of a profile. For more information see HEMP / Hereditary Erythrocytosis Mutations, Whole Blood.

An interpretive report will be provided.

Interpretation

An interpretive report will be provided as a part of the HEMP / Hereditary Erythrocytosis Mutations, Whole Blood and will include specimen information, assay information, and whether the specimen was positive for any variants 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.

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. doi: 10.1038/leu.2009.54
2. McMullin MF: The classification and diagnosis of erythrocytosis. Int J Lab Hematol. 2008 Dec;30(6):447-459
3. Percy MJ, Lee FS: Familial erythrocytosis: molecular links to red blood cell control. Haematologica. 2008 Jul;93(7):963-967. doi: 10.3324/haematol.13250
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
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7. Merchant SH, Oliveira JL, Hoyer JD, Viswanatha DS: Erythrocytosis. In: His ED, ed. Hematopathology. 2nd ed. Elsevier Saunders; 2012:22-723

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
11. Tarade D, Robinson CM, Lee JE, Ohh M: HIF-2alpha-pVHL complex reveals broad genotype-phenotype correlations in HIF-2aalpha-driven disease. Nat Commun. 2018 Aug 22;9(1):3359
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Performance

Method Description
DNA is extracted from whole blood and amplified in 7 separate polymerase chain reaction (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 Feb;100(2):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. Proc Natl Acad Sci U S A.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.25150)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
10 to 25 days

Specimen Retention Time
Whole blood: 2 weeks; Extracted DNA: 3 months

Performing Laboratory Location
Rochester

Test Definition: PHD2

Prolyl Hydroxylase Domain-2 (PHD2/EGLN1)
Gene Sequencing, Whole Blood

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. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81479

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PHD2	PHD2 Gene, Mutation Analysis, B	In Process

Result ID	Test Result Name	Result LOINC® Value
34646	PHD2 Gene Sequencing Result	82939-0