



# Test Definition: HAEV1

Hemolytic Anemia Evaluation, Blood

## Overview

### Useful For

Evaluation of lifelong or inherited hemolytic anemias, including red blood cell membrane disorders, unstable or abnormal hemoglobin variants, and red blood cell enzyme disorders

This evaluation is **not suitable** for acquired causes of hemolysis.

### Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
HAEVI	Hemolytic Anemia Interpretation	No	Yes
HGBCE	Hb Variant, A2 and F Quantitation, B	Yes	Yes
HPLC	HPLC Hb Variant, B	No	Yes
UNHB	Hb Stability, B	No	Yes
FRAGO	Osmotic Fragility	Yes, (Order FRAG)	Yes
SCTRL	Shipping Control Vial	No	Yes
BND3	Band 3 Fluorescence Staining, RBC	No	Yes
G6PDC	G6PD Enzyme Activity, B	Yes, (Order G6PD1)	Yes
PKC	PK Enzyme Activity, B	Yes, (Order PK1)	Yes
GPIC	Glucose Phosphate Isomerase, B	Yes, (Order GPI1)	Yes
HKC	Hexokinase, B	Yes, (Order HK1)	Yes
AKC	Adenylate Kinase, B	Yes, (Order AK1)	Yes
PFKC	Phosphofructokinase, B	Yes, (Order PFK1)	Yes
PGKC	Phosphoglycerate Kinase, B	Yes, (Order PGK1)	Yes
TPIC	Triosephosphate Isomerase, B	Yes, (Order TPI1)	Yes
GSH	Glutathione, B	Yes	Yes
P5NT	Pyrimidine 5' Nucleotidase, B	Yes	Yes
PBSM	Morphology Review	No	Yes

### Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
SDEX	Sickle Solubility, B	Yes	No
IEF	Isoelectric Focusing, B	No	No
MASS	Hb Variant by Mass Spec, 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
HAEV0	Hemolytic Anemia Summary Interp	No	No
WAGDR	Alpha Globin Clustr Locus Del/Dup,B	Yes, (Order AGDD)	No
WBGDR	Beta Globin Gene Cluster, Del/Dup,B	Yes, (Order WBGDD)	No

## Testing Algorithm

This is a consultative evaluation in which the case will be evaluated, the appropriate tests performed at an additional charge, and the results interpreted. If a peripheral blood smear is provided, the morphologic features will be incorporated into the interpretation.

Red blood cell enzymes will always be performed. Capillary electrophoresis, cation exchange high-performance liquid chromatography, and hemoglobin stability studies will always be performed. Reflex testing required to positively identify a hemoglobin abnormality may be added as the case requires. Osmotic fragility (OF) and eosin-5-maleimide binding (band 3) flow cytometry will be performed on all cases. A normal shipping control for OF is necessary to exclude false-positive results due to preanalytic artifact. Testing will be canceled if no shipping control is received or if the shipping control is abnormal.

The protein and molecular test results will be reported separately, which may result in incomplete data until all testing has been finalized.

One or more of the following molecular tests may be performed:

- 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

An additional comprehensive consultative interpretation that summarizes all results will be provided after all tests are completed to incorporate results into an overall evaluation.

For more information see [Hereditary Hemolytic Anemia Evaluation Testing Algorithm](#).

## Special Instructions

- [Informed Consent for Genetic Testing](#)
- [Metabolic Hematology Patient Information](#)
- [Benign Hematology Evaluation Comparison](#)

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- [Informed Consent for Genetic Testing \(Spanish\)](#)
  - [Hereditary Hemolytic Anemia Evaluation Testing Algorithm](#)
  - [Specimen Collection and Labeling Instructions for Osmotic Fragility Testing of Erythrocytes](#)

**Method Name**

HAEVI: Medical Interpretation

HGBCE: Capillary Electrophoresis

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

UNHB: Isopropanol and Heat Stability

FRAGO, SCTRL: Osmotic Lysis

BND3: Flow Cytometry

G6PDC, PKC, GPIC, HKC, AKC, PFKC, PGKC, TPIC, GSH, P5NT: Kinetic Spectrophotometry (KS)

PBSM: Consultant Review

MASS: Mass Spectrometry (MS)

HPFH: Flow Cytometry

IEF: Isoelectric Focusing

**NY State Available**

Yes

**Specimen****Specimen Type**

Whole Blood ACD-B

Control

Whole Blood EDTA

Whole Blood Slide

**Ordering Guidance**

Preliminary screening tests, such as complete blood cell count with peripheral smear and direct Coombs test with a negative result, should be performed before ordering this evaluation.

