MAYO CLINIC LABORATORIES

Test Definition: VHLE

VHL Gene, Erythrocytosis, Mutation Analysis, Varies

Overview

Useful For

Diagnosis of suspected *JAK2*-negative VHL-related erythrocytosis associated with lifelong sustained increased RBC mass, elevated RBC count, hemoglobin, or hematocrit

Method Name

Polymerase Chain Reaction (PCR) Followed by DNA Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Specimen Required

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

This test is only available as a reflex from the HEMP / Hereditary Erythrocytosis Mutations. VHLE is not a single orderable test.

Specimen Minimum Volume

Blood: 1 mL

Reject Due To

All specimens will be evaluated by Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Refrigerated		
	Frozen		

Clinical & 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, or secondary, in response to increased serum erythropoietin 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 paraganglioma formation. It is caused by mutations in several genes, including *VHL*, 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 mutations to arise in an individual.

The genes coding for hemoglobin, hemoglobin-stabilization proteins (2,3 bisphosphoglycerate mutase: *BPGM*), the erythropoietin receptor (*EPOR)*, and oxygen-sensing pathway enzymes (hypoxia-inducible factor: *HIF/EPAS1*, prolyl hydroxylase domain: *PHD2/EGLN1*, and *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* typically have normal p50 results. The true prevalence of hereditary erythrocytosis causing mutations 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	Normal to 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 proteasomal degradation, which requires prolyl hydroxylation of HIF proline residues. Mutations resulting in altered VHL proteins can lead to familial erythrocytosis, type 2 (ECYT2; OMIM 263400). ECYT2 is a clinically heterogeneous disorder characterized by congenital erythrocytosis with or without high serum EPO levels, venous and arterial thrombosis, and pulmonary hypertension that can manifest as early as infancy but more typically into adulthood. An increased risk for tumors associated with von

Hippel-Lindau syndrome, which is also caused by mutations in the *VHL* gene, has not been observed.

Reference Values

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

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.(1) 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

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

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

This test is not intended for prenatal diagnosis.

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.

Some individuals who have involvement of the *VHL* gene may have a pathogenic variant that is not identified by the methods performed (eg, promoter variants, deep intronic variants). The absence of a variant, therefore, does not eliminate the possibility of *VHL* disease. For predictive testing of asymptomatic individuals, it is important to first document the presence of a pathogenic gene variant in an affected family member.

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.

In some cases, DNA variants of undetermined significance may be identified. Rarely, sequence variants in primer- or probe-binding sites can result in false-negative test results (DNA sequencing) or either false-positive or false-negative results (multiplex ligation-dependent probe amplification [MLPA]; deletion screening), due to selective allelic drop-out. False-negative or false-positive results can occur in MLPA deletion screening assays due to poor DNA quality. If results obtained do not match the clinical findings, additional testing should be considered.

Unless reported or predicted to cause disease, alterations found deep in the intron or alterations that do not result in an amino acid substitution are not reported. These and common benign variants identified for this patient are available

upon request.

Supportive Data

Accuracy of this assay was assessed by sequencing 25 specimens from patients with clear-cell renal cell carcinoma (cRCC) of which 6 (24%) showed pathogenic variants. These results are in agreement with published estimates of pathogenic variant rates of 29% to 61% for von Hippel-Lindau (*VHL*) in cRCC. Additionally, 2 specimens with known variants were tested. Sequences were 100% concordant with published data. Both inter- and intra-assay testing showed 100% consistency in sequencing. Fifteen normal specimens tested; all showed 100% normal sequences.

Clinical Reference

1. 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;17:405-423
2. Online Mendelian inheritance in Man-OMIM. Available at http://www.omim.org/entry/263400
3. Bento C, Percy M, Gardie B, et al: Genetic basis of congenital erythrocytosis: mutation update and online databases. Hum Mutat 2014;35(1):15-26
4. Pastore Y, Jedlickova K, Guan Y, et al: Mutations of von Hippel-Lindau tumor-suppressor gene and congenital polycythemia. Am J Hum Genet 2003;73(2):412-419
5. Merchant SH, Oliveira JL, Hoyer JD, et al: Molecular Diagnosis. In Hematopathology. Second edition, Series editor John Goldblum. Edited by ED His. Churchill Livingstone. Hematopathology: A Volume in Foundations in Diagnostic Pathology Series. 2012

Performance

Method Description

Bidirectional sequence analysis was performed to test for the presence of sequence variants in the three coding exons and intron/exon boundaries of the VHL gene (GenBank accession number NM_000551; build GRCh37 [hg19]).

PDF Report

No

Day(s) Performed

Varies

Report Available

14 to 20 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-*VHL* (von Hippel-Lindau tumor suppressor) (eg, von Hippel-Lindau familial cancer syndrome), full gene sequence

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
VHLE	VHL Gene Erythrocytosis Mutations	82528-1

Result ID	Test Result Name	Result LOINC® Value
37886	Known Mut Reason for Referral	42349-1
37840	Result Summary	50397-9
37841	Result	82939-0
37842	Interpretation	69047-9
37843	Additional Information	48767-8
37844	Specimen	31208-2
37845	Source	31208-2
37846	Released By	18771-6