

## Overview

### Useful For

Definitive, comprehensive, and economic 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
REVE1	Erythrocytosis Interpretation	No	Yes
HGBCE	Hb Variant, A2 and F Quantitation, B	Yes	Yes
HPLC	HPLC Hb Variant, B	No	Yes
MASS	Hb Variant by Mass Spec, B	No	Yes

### Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
SDEX	Sickle Solubility, B	Yes	No
HEMP	Hereditary Erythrocytosis Mut, B	Yes	No
IEF	Isoelectric Focusing, B	No	No
UNHB	Hb Stability, B	No	No
HPFH	Hb F Distribution, B	No	No
WASQR	Alpha Globin Gene Sequencing, B	Yes, (Order WASEQ)	No
WBSQR	Beta Globin Gene Sequencing, B	Yes, (Order WBSEQ)	No
WGSQR	Gamma Globin Full Gene Sequencing	Yes, (Order WGSEQ)	No
BPGMM	BPGM Full Gene Sequencing	Yes	No
REVEO	Erythrocytosis Summary Interp	No	No
WAGDR	Alpha Globin Clstr Locus Del/Dup, B	Yes, (Order AGDD)	No
WBGDR	Beta Globin Gene Cluster, Del/Dup, B	Yes, (Order WBGDD)	No
VHLE	VHL Gene Erythrocytosis Mutations	No	No

**Testing Algorithm**

This is a consultative evaluation in which the case will be evaluated at Mayo Clinic Laboratories, the appropriate tests will be 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 hemoglobin variants (cation exchange high performance liquid chromatography, 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. For more information see [Erythrocytosis Evaluation Testing Algorithm](#).

If any of the following molecular tests are performed, an additional consultative interpretation that summarizes all testing will be provided to incorporate subsequent results into an overall evaluation:

- WAGDR / Alpha Globin Cluster Locus Deletion/Duplication, Blood
- WASQR / Alpha -Globin Gene Sequencing, Blood
- WBSQR / Beta-Globin Gene Sequencing, Blood
- WBGDR / Beta-Globin Gene Cluster Deletion/Duplication, Blood
- WGSQR / Gamma-Globin Full Gene Sequencing, Varies

Additional reflex tests are performed if the hemoglobin testing does not explain the patient's phenotype/hereditary erythrocytosis. Each of the following reflex tests contains an individual interpretative report.

- BPGMM / 2,3-Bisphosphoglycerate Mutase, Full Gene Sequencing Analysis, Varies
- HEMP / Hereditary Erythrocytosis Mutations, Whole Blood
- VHLE / *VHL* Gene, Erythrocytosis, Mutation Analysis, Varies

For more information, see:

- [-Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation](#)
- [-Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation](#)
- [-Benign Hematology Evaluation Comparison](#)

**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**

REVE1, REVE0: Medical Interpretation

HGBCE: Capillary Electrophoresis

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

MASS: Mass Spectrometry (MS)

IEF: Isoelectric Focusing

HPFH: Flow Cytometry

UNHB: Isopropanol and Heat Stability

VHLE: Polymerase Chain Reaction (PCR) followed by DNA Sequence Analysis

**NY State Available**

Yes

**Specimen****Specimen Type**

Whole Blood EDTA

**Ordering Guidance**

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

**Necessary Information**

Send the following information with the specimen:

- Recent transfusion information
- Most recent complete blood cell count (CBC) results and serum erythropoietin (EPO) levels, if known

**Metabolic Hematology Patient Information (T810)** is strongly recommended and should include clinical and family history, CBC results, EPO levels, and JAK2 testing results, if known. Testing may proceed without this information; however, it allows for a more complete interpretation.

**Specimen Required****Container/Tube:**

**Preferred:** Lavender top (EDTA)

**Acceptable:** Yellow top (ACD solution B)

**Specimen Volume:** 5 mL

**Collection Instructions:** Send whole blood specimen in original tube. **Do not aliquot.**

**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:

- [Informed Consent for Genetic Testing](#) (T576)
- [Informed Consent for Genetic Testing-Spanish](#) (T826)

2. [Metabolic Hematology Patient Information](#) (T810)

3. If not ordering electronically, complete, print, and send a [Benign Hematology Test Request](#) (T755) with the specimen.