Cold agglutinin disorders and autoimmune disorders should be excluded prior to testing. This evaluation **is not suitable for** acquired causes of hemolysis.

**Shipping Instructions**

**Specimens must arrive within 72 hours of collection.**

**Necessary Information**

1. At minimum, include recent transfusion information and most recent complete blood cell count results.
2. [Metabolic Hematology Patient Information \(T810\)](#) **is strongly recommended.** Testing may proceed without this information, however if the information requested is received, any pertinent reported clinical features and data will drive the focus of the evaluation and be considered in the interpretation.
3. The laboratory has extensive experience in hemoglobin variant identification and many cases can be confidently

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classified without molecular testing. However, molecular confirmation is always available, subject to sufficient sample quantity (eg, multiplex ligation-dependent probe amplification testing requires at least 2 mL of specimen in addition to protein testing requirements). If no molecular testing or specific molecular tests are desired, utilize the appropriate check boxes on the form. If the form or other communication is not received, the reviewing hematopathologist will select appropriate tests to sufficiently explain the protein findings, which may or may not include molecular testing.

**Specimen Required**

The following specimens are required for testing:

**2 Patient whole blood EDTA specimens**

**2 Patient whole blood ACD specimens**

**1 Shipping control whole blood EDTA specimen** (collected from a healthy individual)

**2 Well-made peripheral blood smears** (Wright stained or fixed in absolute methanol)

For complete instructions, refer to the [Specimen Collection and Labeling Instructions for Osmotic Fragility Testing of Erythrocytes](#).

**Shipping Control Specimen:**

**Specimen Type:** Whole blood (non-patient)

**Container/Tube:** Lavender top (EDTA)

**Specimen Volume:** 4 mL

**Collection Instructions:**

1. Collect a shipping control specimen from a healthy person unrelated to the patient at the same time (or within 4 hours) as the patient specimen.

**Note:** The shipping control specimen can be collected from a phlebotomist, volunteer or another healthy patient.

2. Clearly handwrite "CONTROL" on the outermost label of the tube.

3. Refrigerate (or place on cold gel pack/small amount of wet ice) specimen immediately after collection.

4. Send shipping control in the original tube. **Do not aliquot.**

5. Keep the shipping control and the patient specimens **together**, either rubber banded or in a bag.

**Additional Information:**

1. The shipping control and patient specimens **must be handled identically** from the time of collection through receipt in the testing laboratory.

2. **If the shipping control is not sent with the patient specimen, test cancellation is likely.**

3. The shipping control specimen evaluates whether the patient result has been compromised by handling conditions such as temperature, motion, or other transportation interferences, as temperature and handling extremes can adversely impact the integrity of the specimen.

**Patient Specimen:**

**Specimen Type:** Whole blood

**Container/Tube:** Lavender top (EDTA) and yellow top (ACD)

**Specimen Volume:** Two 4-mL EDTA tubes AND two 6-mL ACD tubes

**Collection Instructions:**

1. Collect and label patient specimens.

2. Refrigerate (or place on cold gel pack/small amount of wet ice) specimen immediately after collection.

3. Send whole blood specimens in the original tubes. **Do not aliquot.**

4. Keep the shipping control and the patient specimens together, either rubber banded or in a bag.

**Additional Information:** The shipping control and patient specimens must be handled identically from the time of collection through receipt in the testing laboratory.

**Patient Specimen:**

**Specimen Type:** Slides

**Container/Tube:** Blood smears

**Specimen Volume:** 2 Well-made peripheral blood smears

**Collection Instructions:**

1. Prepare 2 peripheral blood smears from the EDTA tube collected from the patient.
2. Either stain the smear with Wright stain or fix the smear with absolute methanol prior to shipping.

