Overview

Useful For
The definitive evaluation of an individual with JAK2-negative erythrocytosis associated with lifelong sustained increased RBC mass, elevated RBC count, hemoglobin, or hematocrit

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

<table>
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<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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<tbody>
<tr>
<td>MINT</td>
<td>Molecular Interpretation</td>
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<td>Yes</td>
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<tr>
<td>EPOR</td>
<td>EPOR Gene, Mutation Analysis, B</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>HIF2A</td>
<td>HIF2A Gene, Mutation Analysis, B</td>
<td>No</td>
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<tr>
<td>PHD2</td>
<td>PHD2 Gene, Mutation Analysis, B</td>
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Reflex Tests

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<tr>
<td>VHLE</td>
<td>VHL Gene Erythrocytosis Mutations</td>
<td>No</td>
<td>No</td>
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<tr>
<td>BPGM</td>
<td>BPGM Full Gene Sequencing</td>
<td>Yes</td>
<td>No</td>
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</tbody>
</table>

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 mutation should not be present. Additionally, p50 testing should be performed and a normal result confirmed before ordering this test. For a complete evaluation including p50 testing, hemoglobin electrophoresis testing, and hereditary erythrocytosis mutation analysis in an algorithmic fashion, order REVE / Erythrocytosis Evaluation. Please note that this test (HEMP) is a reflex test for REVE and should not be ordered if REVE is ordered.

See Erythrocytosis Evaluation Testing Algorithm in Special Instructions.

Special Instructions
- [Informed Consent for Genetic Testing](#)
- [Erythrocytosis Patient Information](#)
- [Erythrocytosis Evaluation Testing Algorithm](#)
Test Definition: HEMP
Hereditary Erythrocytosis Mut, B

- 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 mutations. For a complete evaluation in an algorithmic fashion, order REVE/Erythrocytosis Evaluation.

Specimen Required

Container/Tube: EDTA (lavender)

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

<table>
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<tr>
<th>Reason</th>
<th>Status</th>
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<tbody>
<tr>
<td>Hemolysis</td>
<td>Mild OK; Gross reject</td>
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<tr>
<td>Lipemia</td>
<td>Mild reject; Gross reject</td>
</tr>
<tr>
<td>Icterus</td>
<td>Mild reject; Gross reject</td>
</tr>
<tr>
<td>Other</td>
<td>Moderately to severely clotted</td>
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Specimen Stability Information
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 mutations 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 mutations 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 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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<tbody>
<tr>
<td>JAK2 V617F</td>
<td>Acquired</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>JAK2 exon 12</td>
<td>Acquired</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>EPOR</td>
<td>Dominant</td>
<td>Decreased to normal level</td>
<td>Normal</td>
</tr>
<tr>
<td>PHD2/EGLN1</td>
<td>Dominant</td>
<td>Normal level</td>
<td>Normal</td>
</tr>
<tr>
<td>BPGM</td>
<td>Recessive</td>
<td>Normal level</td>
<td>Decreased</td>
</tr>
<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>
</tr>
<tr>
<td>HIF2A/EPAS1</td>
<td>Dominant</td>
<td>Normal level to increased</td>
<td>Normal</td>
</tr>
<tr>
<td>VHL</td>
<td>Recessive</td>
<td>Markedly Increased</td>
<td>Normal</td>
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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

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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. Mutations resulting in altered HIF-alpha, PHD2, and VHL proteins can lead to clinical erythrocytosis. A small subset of mutations, in PHD2 and HIF2A, has also been detected in erythrocytic patients presenting with paragangliomas or pheochromocytomas.

Truncating mutations 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 mutations have been localized to exon 8, are mainly missense or small deletion and insertions resulting in stop codons, and are heterozygous. EPOR mutations 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 mutations in the gene. If positive, the mutation will be correlated with clinical significance, if known.

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. The p50 value should be normal.

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.

Clinical Reference


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 polymorphisms known to occur in the genes. If a mutation 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)

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
Test Definition: HEMP
Hereditary Erythrocytosis Mut, B

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.

CPT Code Information
81479-Unlisted molecular pathology procedure

LOINC® Information

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