Overview

Useful For
Definitive, comprehensive, and economical evaluation of an individual with JAK2-negative erythrocytosis associated with lifelong sustained increased hemoglobin or hematocrit

Profile Information

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<td>A2F</td>
<td>Hemoglobin A2 and F</td>
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<td>HBEL</td>
<td>Hemoglobin Electrophoresis, B</td>
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<td>P50P</td>
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Reflex Tests

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Testing Algorithm

This is a consultative evaluation in which the case will be evaluated at Mayo Clinic Laboratories, the appropriate tests performed at an additional charge, and the results interpreted.

This profile evaluates for hereditary (congenital) causes of erythrocytosis. Symptoms should be long-standing or familial in nature. All cases will be tested for p50 (if shipping control is received) and hemoglobin variants (cation exchange HPLC, capillary electrophoresis and mass spectrometry) with an interpretative report. Additional testing is guided in a reflexive manner, and may include molecular testing of the HBA1/HBA2, HBB, EPOR, VHL, EGLN1(PHD2), EPAS1(HIF2a), and BPGM genes, among others, as appropriate. See Erythrocytosis Evaluation Testing Algorithm in Special Instructions. An information sheet relaying clinical history, erythropoietin (EPO) levels, and JAK2 results, if known, allows more complete interpretation.

An additional consultative interpretation that summarizes all testing, will be provided after test completion to incorporate subsequent results into an overall evaluation if any of the following molecular tests are reflexed:

- ATHAL / Alpha-Globin Gene Analysis, Varies
- WASQR / Alpha Globin Gene Sequencing, Blood
- WBSQR / Beta-Globin Gene Sequencing, Blood
- WBDDR / Beta-Globin Cluster Locus Deletion/Duplication, Blood
- WGSQR / Gamma-Globin Full Gene Sequencing, Varies
- BPGMM / 2,3-Bisphosphoglycerate Mutase, Full Gene Sequencing Analysis, Varies
- HEMP / Hereditary Erythrocytosis Mutations, Whole Blood
- VHLE / VHL Gene, Erythrocytosis Mutation Analysis

The following algorithms are available in Special Instructions:

- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation
- Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation

See Benign Hematology Evaluation Comparison in Special Instructions.

Special Instructions

- Informed Consent for Genetic Testing
- Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation
- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation
- Erythrocytosis Evaluation Testing Algorithm
- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison
- Informed Consent for Genetic Testing (Spanish)

Method Name
REV: Consultative Interpretation

A2F: Cation Exchange/High-Performance Liquid Chromatography (HPLC)

HBEL: Capillary Electrophoresis

P50P: Hemox-Analyzer Measures and Plots O(2) Saturation

IEF: Electrophoresis

MASS: Mass Spectrometry (MS)

HPFH: Flow Cytometry

UNHB: Isopropanol and Heat Stability

REVB: Consultative Interpretation

NY State Available

Yes

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<th>Specimen Type</th>
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<tr>
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<tr>
<td>WB Sodium Heparin</td>
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<tr>
<td>Whole Blood EDTA</td>
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</table>

**Advisory Information**

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

**Shipping Instructions**

All 3 specimens must arrive within 72 hours of collection.

**Necessary Information**

Include recent transfusion information.

Include most recent CBC results.

**Specimen Required**

A total of 3 specimens are required to perform this profile; all 3 specimens must arrive within 72 hours of collection:

- Whole blood EDTA for A2F, HBEL, MASS

- Whole blood sodium heparin for P50*

- Normal shipping control: whole blood sodium heparin for P50*

*Please note: If sodium heparin patient and control specimens are not received, the P50 test cannot be performed.
Test Definition: REVE
Erythrocytosis Evaluation

Patient:

**Container/Tube:** Lavender top (EDTA) and green top (sodium heparin)

**Specimen Volume:**

EDTA: 5 mL
Sodium heparin: 4 mL

**Collection Instructions:**

1. Immediately refrigerate specimens after collection.
2. Send specimen in original tube. **Do not aliquot.**
3. Rubber band patient specimen and control vial together.

**Normal Shipping Control:**

**Container/Tube:** Green top (sodium heparin)

**Specimen Volume:** 4 mL

**Collection Instructions:**

1. Collect a control specimen from a normal (healthy), unrelated, nonsmoking person at the same time as the patient.
2. Label clearly on outermost label **normal control.**
3. Immediately refrigerate specimen after collection.
4. Send specimen in original tube. **Do not aliquot.**
5. Rubber band patient specimen and control vial together.

**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. [Metabolic Hematology Patient Information](T810) is available in Special Instructions.

**Specimen Minimum Volume**

EDTA blood: 2.5 mL
Sodium heparin blood: 1 mL

**Reject Due To**
Erythrocytosis Evaluation

Specimen Stability Information

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<td>Whole Blood EDTA</td>
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Clinical and Interpretive

Clinical Information

Erythrocytosis (polycythemia) is identified by a sustained increase in hemoglobin or hematocrit. An isolated increase in RBC count (in the absence of chronic phlebotomy or coincident iron deficiency) is not within the definition of erythrocytosis and may occur in thalassemia or other causes. Erythrocytosis may occur as a primary disorder, due to an intrinsic defect of bone marrow stem cells, 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, 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 present. It is important to differentiate polycythemia vera (PV) from heritable causes of erythrocytosis, the latter of which can be passed to progeny but do not carry the risks of clonal evolution associated with PV.