**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**

EDTA whole blood patient/Shipping control: 3 mL; ACD whole blood patient: 5 mL; Slides: see Specimen Required

**Reject Due To**

Gross hemolysis	Reject
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**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Whole Blood ACD-B	Refrigerated	72 hours	
Control	Refrigerated	72 hours	PURPLE OR PINK TOP/EDTA
Whole Blood EDTA	Refrigerated	72 hours	
Whole Blood Slide	Refrigerated		CARTRIDGE

**Clinical & Interpretive**

**Clinical Information**

Hemolytic anemia (HA) is characterized by increased red blood cell (RBC) destruction and a decreased RBC life span. Patients usually have decreased hemoglobin concentration, hematocrit, and RBC count, but some can have compensated disorders, and symptoms such as reticulocytosis, pigmented gallstones, and decreased haptoglobin are factors that raise clinical suspicion. Blood smear abnormalities may include variable amounts of poikilocytosis including spherocytes, elliptocytes, schistocytes, stomatocytes, echinocytes, polychromasia, basophilic stippling, and target cells. Osmotic fragility can be increased due to the presence of spherocytes. These are all nonspecific features that can be

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present in both hereditary and acquired hemolytic disorders.

Inherited hemolytic disorders may include RBC membrane disorders, RBC enzyme defects, or abnormalities in the hemoglobin molecule in the RBC. This panel assesses for possible causes of congenital/hereditary causes of HA and does not evaluate for acquired causes. Therefore, the anemia should be lifelong or familial in nature. Examples of acquired HA include autoimmune HA (Coombs-positive HA, Coombs-negative autoimmune HA), cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, paroxysmal cold hemoglobinuria, mechanical hemolysis (aortic stenosis or prosthetic heart valves), disseminated intravascular coagulation/thrombotic microangiopathy, and drug-induced HA.

This consultation evaluates for a hereditary cause of increased RBC destruction and includes testing for RBC membrane disorders, such as hereditary spherocytosis and hereditary pyropoikilocytosis, hemoglobinopathies, and red blood cell enzyme abnormalities.

This panel is of limited use in patients with a history of recent transfusion and should be ordered as remote a date from transfusion as possible in those patients who are chronically transfused.

**Reference Values**

Hemoglobin Variant, A2 and F Quantitation

**HEMOGLOBIN A**

0-30 days: 5.9-77.2%

1-2 months: 7.9-92.4%

3-5 months: 54.7-97.1%

6-8 months: 80.0-98.0%

9-12 months: 86.2-98.0%

13-17 months: 88.8-98.0%

18-23 months: 90.4-98.0%

> or =24 months: 95.8-98.0%

**HEMOGLOBIN A2**

0-30 days: 0.0-2.1%

1-2 months: 0.0-2.6%

3-5 months: 1.3-3.1%

> or =6 months: 2.0-3.3%

**HEMOGLOBIN F**

0-30 days: 22.8-92.0%

1-2 months: 7.6-89.8%

3-5 months: 1.6-42.2%

6-8 months: 0.0-16.7%

9-12 months: 0.0-10.5%

13-17 months: 0.0-7.9%

18-23 months: 0.0-6.3%

> or =24 months: 0.0-0.9%

**VARIANT 1**

0.0

VARIANT 2

0.0

VARIANT 3

0.0

HEMOGLOBIN VARIANT, HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Not applicable

Hemoglobin Stability

Normal (reported as normal [stable] or abnormal [unstable])

OSMOTIC FRAGILITY

<12 months: Not established

> or =12 months:

0.50 g/dL NaCl (unincubated): 3-53% hemolysis

0.60 g/dL NaCl (incubated): 14-74% hemolysis

0.65 g/dL NaCl (incubated): 4-40% hemolysis

0.75 g/dL NaCl (incubated): 1-11% hemolysis

NaCl = sodium chloride

BAND 3 FLUORESCENCE STAINING RED BLOOD CELLS (RBC)

<12 months: Not established

> or =12 months: Normal (reported as normal, decreased, or equivocal)

