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

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
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<td>Varies</td>
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<tr>
<td></td>
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</table>

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, 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Serum Epo</th>
<th>p50</th>
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</thead>
<tbody>
<tr>
<td>JAK2 V617F</td>
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<td>Decreased</td>
<td>Normal</td>
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<tr>
<td>JAK2 exon 12</td>
<td>Acquired</td>
<td>Decreased</td>
<td>Normal</td>
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<tr>
<td>EPOR</td>
<td>Dominant</td>
<td>Decreased to normal level</td>
<td>Normal</td>
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<tr>
<td>PHD2/EGLN1</td>
<td>Dominant</td>
<td>Normal level</td>
<td>Normal</td>
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<tr>
<td>BPGM</td>
<td>Recessive</td>
<td>Normal level</td>
<td>Decreased</td>
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<tr>
<td>Beta Globin</td>
<td>Dominant</td>
<td>Normal level to increased</td>
<td>Decreased</td>
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<tr>
<td>Alpha Globin</td>
<td>Dominant</td>
<td>Normal level to increased</td>
<td>Decreased</td>
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<td>HIF2A/EPAS1</td>
<td>Dominant</td>
<td>Normal level to increased</td>
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<tr>
<td>VHL</td>
<td>Recessive</td>
<td>Normal to increased</td>
<td>Normal</td>
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</table>

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. 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**

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


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) and Time(s) Test Performed

Performed weekly, Varies

Analytic Time

14 days

Maximum Laboratory Time

20 days

Specimen Retention Time

Whole Blood: 2 weeks (if available) Extracted 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.
Test Definition: VHLE
VHL Gene Erythrocytosis Mutations

CPT Code Information
81404- VHL (von Hippel-Lindau tumor suppressor) (eg, von Hippel-Lindau familial cancer syndrome), full gene sequence

LOINC® Information

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