Overview

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

This evaluation is not suitable for acquired causes of hemolysis.

Profile Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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</thead>
<tbody>
<tr>
<td>HAEV</td>
<td>Hemolytic Anemia Interpretation</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>A2F</td>
<td>Hemoglobin A2 and F</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>HBEL</td>
<td>Hemoglobin Electrophoresis, B</td>
<td>No</td>
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<tr>
<td>UNHB</td>
<td>Unstable Hemoglobin, B</td>
<td>No</td>
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<tr>
<td>FRAGO</td>
<td>Osmotic Fragility</td>
<td>Yes, (Order FRAG)</td>
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<tr>
<td>SCTRL</td>
<td>Shipping Control Vial</td>
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<tr>
<td>G6PD</td>
<td>G-6-PD, QN, RBC</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>PK</td>
<td>Pyruvate Kinase, RBC</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>GPI</td>
<td>Glucose Phosphate Isomerase, B</td>
<td>Yes</td>
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<tr>
<td>HEXK</td>
<td>Hexokinase, B</td>
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<td>Yes</td>
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<tr>
<td>PBSM</td>
<td>Morphology Review</td>
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Reflex Tests

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<td>SDEX</td>
<td>Hemoglobin S, Scrn, B</td>
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<td>IEF</td>
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<td>MASS</td>
<td>Hb Variant by Mass Spec, B</td>
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<td>RBCE</td>
<td>Reflexed RBC Enzymes</td>
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<tr>
<td>HPFH</td>
<td>Hemoglobin F, Red Cell Distrib, B</td>
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<td>ATHAL</td>
<td>Alpha-Globin Gene Analysis</td>
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<td>WASQR</td>
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<tr>
<td>WBSQR</td>
<td>Beta Globin Gene Sequencing, B</td>
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### Test Definition: HAEVP

**Hemolytic Anemia Evaluation**

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<td>WGSQR</td>
<td>Gamma Globin Full Gene Sequencing</td>
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<td>HAEVA</td>
<td>Hemolytic Anemia Summary Interp</td>
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### Additional Tests

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<tbody>
<tr>
<td>BND3</td>
<td>Band 3 Fluorescence Staining, RBC</td>
<td>No</td>
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</table>

### Testing Algorithm

This is a consultative evaluation in which the case will be evaluated and 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. If a Hemolytic Anemia Patient Information sheet (T705) is received with the sample, the reported clinical features or clinical impression will be incorporated into the interpretation. The most common RBC enzymes (G6PD, pyruvate kinase, glucose phosphate isomerase, and hexokinase) will always be performed. If these are normal, the second-tier enzymes will be performed (provided sufficient sample volume is available). If second-tier enzymes are desired, even if the first-tier testing is abnormal, fill out the Hemolytic Anemia Patient Information sheet (T705) and indicate this desire. Cation exchange HPLC, capillary electrophoresis, and hemoglobin stability studies will always be performed. Reflex testing required to identify a hemoglobin abnormality can be added as the case requires. Osmotic fragility and eosin-5-maleimide (EMA) binding (band 3) flow cytometry will be performed on all cases. A normal shipping control for osmotic fragility (OF) is necessary to exclude false-positive results due to preanalytical artifact.

OF and EMA binding testing will be canceled if no shipping control is received or if the shipping control is abnormal.

HAEVA / Hemolytic Anemia Summary Interpretation, an additional consultative interpretation that summarizes all testing, will be provided after test completion to incorporate subsequent results into an overall evaluation if 1 or more of the following molecular tests are reflexed on the Hemolytic Anemia Evaluation:

- ATHAL / Alpha-Globin Gene Analysis
- 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

**Note:** RBCE / Reflexed RBC Enzymes, Blood (second-tier enzymes) includes: adenylate kinase,
phosphofructokinase, phosphoglycerate kinase, triosephosphate isomerase, and pyrimidine 5’ nucleotidase.

See Benign Hematology Evaluation Comparison in Special Instructions.