Glucose 6 Phosphate Dehydrogenase Enzyme Activity

<12 months: Not established

> or =12 months of age: 8.0-11.9 U/g Hb

Pyruvate Kinase Enzyme Activity

<12 months: Not established

> or =12 months of age: 5.5-12.4 U/g Hb

Glucose Phosphate Isomerase Enzyme Activity

<12 months: Not established

> or =12 months of age: 40.0-58.0 U/g Hb

Hexokinase Enzyme Activity

<12 months: Not established

> or =12 months: 0.7-1.7 U/g Hb

Adenylate Kinase Enzyme Activity

<12 months: Not established

> or =12 months: 195-276 U/g Hb

Phosphofructokinase Enzyme Activity

<12 months: Not established

> or =12 months of age: 5.8-10.9 U/g Hb

Phosphoglycerate Kinase Enzyme Activity

<12 months: Not established

> or =12 months: 142-232 U/g Hb

Triosephosphate Isomerase Enzyme Activity

<12 months: Not established

> or =12 months of age: 1033-1363 U/g Hb

Glutathione

<12 months: Not established

> or =12 months: 46.9-90.1 mg/dL RBC

Pyrimidine 5' Nucleotidase

Normal

### Interpretation

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

### Cautions

Recent transfusion may cause unreliable results.

A normal shipping control for osmotic fragility (OF) is necessary to exclude false-positive results due to preanalytical artifact. OF and eosin-5-maleimide binding (band 3) testing will be canceled if no shipping control is received or if the shipping control result is abnormal.

This panel is most effectively interpreted in the context of clinical information and the peripheral blood morphology. Fill out the [Metabolic Hematology Patient Information](#) (T810) to maximize the interpretive capabilities of the panel.

This group of tests should not ordinarily be requested in patients who are likely to have immune hemolytic anemia (HA), such as that due to either warm or cold antibodies or to paroxysmal nocturnal hemoglobinurias. Coombs tests, tests for cold agglutinins, sucrose hemolysis, and Hams and Crosby tests are not part of the HA evaluation. In general, the foregoing tests should have been performed and found to be negative prior to requesting an HA evaluation. Since Wilson disease is another rare cause for acute intermittent hemolysis, testing for Wilson disease also may be appropriate prior to requesting an HA evaluation.

### Clinical Reference

1. Steiner LA, Gallagher PG. Erythrocyte disorders in the perinatal period. *Semin Perinatol.* 2007;31(4):254-261
2. Beutler E: Glucose-6-phosphate dehydrogenase deficiency and other enzyme abnormalities. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds. *Hematology.* 5th ed. McGraw-Hill Book Company; 1995:564-581

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6. Gallagher PG. Abnormalities of the erythrocyte membrane. *Pediatr Clin North Am*. 2013;60(6):1349-1362
7. Bianchi P, Fermo E, Vercellati C, et al. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. *Haematologica*. 2012;97(4):516-523
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10. Gallagher PG. Diagnosis and management of rare congenital nonimmune hemolytic disease. *Hematology Am Soc Hematol Educ Program*. 2015;2015:392-399
11. Koralkova P, van Solinge WW, van Wijk R. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia - pathophysiology, clinical aspects, and laboratory diagnosis. *Int J Lab Hematol*. 2014;36(3):388-397

## 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 hemolyzing solution is injected by aspiration. A high-voltage protein separation occurs with direct detection of the hemoglobin protein fractions at 415 nm, which is specific to hemoglobins. The resulting electrophoregram 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: HbA<sub>2</sub>, C, A<sub>2</sub>/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 =9.15. Sebia; 12/2020; Poventud-Fuentes I, Granett E, Vispo B, Elghetany MT, Devaraj S. Hemoglobin fractionation by Sebia Capillarys 2 Flex Piercing System as primary method for evaluation of hemoglobinopathies. *Clin Chim Acta*. 2021;519:193-197)

#### High-Performance Liquid Chromatography Hemoglobin Variant:

Hemolysate of whole blood is injected into an analysis stream passing through 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, Schroeder WA, Brodie AN, Mayson SM, Jakway J. Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A<sub>2</sub>. *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

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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; Sani A, Khan M, Shah S, et al. Diagnosis and screening of abnormal hemoglobins. *Clin Chim Acta.* 2024;552:117685)

**Unstable Hemoglobin:**

Two different hemoglobin stability tests are performed: isopropanol and heat stability.