**Specimen Minimum Volume**

2.5 mL

**Reject Due To**

Gross hemolysis	Reject
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**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated	7 days	

**Clinical & Interpretive**
**Clinical Information**

Erythrocytosis (polycythemia) is identified by a sustained increase in hemoglobin or hematocrit. An isolated increase in red blood cell count (in the absence of chronic phlebotomy or coincident iron deficiency) may occur in thalassemia or other causes and does not indicate erythrocytosis. 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, kidney cysts and tumors, hepatoma, and other EPO-secreting tumors. Rare plasma cell dyscrasia-associated syndromes such as POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes) and TEMPI (telangiectasias, elevated EPO and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting) can be associated with increased hemoglobin levels. When these 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 does not carry the risks of clonal evolution or marrow fibrosis associated with PV.

The most common cause of hereditary erythrocytosis is the presence of a high-oxygen-affinity (HOA) hemoglobin variant. A subset of hemoglobins with increased oxygen (O<sub>2</sub>) affinity results in clinically evident erythrocytosis caused by decreased O<sub>2</sub> unloading at the tissue level. Many are asymptomatic; however, some patients have recurrent headaches, dizziness, fatigue, and restless legs. A subset of patients experience thrombotic episodes. Affected individuals can be plethoric, and many are misclassified as polycythemia vera, particularly prior to more recent genetic testing availability. The O<sub>2</sub>-dissociation curve is left-shifted (p50 values are decreased) in HOA variants. Changes to the amino acid sequence of the hemoglobin molecule may distort the protein structure, affecting O<sub>2</sub> transport or unloading and the binding of 2,3-bisphosphoglyceric acid (2,3-BPG). 2,3-BPG stabilizes the deoxygenated state of hemoglobin. Therefore, a decrease in the 2,3-BPG concentration results in greater O<sub>2</sub> affinity of the normal hemoglobin molecule. Rare cases of erythrocytosis have been associated with a reduction in 2,3-BPG formation. This is due to variants in the converting enzyme, bisphosphoglycerate mutase (BPGM). Truncating variants in the erythropoietin receptor gene, *EPOR*, have been shown to be a cause of the autosomal dominant primary familial and congenital polycythemia (OMIM 133100).

In addition, O<sub>2</sub>-sensing pathway variants, *EPAS1(HIF2A)* (OMIM 611783); *EGLN1(PHD2)* (OMIM 609820), and *VHL* (OMIM 263400) cause hereditary erythrocytosis and a subset are associated with pheochromocytoma and paragangliomas. All have shown an autosomal dominant pattern of inheritance, except *VHL*-associated erythrocytosis, which is an autosomal recessive disorder. Homozygous *VHL* R200W alterations have been shown to be causative of Chuvash polycythemia, an endemic heritable erythrocytic disorder first described in Russia but subsequently found in other ethnic groups. The prevalence of causative variants in *EPOR* and the O<sub>2</sub>-sensing pathway genes is unknown; however, in our experience, they are less prevalent than genetic variants that cause HOA hemoglobin variants and are much less prevalent than polycythemia vera. Because there are many causes of erythrocytosis, an algorithmic and

reflexive testing strategy is useful for evaluating these disorders. Initial *JAK2* V617F alteration testing and serum EPO levels are useful. Importantly, a significant subset of HOA hemoglobin variants can be electrophoretically silent on multiple routine screening platforms; however, most, and possibly all, HOA hemoglobin variants can be identified with addition of the intact 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. Reflex testing for *EPOR*, *EGLN1 (PHD2)*, *EPAS1 (HIF2a)*, *VHL*, and *BPGM* will be performed as needed.

A hematopathology expert in these disorders will evaluate the case, have the appropriate tests performed, and issue an interpretive report.

## Cautions

An isolated increase in red blood cell count in the setting of normal hemoglobin levels (in the absence of chronic phlebotomy or coincident iron deficiency) may occur in thalassemia or other causes and is not an indication for a thorough erythrocytosis evaluation.