**Special Instructions**
- Informed Consent for Genetic Testing
- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison
- Informed Consent for Genetic Testing (Spanish)

**Method Name**
- HAEV: Consultative Interpretation
- A2F: Cation Exchange/High-Performance Liquid Chromatography (HPLC)
- HBEL: Capillary Electrophoresis
- UNHB: Isopropanol and Heat Stability
- FRAGO: Osmotic Lysis
- G6PD, PK, GPI, HEXK, RBCE, GLTI: Kinetic Spectrophotometry (KS)
- PBSM: Consultant Review
- BND3 (eosin-5-maleimide (EMA) binding): Flow Cytometry
- MASS: Mass Spectrometry (MS)
- IEF: Electrophoresis
- HPFH: Flow Cytometry
- HAEVA: Consultative Interpretation

**NY State Available**
- Yes

**Specimen**

**Specimen Type**
- Control
- Whole Blood ACD-B
- Whole Blood EDTA
- Whole Blood Slide

**Advisory Information**

Preliminary screening tests, such as complete blood count with peripheral smear and direct Coombs test with a negative result, should be run 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 draw.

**Necessary Information**
Include recent transfusion information.
Include most recent CBC results.

**Specimen Required**
Two whole blood EDTA specimens, 2 whole blood ACD specimens, an EDTA control specimen, and 2 well-made peripheral blood smears (Wright stained or fixed in absolute methanol) are required for testing.

**Patient:**

**Specimen Type:** Blood

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

**Specimen Volume:**

**EDTA:** Two 4-mL vials

**ACD:** Two 6-mL vials

**Collection Instructions:**
1. Immediately refrigerate specimens after draw.
2. Send specimens in original tubes. **Do not aliquot.**
3. Prepare smears.
4. Rubber band patient specimen and control vial together.

**Patient:**

**Specimen Type:** Slides

**Container/Tube:** Blood smears

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

**Collection Instructions:** Collect 2 well-made peripheral blood smears (Wright stained or fixed in absolute methanol).

**Shipping Normal Control:**

**Specimen Type:** Whole blood

**Container/Tube:** Lavender top (EDTA)
**Test Definition: HAEVP**

**Hemolytic Anemia Evaluation**

**Specimen Volume:** 4 mL

**Collection Instructions:**

1. Draw 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 draw.

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) in Special Instructions. Please fill out for a more complete evaluation by the signing Hematopathologist.

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

**Specimen Minimum Volume**

- EDTA Blood: 3 mL
- ACD Blood: 5 mL

**Reject Due To**

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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**Specimen Stability Information**

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<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>Control</td>
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<td>72 hours</td>
<td>PURPLE OR PINK TOP/EDTA</td>
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<tr>
<td>Whole Blood ACD-B</td>
<td>Refrigerated</td>
<td>72 hours</td>
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<tr>
<td>Whole Blood EDTA</td>
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<td>72 hours</td>
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<tr>
<td>Whole Blood Slide</td>
<td>Refrigerated</td>
<td></td>
<td>CARTRIDGE</td>
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**Clinical and Interpretive**
Clinical Information

Hemolytic anemia (HA) is characterized by increased red cell destruction and a decreased red cell life span. Patients usually have decreased hemoglobin concentration, hematocrit, and red blood cell 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 spherocytes, schistocytes, stomocytes, 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 present in both hereditary and acquired hemolytic disorders.

Inherited hemolytic disorders may include red cell membrane disorders, red cell enzyme defects, or abnormalities in the hemoglobin molecule in the red cell. This panel assesses for possible causes of congenital/hereditary causes of hemolytic anemia and does not evaluate for acquired causes. Therefore, the anemia should be lifelong or familial in nature. Examples of acquired HA (which should be excluded prior to ordering this panel) include: autoimmune HA (direct 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 red cell destruction and includes testing for red cell membrane disorders, such as hereditary spherocytosis and hereditary pyropoikilocytosis, hemoglobinopathies, and red 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

Definitive results and an interpretive report will be provided.

Interpretation

An interpretive report will be provided.

Cautions

A normal shipping control for osmotic fragility (OF) is necessary to exclude false-positive results due to preanalytical artifact. OF and eosin-5-maleimide (EMA) binding testing will be canceled if no shipping control is received or if the shipping control 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 sheet (T705) available in Special Instructions 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


2. Beutler E: Glucose-6-phosphate dehydrogenase deficiency and other enzyme abnormalities. In Hematology. Fifth
Test Definition: HAEVP
Hemolytic Anemia Evaluation

Performance
Method Description
Hemolytic Anemia Evaluation:

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

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, Schroeder WA, Brodie AN, et al: 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)

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 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

Unstable Hemoglobin:

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


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 (NaCl) solution, 0.50 g/dL. An incubated fragility test is performed following 24-hour incubation at 37 degrees C at the following NaCl 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)

Glucose-6-Phosphate Dehydrogenase (G6PD):


Band 3/Eosin-5-maleimide (EMA) 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 RBC, is assessed and compared to normal-value patients.(King MJ, Behrens J, Rogers C, et al: Rapid flow cytometric test for the diagnosis of membrane cytoskeletal associated hemolytic anemia. Br J Haematol 2000;111:924-933)

Pyruvate Kinase:

A red cell hemolysate is incubated with adenosine diphosphate and phosphoenolpyruvate. The amount of pyruvate formed is quantitated by adding lactic dehydrogenase and reduced nicotinamide adenine di-nucleotide and measuring the rate of decrease in absorbance at 340 nm.(Beutler E: Red Cell Metabolism: A Manual of Biochemical Methods. Third edition. New York, Grune and Stratton, 1984, pp 68-71)

Glucose Phosphate Isomerase:
Test Definition: HAEVP
Hemolytic Anemia Evaluation

Washed erythrocytes are hemolyzed and the hemolysate is mixed with glucose, adenosine triphosphate (ATP), glucose-6-phosphate dehydrogenase, and nicotinamide adenine dinucleotide phosphate (NADP). The reduction of NADP is measured spectrophotometrically and is proportional to the enzymatic conversion of ATP and glucose to glucose-6-phosphate. (Beutler E: Red Cell Metabolism: A Manual of Biochemical Methods. Third edition. New York, Grune and Stratton, 1984, pp 40-42)

Hexokinase:

Hexokinase (in the presence of magnesium) catalyzes the reaction of ATP and glucose to G-6-P and ADP. In this assay the formation of glucose-6-phosphate (G-6-P) is measured by linking its further oxidation to 6-phosphogluconate (6-PG) to the reduction of NADP through the glucose-6-phosphate dehydrogenase (G-6-PD) reaction. The increase in absorbance which occurs as NADP+ is reduced is measured at 340 nm. (Beutler E: Red cell metabolism: A Manual of Biochemical Methods. Third edition. Grune and Stratton, New York, 1984, pp 38-40)

Morphology Review:

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

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 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

See Individual Test IDs

CPT Code Information

Hemolytic Anemia Evaluation

82657-Hexokinase, B
Test Definition: HAEVP
Hemolytic Anemia Evaluation

82955-G-6-PD
83020-Hemoglobin electrophoresis
83021-Hemoglobin A2 and F
83068-Hemoglobin stability
84087-Glucose phosphate isomerase
84220-Pyruvate kinase
85060-Morphology review
85557-Osmotic fragility
Band 3 Fluorescence Staining, RBC
88184
Reflexed RBC Enzymes
83915 (if appropriate)
Glutathione, Blood
82978 (if appropriate)
Hemoglobin Variant by Mass Spectrometry (MS), Blood
83789 (if appropriate)
IEF Confirms
82664 (if appropriate)
Hemoglobin F, Red Cell Distribution, Blood
88184 (if appropriate)

**LOINC® Information**

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9064        | Osmotic Fragility, RBC              | 34964-7            |
G6PD_       | G-6-PD, QN, RBC                     | 32546-4            |
GPI_        | Glucose Phosphate Isomerase, B      | 44050-3            |
2380        | Hemoglobin A                        | 20572-4            |
13082       | Morphology Review                   | 59466-3            |
2381        | Hemoglobin A2                       | 42245-1            |
SCTRL       | Shipping Control Vial               | 40431-9            |
9992        | Hemolytic Anemia Interpretation     | 59466-3            |
37437       | Reviewed By                         | 19139-5            |
2382        | Hemoglobin F                        | 42246-9            |
2383        | Variant                             | 32017-6            |
3306        | Osmotic Fragility, 0.50 g/dL NaCl   | 23915-2            |
3307        | Osmotic Fragility, 0.60 g/dL NaCl   | 23918-6            |
29224       | Variant 2                           | 32017-6            |
29225       | Variant 3                           | 32017-6            |
3308        | Osmotic Fragility, 0.65 g/dL NaCl   | 23920-2            |
3309        | Osmotic Fragility, 0.75 g/dL NaCl   | 23921-0            |
2101        | Interpretation                      | 78748-1            |
3310        | Osmotic Fragility Comment           | 59466-3            |