Unstable hemoglobins will precipitate in dilute solutions of isopropanol. Washed erythrocytes are hemolyzed and cleared by centrifugation. Isopropanol is added. The hemolysate is incubated at 37 degrees C for 20 minutes and examined for turbidity. There is no turbidity with normal hemoglobins. (Fairbanks VF, Klee GG. *Biochemical aspects of hematology.* In: Burtis CA, Ashwood ER, eds. *Tietz Textbook of Clinical Chemistry.* 3rd ed. WB Saunders Company; 1999:1685-1687; Greene DN, Vaughn CP, Crews BO, Agarwal AM. *Advances in the detection of hemoglobinopathies.* *Clinica Chimica Acta.* 2015;439:50-57)

Unstable hemoglobins can also be precipitated by heating to 50 degrees C. Washed erythrocytes are hemolyzed and cleared by centrifugation. The hemolysate is incubated at 50 degrees C for 90 minutes and examined for turbidity. There is no turbidity with normal hemoglobins.

**Osmotic Fragility:**

Specimens for erythrocyte osmotic fragility tests are anticoagulated with EDTA. Osmotic lysis is performed using sodium chloride solution, 0.5 g/dL. An incubated fragility test is performed following 24-hour incubation at 37 degrees C at the following sodium chloride concentrations: 0.60, 0.65, and 0.75 g/dL. Results are reported and interpreted. (Larson CJ, Scheidt R, Fairbanks VF. *The osmotic fragility test for hereditary spherocytosis: use of EDTA-anticoagulated blood stored at 4 degrees C for up to 96 hours.* *Am Soc Clin Pathol Meeting Abstract,* 1988; Larson CJ, Scheidt R, Fairbanks VF. *The osmotic fragility test for hereditary spherocytosis: objective criteria for test interpretation.* *Am Soc Clin Pathol Meeting Abstract,* 1988; King MJ, Zanella A. *Hereditary red cell membrane disorders and laboratory diagnostic testing.* *Int J Lab Hematol.* 2013;35:237-243)

**Band 3/Eosin-5-Maleimide Binding Assay:**

Eosin-5-maleimide (EMA) is a fluorescent dye that binds to Lys-430 of the extracellular loop of the band 3 protein. Using a 1-color flow cytometry method (number of events plotted against fluorescence), the fluorescent intensity of EMA-stained red blood cells is assessed and compared to normal-value patients. (King MJ, Behrens J, Rogers C, Flynn C, Greenwood D, Chambers K. *Rapid flow cytometric test for the diagnosis of membrane cytoskeletal associated hemolytic anemia.* *Br J Haematol.* 2000;111:924-933; King MJ, Zanella A. *Hereditary red cell membrane disorders and laboratory diagnostic testing.* *Int J Lab Hematol.* 2013;35:237-243; Farias MG. *Advances in laboratory diagnosis of hereditary spherocytosis.* *Clin Chem Lab Med.* 2017;55[7]:944-948)

**Glucose-6-phosphate dehydrogenase:**

Glucose-6-phosphate dehydrogenase in a hemolysate catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate. Concomitantly, nicotinamide adenine dinucleotide phosphate (NADP[+]) is changed to its reduced form (NADPH) and the reaction is measured spectrophotometrically on an automated chemistry analyzer. (Beutler E. *Red Cell Metabolism: A Manual of Biochemical Methods.* 3rd ed. Grune and Stratton: 1984:68-71; van Solinge WW, van Wijk. *Enzymes of the red blood cell.* In: Rifai N, Horvath AR, Wittwer CT: eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 6th ed. Elsevier; 2018:chap 30)

**Pyruvate Kinase:**

Pyruvate kinase catalyzes the phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) by converting phosphoenolpyruvate to pyruvate. The amount of pyruvate formed is quantitated by adding lactate dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH) and measuring the rate of decrease in absorbance spectrophotometrically at 340 nm as the NADH is oxidized to NAD(+) on an automated chemistry analyzer. (Beutler E: Red Cell Metabolism. A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Glucose Phosphate Isomerase:**

Glucose phosphate isomerase interconverts glucose-6-phosphate (G6P) and fructose-6-P (F6P). In this assay, the F6P is then further converted to 6-phosphogluconate (6-PG) through the G6PD reaction resulting in the reduction of NADP(+) to NADPH. The reduction of NADP(+) is measured spectrophotometrically by the increase in absorbance at 340 nm on an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:40-42; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Hexokinase:**

