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

Test ID	Reporting Name	Available Separately	Always Performed
REV	Erythrocytosis Interpretation	No	Yes
A2F	Hemoglobin A2 and F	No	Yes
HBEL	Hemoglobin Electrophoresis, B	No	Yes
P50P	Oxygen Dissociation P50	No	Yes
CTRL	P50 Shipping Control Vial	No	Yes
MASS	Hb Variant by Mass Spec, B	No	Yes

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
SDEX	Hemoglobin S, Scrn, B	Yes	No
HEMP	Hereditary Erythrocytosis Mut, B	Yes	No
IEF	IEF Confirms	No	No
UNHB	Unstable Hemoglobin, B	No	No
HPFH	Hemoglobin F, Red Cell Distrib, B	No	No
ATHAL	Alpha-Globin Gene Analysis	Yes	No
WASQR	Alpha Globin Gene Sequencing, B	Yes, (Order WASEQ)	No
WBSQR	Beta Globin Gene Sequencing, B	Yes, (Order WBSEQ)	No
WBDDR	Beta Globin Cluster Locus Del/Dup,B	Yes, (Order WBDD)	No
BPGMM	BPGM Full Gene Sequencing	Yes	No
REVB	Erythrocytosis Summary Interp	No	No
VHLE	VHL Gene Erythrocytosis Mutations	Yes, (Order VHLZ)	No
WGSQR	Gamma Globin Full Gene Sequencing	No	No

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* result s, 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

Specimen

Specimen Type

Control
WB Sodium Heparin
Whole Blood EDTA

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.

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

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Control	Refrigerated	72 hours	GREEN TOP/HEP
WB Sodium Heparin	Refrigerated	72 hours	GREEN TOP/HEP
Whole Blood EDTA	Refrigerated	72 hours	

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 variantations, 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 variantations 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

1. Patnaik MM, Tefferi A: The complete evaluation of erythrocytosis: congenital and acquired. Leukemia 2009 May;23(5):834-844

2. McMullin MF: The classification and diagnosis of erythrocytosis. Int J Lab Hematol 2008;30:447-459

3. Percy MJ, Lee FS: Familial erythrocytosis: molecular links to red blood cell control. Haematologica 2008 Jul;93(7):963-967

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

5. Maran J, Prchal J: Polycythemia and oxygen sensing. Pathol Biol 2004;52:280-284

6. Lee F: Genetic causes of erythrocytosis and the oxygen-sensing pathway. Blood Rev 2008;22:321-332

7. Merchant SH, Oliveira JL, Hoyer JD, Viswanatha DS: Erythrocytosis. In Hematopathology. Second edition. Edited by ED His. Philadelphia, Elsevier Saunders, 2012, pp 722-723

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. 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 PMID: 29790589

Performance

Method Description

Hemoglobin A2 and F:

Hemolysate 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;Â Ou CN, Buffone GJ, Reimer GL, Alpert AJ: High-performance liquid

chromatography of human hemoglobins on a new cation exchanger. J Chromatogr 1983;266:197-205)

Hemoglobin Electrophoresis:

The CAPILLARYS System is an automated system that uses capillary electrophoresis to separate charged molecules by their electrophoretic mobility in an alkaline buffer. Separation occurs according to the electrolyte pH and electro-osmotic flow. A sample dilution with hemolysing solution is injected by aspiration. A high-voltage protein separation occurs and direct detection of the hemoglobin protein fractions is at 415 nm, which is specific to hemoglobins. The resulting electrophoregrams peaks are evaluated for pattern abnormalities and are quantified as a percentage of the total hemoglobin present. Examples of position of commonly found hemoglobin fractions are, from cathode to anode: Hb A2', C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Bart, J, N-Baltimore, and H.(Riou J, Szuberski J, Godart C, Wajcman H, Oliveira JL, Hoyer JD, Bardakdjian-Michau J. Precision of CAPILLARYS 2 for the Detection of Hemoglobin Variants Based on Their Migration Positions. Am J Clin Pathol. 2018 Jan 29;149(2):172-180; Louahabi A, Philippe M, et al: Evaluation of a new Sebia kit for analysis of hemoglobin fractions and variants on the Capillarys system. Clin Chem Lab Med 2006;44[3]:340-345)

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 MSÂ and results are analyzed with Waters BioPharmalynx 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 to 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

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.

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

Test ID	Test Order Name	Order LOINC Value
REVE	Erythrocytosis Evaluation	In Process

Result ID	Test Result Name	Result LOINC Value
2380	Hemoglobin A	20572-4
60286	Hb Variant by Mass Spec, B	No LOINC Needed
2381	Hemoglobin A2	42245-1
174	Erythrocytosis Interpretation	59466-3
37951	P50 Shipping Control Vial	No LOINC Needed

Result ID	Test Result Name	Result LOINC Value
37950	Oxygen Dissociation P50, RBC	65343-6
37948	Reviewed By	59465-5
2382	Hemoglobin F	42246-9
2383	Variant	32017-6
29224	Variant 2	32017-6
29225	Variant 3	32017-6
2101	Interpretation	78748-1