Test Definition: HBELC

HGB Electrophoresis Cascade

Overview

Useful For

Diagnosis and comprehensive classification of thalassemias and hemoglobin variants

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
A2F	Hemoglobin A2 and F	No	Yes
HBEL	Hemoglobin Electrophoresis, B	No	Yes

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
SDEX	Hemoglobin S, Scrn, B	Yes	No
IEF	IEF Confirms	No	No
MASS	Hb Variant by Mass Spec, B	No	No
UNHB	Unstable Hemoglobin, 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
HBELA	HGB Electrophoresis Summary Interp	No	No
HPFH	Hemoglobin F, Red Cell Distrib, B	No	No
WGSQR	Gamma Globin Full Gene Sequencing	No	No

Testing Algorithm

Hemoglobin electrophoresis cascade will always include hemoglobin A(2) and F and hemoglobin electrophoresis utilizing cation exchange HPLC and capillary electrophoresis methods.

Hemoglobin electrophoresis reflex testing, performed at additional charge, may include any or all of the following to identify rare hemoglobin variants present: sickle solubility (hemoglobin S screen), hemoglobin heat and isopropanol stability studies (unstable hemoglobin), isoelectric focusing, intact globin chain mass spectrometry (hemoglobin variant by mass spectrometry), Hb F distribution by flow cytometry (hemoglobin F red cell distribution), DNA (Sanger)

testing for beta chain variants and the most common beta thalassemias (beta-globin gene sequencing), multiplex ligation-dependent probe amplification (MLPA) testing for beta cluster locus large deletions and duplications, including large deletional hereditary persistence of fetal hemoglobin (HPFH), delta-beta (DBT), delta thalassemias, gamma-delta-beta (GDBT), and epsilon-gamma-delta-beta (EGDBT) thalassemias (beta globin cluster locus del/dup), large deletional alpha thalassemias and alpha gene duplications (alpha-globin gene analysis), alpha chain variants and non-deletional alpha thalassemias (alpha-globin gene sequencing), and gamma chain variants and non-deletional HPFH (gamma globin full gene sequencing).

If a Thalassemia/Hemoglobinopathy Patient Information sheet (T358) is received with the sample, the reported clinical features or clinical impression will be considered in the interpretation and focus of the evaluation. Our laboratory has extensive experience in hemoglobin variant identification and many cases can be confidently classified without molecular testing. However, molecular confirmation is always available. If no molecular testing or, conversely, specific molecular tests are desired, utilize the appropriate check boxes on the information sheet. If the information sheet or other communication is not received, the reviewing hematopathologist will select appropriate tests to sufficiently explain the clinical impression or reported CBC results, which may or may not include molecular testing.

Hemoglobin (HGB) Electrophoresis 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 HBELC / Hemoglobin Electrophoresis Cascade, Blood:

-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

See Benign Hematology Evaluation Comparison in Special Instructions.

Special Instructions

- Thalassemia/Hemoglobinopathy Patient Information
- Informed Consent for Genetic Testing
- Metabolic Hematology Patient Information
- Benign Hematology Evaluation Comparison
- Informed Consent for Genetic Testing (Spanish)

Method Name

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

HBEL: Capillary Electrophoresis

IEF: Isoelectric Focusing

MASS: Mass Spectrometry (MS)

HPFH: Flow Cytometry

UNHB: Isopropanol and Heat Stability

HBELA: Consultative Interpretation

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Advisory Information

Alpha-thalassemias with only 1 or 2 alpha-globin gene deletions are not recognized by this testing protocol. ATHAL / Alpha-Globin Gene Analysis is required to identify 1 or 2 globin gene deletions.

Necessary Information

Include recent transfusion information.

Include most recent CBC results.