Hexokinase catalyzes the reaction of ATP and glucose to G6P and ADP. In this assay the formation of G6P is measured by linking its further oxidation to 6-PG to the reduction of NADP(+) through the G6PD reaction. The increase in absorbance which occurs as NADP(+) is reduced to NADPH is measured spectrophotometrically at 340 nm on an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:38-40; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Adenylate Kinase:**

Adenylate kinase (myokinase) catalyzes the dismutation of ADP into adenosine-5'-monophosphate (AMP) and ATP. In this assay, the reverse reaction is measured by following the formation of ADP with pyruvate kinase (PK) and lactate dehydrogenase reactions resulting in NADH being oxidized to NAD(+). The decrease in absorbance that occurs as NADH is oxidized is measured spectrophotometrically at 340 nm by an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:93-95; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Phosphofructokinase:**

Phosphofructokinase catalyzes the phosphorylation of F6P by ATP to fructose-1,6-diphosphate (FDP). FDP is then converted to dihydroxyacetone phosphate (DHAP) through subsequent aldolase and triosephosphate isomerase (TPI) catalyzed reactions. The rate of formation of DHAP is measured by linking its reduction to alpha-glycerophosphate by alpha-glycerophosphate dehydrogenase, which results in the oxidation of NADH to NAD(+). The decrease in absorbance at 340 nm is measured spectrophotometrically as the NADH is oxidized on an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:68-71; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

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**Phosphoglycerate Kinase:**

Phosphoglycerate kinase catalyzes the phosphorylation of ADP to ATP by conversion of 1,3-diphosphoglycerate (1,3-DPG) to 3-phosphoglyceric acid (3-PGA). In this assay, the reaction is driven in the reverse direction. The formation of 1,3-DPG is then measured through the glyceraldehyde phosphate dehydrogenase reaction as 1,3-DPG is converted to glyceraldehyde-3-phosphate (GAP) resulting in the oxidation of NADH to NAD(+). The decrease in absorbance that occurs as NADH is oxidized is measured spectrophotometrically at 340 nm on an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:53-55; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Triosephosphate Isomerase:**

TPI interconverts GAP and DHAP. The rate of DHAP formation is measured by further converting it to alpha-glycerophosphate by alpha-glycerophosphate dehydrogenase which results in the oxidation of NADH to NAD(+). The oxidation of NADH is measured spectrophotometrically by the decrease in absorbance at 340 nm on an automated chemistry analyzer. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Glutathione:**

Virtually all of the nonprotein sulfhydryl of red cells are in the form of reduced glutathione. 5,5'-Dithiobis (2-nitrobenzoic acid) is a disulfide compound that is readily reduced by sulfhydryl compounds, forming a highly colored yellow anion. The absorbance of this resultant yellow substance is measured by 412 nm and compared to that of a known standard. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods; 3rd ed. Grune and Stratton: 1984; Alisik M, Neselioglu S, Erel O. A colorimetric method to measure oxidized, reduced and total glutathione levels in erythrocytes, J Lab Med. 2019;43[5], 269-277. doi:10.1515/labmed-2019-0098)

**Pyrimidine 5' Nucleotidase:**

Pyrimidine nucleotides have a spectral absorption curve that is markedly different from that exhibited by (normally present) adenine nucleotides, eg, adenosine triphosphate. The former have a peak at about 270 nm; the latter at about 257 nm. Thus, pyrimidine 5' nucleotidase deficiency may be ascertained by demonstrating a very high spectral absorption maximum of 270 nm in erythrocyte extracts. (Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton; 1984:100-102; van Solinge WW, van Wijk. Enzymes of the red blood cell. In: Rifai N, Horvath AR, Wittwer CT: eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:chap 30)

**Morphology Review:**

A hematopathologist who is an expert in these disorders evaluates the slides and an interpretive report is issued.