The most common cause of hereditary erythrocytosis is the presence of a high-oxygen-affinity (HOA) hemoglobin. A subset of hemoglobins with increased oxygen (O2) affinity result in clinically evident erythrocytosis caused by decreased O2 unloading at the tissue level. The most common symptoms are headache, dizziness, tinnitus, and memory loss. The affected individuals are plethoric, but not cyanotic. Patients with a HOA hemoglobin may present with an increased hemoglobin concentration, and hematocrit, but normal leukocyte and platelet counts. The p50 values are low. Changes to the amino acid sequence of the hemoglobin molecule may distort the molecular structure, affecting O2 transport and the binding of 2,3-bisphosphoglyceric acid (2,3-BPG). 2,3-BPG is critical to O2 transport of erythrocytes because it regulates the O2 affinity of hemoglobin. A decrease in the 2,3-BPG concentration within erythrocytes results in greater O2 affinity of hemoglobin and reduction in O2 delivery to tissues. A few cases of erythrocytosis have been described as being due to a reduction in 2,3-BPG formation. This is most commonly due to variants in the converting enzyme, bisphosphoglycerate mutase (BPGM). Variants in the genes EPOR, EPAS1(HIF2A), EGLN1(PHD2), and VHL also cause hereditary erythrocytosis and a subset are associated with pheochromocytoma and paragangliomas. The prevalence of these variants is unknown, but they appear less prevalent than variants that cause high-oxygen-affinity hemoglobin variatations, and much less prevalent than polycythemia vera. Because there are many causes of erythrocytosis, an algorithmic and reflexive testing strategy is useful. Initial JAK2 V617F variant testing and serum EPO levels are important with p50 results further stratifying JAK2-negative cases. A significant subset of HOA hemoglobin variatations can be electrophoretically silent; however, most if not all of these can be isolated with addition of the mass spectrometry method. Our extensive experience with these disorders allows an economical, comprehensive evaluation with high sensitivity.

Reference Values

Definitive results and an interpretive report will be provided.

Interpretation
The evaluation includes testing for a hemoglobinopathy and oxygen (O2) affinity of the hemoglobin molecule. An increase in O2 affinity is demonstrated by a shift to the left in the O2 dissociation curve (decreased p50 result). Reflex testing for EPOR, EGLN1 (PHD2), EPAS1 (HIF2a), VHL, and BPGM will be performed as needed.

A hematopathologist expert in these disorders will evaluate the case, appropriate tests are performed, and an interpretive report is issued.

Cautions
The shipping control specimen is required to adequately interpret these cases, as temperature extremes can impact the integrity of the specimen.

Clinical Reference

Performance

Method Description
Hemoglobin A2 and F:

Hemolsate of whole blood is injected into an analysis stream passing through a cartridge containing diethylaminoethyl-resin using high-performance liquid chromatography (HPLC). A preprogrammed gradient controls the elution buffer mixture that also passes through the analytical cartridge. The ionic strength of the elution buffer is raised by increasing the percentage of a second buffer. As the ionic strength of the buffer increases the more strongly retained hemoglobins elute from the cartridge. Absorbance changes are detected by a dual-wavelength filter photometer. Changes in absorbances are displayed as a chromatogram of absorbances versus time. (Huismann TH, Scroeder WA, Brodie AN, et al: Microchromotography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A2. J Lab Clin Med 1975;86:700-702; Szuberski J, Oliveira JL, Hoyer JD. A comprehensive analysis of hemoglobin variants by high-performance liquid chromatography (HPLC). Int J Lab Hematol. 2012 Dec;34(6):594-604; Ou CN, Buffone GJ, Reimer GL, Alpert AJ: High-performance liquid
Test Definition: REVE
Erythrocytosis Evaluation


Hemoglobin Electrophoresis:


Oxygen Dissociation, P50:

The operating principle of the Hemox-Analyzer is based on dual wave-length spectrophotometry for the measurement of the oxygen saturation of hemoglobin (in percent) and a Clark electrode for measuring the oxygen partial pressure in millimeters of mercury. The resulting output signals from both measuring systems are fed into a computer and analyzed.(VanHille DL, Nussenzveig RH, Glezos C, Perkins S, Agarwal AM. Best practices for use of the HEMOX analyzer in the clinical laboratory: quality control determination and choice of anticoagulant. Lab Hematol. 2012 Sep;18(3):17-9; Guarnone R, Centenara E, Barosi G: Performance characteristics of Hemox-Analyzer for assessment of the hemoglobin dissociation curve. Haematologica 1995;80:426-430)

Hemoglobin Variant by Mass Spec, Blood

Mass spectrometry (MS) is performed using a quadrupole-time-of-flight MS and results are analyzed with Waters BioPharmalyx software. Whole blood is diluted 1:50 with purified water and cell debris removed by centrifugation. The supernatant is then diluted 1:10 with running buffer (1:1 water:methanol, 1% formic acid) and analyzed on a Q-TOF MS in MS mode using flow injection and a myoglobin lockmass. A calculated mass for each variant has been integrated into a database containing historic data of multiple method measurements and empiric MS mass peaks were used as a search criterion.(Zanella-Cleon I, Joly P, Becchi M, Francina A: Phenotype determination of hemoglobinopathies by mass spectrometry. Clin Biochem 2009;42[18]:1807-1817)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Saturday

Analytic Time
3-25 days if structural and/or molecular studies are required. (Not reported on Saturday or Sunday)

Maximum Laboratory Time
25 days

Specimen Retention Time
30 days

Performing Laboratory Location
Test Definition: REVE
Erythrocytosis Evaluation

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
Erythrocytosis Evaluation

82820-Hemoglobin O2 affinity (p50)
83020-Hemoglobin electrophoresis
83021-Hemoglobin A2 and F
83789-Hemoglobin Variant by Mass Spectroscopy (MS), Blood
Hemoglobin, Unstable, Blood
83068 (if appropriate)
IEF confirms
82664 (if appropriate)
Hemoglobin F, Red Cell Distribution, Blood
88184 (if appropriate)

LOINC® Information

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