Specimen Required

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: ACD (solution B), green top (sodium heparin)

Specimen Volume: 10 mL

Collection Instructions: Send 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 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

3. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-General Request (T239)

-Benign Hematology Test Request (T755)

Specimen Minimum Volume

1 mL

Reject Due To

All specimens will be evaluated by Mayo Clinic Laboratories for test suitability

Specimen Stability Information

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

Clinical and Interpretive

Clinical Information

Hemoglobin abnormalities not uncommonly occur as compound disorders (2 or more mutations) that can have complex interactions and variable phenotypes. Although powerful as an adjunct for a complete and accurate diagnosis, genetic methods alone can give incomplete and possibly misleading information due to limitations of the methods. Interpretation of genetic data requires the incorporation of protein analysis results. This profile is well-suited for the classification of hemoglobin disorders.

A large number (>1,000) of variants of hemoglobin (Hb) have been recognized. They are identified by capital letters (eg, Hb A or Hb S), or by the city in which the variant was first discovered (eg, Hb Koln). Clinical symptoms that can be associated with hemoglobin disorders include microcytosis, sickling disorders, hemolysis, erythrocytosis, cyanosis/hypoxia, long-standing or familial anemia, compensated or episodic anemia, and increased methemoglobin or sulfhemoglobin results.

Mayo Clinic Laboratories receives specimens for this test from a wide geographic area and nearly one-half of all specimens received exhibit abnormalities. The most common abnormality is an increase in Hb A2 to about 4% to 8%, which indicates beta-thalassemia minor in the correct clinical context. A wide variety of other hemoglobinopathies also have been encountered. Ranked in order of relative frequency, these are: Hb S (sickle cell disease and trait), C, E, Lepore, G-Philadelphia, H, D-Los Angeles, Koln, Constant Spring, O-Arab, and others. Hb C and S are found mostly in people from west or central Africa and Hb E and H in people from Southeast Asia. Hemoglobin electrophoresis is often used in the evaluation of unexplained microcytosis, thus accounting for the frequent detection of Hb Lepore, which is relatively common in Italians and others of Mediterranean ancestry and in Hb E, which is relatively common in Southeast Asians resettled in the United States; microcytosis is characteristic of both Hb Lepore and Hb E.

Alpha-thalassemia is very common in the United States, occurring in approximately 30% of African Americans and accounting for the frequent occurrence of microcytosis in persons of this ethnic group. Some alpha-thalassemias (ie, hemoglobin variants H, Barts, and Constant Spring) are easily identified in the hemoglobin electrophoresis protocol. However, alpha-thalassemias that are from only 1 or 2 alpha-globin gene deletions are not recognized by protein studies alone. For the diagnosis of alpha-thalassemias, deletion and duplication testing is required.

Reference Values

HEMOGLOBIN A

1-30 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

1-30 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

1-30 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

No abnormal variants

VARIANT 2

No abnormal variants

VARIANT 3

No abnormal variants

Interpretation

The types of hemoglobin present are identified, quantitated, and an interpretive report is issued.

Cautions

No significant cautionary statements

Clinical Reference

Hoyer JD, Hoffman DR: The Thalassemia and hemoglobinopathy syndromes. In Clinical Laboratory Medicine. Second edition. Edited by KD McMlatchey. Philadelphia, Lippincott Williams and Wilkins, 2002, pp 866-895

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; 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 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, 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)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Saturday

Analytic Time

2 to 25 days if structural and/or molecular studies are required

Maximum Laboratory Time

25 days

Specimen Retention Time

7 days; abnormals kept for 14 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 has been modified from the manufacturer's instructions. Its performance characteristics were 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

Hemoglobin Electrophoresis Cascade

83020-Quantitation by electrophoresis

83021-Quantitation by HPLC

IEF Confirms

82664-Electrophoresis, not elsewhere specified (if appropriate)

Hemoglobin, Unstable, Blood

83068 (if appropriate)

Hemoglobin Variant by Mass Spectrometry (MS), Blood

83789 (if appropriate)

Hemoglobin F, Red Blood Cell Distribution, Blood

88184 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HBELC	HGB Electrophoresis Cascade	94538-6

Result ID	Test Result Name	Result LOINC Value
2380	Hemoglobin A	20572-4
2381	Hemoglobin A2	42245-1
2382	Hemoglobin F	42246-9
2383	Variant	32017-6

Result ID	Test Result Name	Result LOINC Value
29224	Variant 2	32017-6
29225	Variant 3	32017-6
2101	Interpretation	78748-1