**Isoelectric Focusing:**

Hemolyzed blood is placed on a polyacrylamide gel containing ampholytes at pH 6 to 8. An electrical current is applied to the gel. When hemoglobin is in this pH gradient, it moves to its isoelectric point, the pH where its net charge is zero. Once this happens, diffusion is counteracted by the electric field and hemoglobin variants are thus separated as bands at their different isoelectric point. (Instruction manual: Resolve Hemoglobin Kit. Perkin Elmer; 04/2019)

**Hemoglobin Variant, Mass Spectrometry:**

Mass spectrometry (MS) is performed using a quadrupole-time-of-flight MS (Q-ToF MS, Agilent) and results are analyzed with Agilent BioConfirm 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)

**Hemoglobin F Distribution:**

This assay uses a flow cytometric method with a monoclonal antibody to hemoglobin (Hb) F. Specimens are analyzed by single-color flow cytometry using fluorescein anti-Hb F. In normal adults, a single peak is seen with minimal fluorescence, which corresponds to Hb A. In neonates, a single peak with bright fluorescence is seen, which corresponds to Hb F. In cases of hereditary persistence of fetal Hb (HPFH) only, a single peak is observed, which has a fluorescence intensity intermediate between the normal Hb A and Hb F peaks. This pattern corresponds to the homocellular (pancellular) pattern obtained by the Kleihauer-Betke (K-B) method. In contrast, specimens from infants, transfused neonates, and cases of beta-thalassemia or delta/beta-thalassemia show both Hb A and Hb F peaks, corresponding to the heterocellular pattern of the K-B method. In patients with Hb S/HPFH, a single peak was observed in contrast to patients with homozygous S in which 2 peaks were observed. (Package insert: Invitrogen Fetal Hemoglobin Test Kit with FITC-conjugated Monoclonal Antibody Directed to HbF. Life Technologies Corporation; MAN 0003641, Rev 3.02, 11/21/2019)

**PDF Report**

No

**Day(s) Performed**

Monday through Friday

**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-Hemolytic Anemia Interpretation  
 82657-Hexokinase, B  
 82955-G6PD Enzyme Activity, B  
 83020-Hemoglobin electrophoresis  
 83021-High-Performance Liquid Chromatography (HPLC)  
 83068-Hemoglobin Stability  
 84087-Glucose phosphate isomerase, B  
 84220-Pyruvate Kinase Enzyme Activity, B  
 82657-Adenylate Kinase, B  
 82657-Phosphofructokinase, B  
 82657-Phosphoglycerate Kinase, B  
 82657-Trisephosphate Isomerase, B  
 85060-Morphology review  
 85557-Osmotic fragility  
 88184-Band 3 Fluorescence Staining, RBC  
 83915-Pyrimidine 5' Nucleotidase  
 82978-Glutathione, B  
 85547 (if appropriate)  
 83789 (if appropriate)  
 82664 (if appropriate)  
 88184 (if appropriate)

### LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HAEV1	Hemolytic Anemia Evaluation	98903-8

Result ID	Test Result Name	Result LOINC® Value
2734	Pyrimidine 5' Nucleotidase, B	2902-5
13082	Morphology Review	59466-3
83141	Band 3 Fluorescence Staining, RBC	98906-1
9095	Hb Stability, B	4639-1
9064	Osmotic Fragility, RBC	34964-7
3306	Osmotic Fragility, 0.50 g/dL NaCl	23915-2
3307	Osmotic Fragility, 0.60 g/dL NaCl	23918-6
3308	Osmotic Fragility, 0.65 g/dL NaCl	23920-2
3309	Osmotic Fragility, 0.75 g/dL NaCl	23921-0
3310	Osmotic Fragility Comment	59466-3
SCTRL	Shipping Control Vial	40431-9
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
AKCL	Adenylate Kinase, B	44051-1
65615	HPLC Hb Variant, B	No LOINC Needed
608409	Glutathione, B	2383-8
608427	Hemolytic Anemia Interpretation	59466-3
608441	Reviewed By	18771-6
TPICL	Triosephosphate Isomerase, B	44054-5
PKCL	PK Enzyme Activity, B	32552-2
PGKCL	Phosphoglycerate Kinase, B	44053-7
PFKCL	Phosphofructokinase, B	72664-6
HKCL	Hexokinase, B	49216-5
GPICL	Glucose Phosphate Isomerase, B	44050-3
G6PCL	G6PD Enzyme Activity, B	32546-4