## Clinical Reference

1. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. *Leukemia*. 2009;23(5):834-844
2. McMullin MF. The classification and diagnosis of erythrocytosis. *Int J Lab Hematol*. 2008;30(6):447-459
3. Percy MJ, Lee FS. Familial erythrocytosis: molecular links to red blood cell control. *Haematologica*. 2008;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;148(6):844-852
5. Maran J, Prchal J. Polycythemia and oxygen sensing. *Pathol Biol*. 2004;52(5):280-284
6. Lee F. Genetic causes of erythrocytosis and the oxygen-sensing pathway. *Blood Rev*. 2008;22(6):321-332
7. Merchant SH, Oliveira JL, Hoyer JD, Viswanatha DS. Erythrocytosis. In: His ED, ed. *Hematopathology*. 2nd ed. Elsevier Saunders; 2012:722-723
8. Zhuang Z, Yang C, Lorenzo F, et al. Somatic *HIF2A* gain-of-function mutations in paraganglioma with polycythemia. *N Engl J Med*. 2012;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. doi:10.1002/ajh.25150
10. Gangat N, Oliveira JL, Hoyer JD, Patnaik MM, Pardanani A, Tefferi A. High-oxygen-affinity hemoglobinopathy-associated erythrocytosis: Clinical outcomes and impact of therapy in 41 cases. *Am J Hematol*. 2021;96(12):1647-1654. doi:10.1002/ajh.26375
11. Gangat N, Oliveira JL, Porter TR, et al. Erythrocytosis associated with *EPAS1(HIF2A)*, *EGLN1(PHD2)*, *VHL*, *EPOR* or *BPGM* mutations: the Mayo Clinic experience. *Haematologica*. 2022;107(5):1201-1204. doi:10.3324/haematol.2021.280516

## Performance

**Method Description****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 a hemolyzing 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 electropherogram 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.(Louahabi A, Philippe M, Lali S, Wallemacq P, Maisin D. 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; instruction manual: CAPI 3 HEMOGLOBIN(E) Phoresis VS  $\geq$ 9.15. Sebia; 12/2020)

**High Performance Liquid Chromatography:**

Hemolysate of whole blood is injected into an analysis stream passing through a cation exchange column using high-performance liquid chromatography. 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 absorbance are displayed as a chromatogram of absorbance versus time.(Huismann TH, Scroeder WA, Brodie AN, Mayson SM, Jakway J. Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A2. J Lab Clin Med. 1975;86:700-702; 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; Szuberski J, Oliveira JL, Hoyer JD. A comprehensive analysis of hemoglobin variants by high-performance liquid chromatography (HPLC). Int J Lab Hematol. 2012;34(6):594-604; instruction manual: Bio-Rad Variant II Beta-thalassemia Short Program Instructions for Use, L70203705. Bio-Rad Laboratories, Inc; 11/2011)

**Mass Spectrometry**

Mass spectrometry (MS) is performed using a quadrupole time-of-flight MS (Q-TOF-MS), and results are analyzed with Agilent MassHunter 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:acetonitrile, 1% formic acid) and analyzed on a Q-TOF MS in MS mode using flow injection. A calculated mass for each variant has been integrated into a database containing historical 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; Helmich F, van Dongen JL, Kuijper PH, Scharnhorst V, Brunsved L, Broeren MA. Rapid phenotype hemoglobin screening by high-resolution mass spectrometry on intact proteins. Clin Chim Acta. 2016;460:220-226. doi:10.1016/j.cca.2016.07.006)

**PDF Report**

No

**Day(s) Performed**

Monday through Saturday

**Report Available**

3 to 25 days

**Specimen Retention Time**

28 days

**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**

83020-26

83020

83021

83789

83068 (if appropriate)

82664 (if appropriate)

88184 (if appropriate)

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
REVE2	Erythrocytosis Evaluation	43113-0

Result ID	Test Result Name	Result LOINC® Value
60286	Hb Variant by Mass Spec, B	No LOINC Needed
41927	Hb A	20572-4
41928	Hb F	32682-7
41929	Hb A2	4552-6
41930	Variant 1	24469-9
41931	Variant 2	24469-9
41932	Variant 3	24469-9
41933	HGBCE Interpretation	78748-1
65615	HPLC Hb Variant, B	No LOINC Needed

608426	Erythrocytosis Interpretation	59466-3
608440	Reviewed By	18771-6