Test Definition: HBEL1

Hemoglobin Electrophoresis Evaluation, Blood

Overview

Useful For

Diagnosis and classification of hemoglobin disorders, including thalassemias and hemoglobin variants

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
HBELI	Hb Electrophoresis Interpretation	No	Yes
HGBCE	Hb Variant, A2 and F Quantitation,B	Yes	Yes
HPLC	HPLC Hb Variant, B	No	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
HPFH	Hb F Distribution, B	No	No
MASS	Hb Variant by Mass Spec, B	No	No
SDEX	Sickle Solubility, B	Yes	No
IEF	Isoelectric Focusing, B	No	No
UNHB	Hb Stability, 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
HBEL0	Hb Electrophoresis Summary Interp	No	No
WBGDR	Beta Globin Gene Cluster, Del/Dup,B	Yes, (Order WBGDD)	No
WAGDR	Alpha Globin Clustr Locus Del/Dup,B	Yes, (Order AGDD)	No

Testing Algorithm

This evaluation will always include hemoglobin (Hb) A2 and HbF and hemoglobin electrophoresis utilizing capillary electrophoresis and cation exchange high-performance liquid chromatography methods.

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);

HbF distribution by flow cytometry; DNA Sanger sequencing assays for: 1) beta-chain variants and the most common beta thalassemias (beta-globin gene sequencing), 2) alpha-chain variants and less common nondeletional alpha thalassemias (alpha-globin gene sequencing), or 3) gamma-chain variants and nondeletional hereditary persistence of fetal hemoglobin (HPFH) (gamma-globin full gene sequencing); multiplex ligation-dependent probe amplification assays for: 1) large deletional alpha thalassemias and alpha-gene duplications (alpha-globin gene analysis), or 2) beta-globin gene cluster locus large deletions and duplications, including large deletional HPFH, delta-beta thalassemia, gamma-delta-beta thalassemia, epsilon-gamma-delta-beta thalassemia and large deletional beta or delta thalassemia (beta-globin cluster locus deletion/duplication).

If test results in the profile are abnormal, results may be reviewed by a hematopathology consultant, and a summary interpretation provided.

One or more of the following molecular tests may be reflexed:
-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

For cases with molecular testing added, a preliminary interpretation will be reported that discusses the protein test results. After all test results are finalized, an additional consultative interpretation that summarizes all testing and incorporates subsequent genetic results will be provided.

Special Instructions

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

Method Name

HBELI, HBEL0: Medical Interpretation
HGBCE: Capillary Electrophoresis
HPLC: Cation Exchange/High-Performance Liquid Chromatography (HPLC)
IEF: Isoelectric Focusing
MASS: Mass Spectrometry (MS)
HPFH: Flow Cytometry
UNHB: Isopropanol and Heat Stability

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

Multiple hematology evaluations are available. For information on testing that is performed with each evaluation, see Benign Hematology Evaluation Comparison.

If monitoring treatment with HbA-T87Q (Lyfgenia for sickle cell disorders or Zynteglo for thalassemia), complete Metabolic Hematology Patient Information (T810) to notify the laboratory and request mass spectrometry testing.

Necessary Information

At minimum, include recent transfusion information and most recent complete blood cell count results.

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.

The laboratory has extensive experience in hemoglobin variant identification and many cases can be confidently 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 sample 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

Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD solution B)
Specimen Volume: 10 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

1 mL (this volume will limit reflex testing possibilities); 3 mL if multiplex ligation-dependent probe amplification is needed

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

A large number of variants of hemoglobin (Hb) have been recognized. Although many do not result in clinical or hematologic effects, clinical symptoms that can be associated with Hb disorders include microcytosis, sickling disorders, hemolysis, erythrocytosis/polycythemia, cyanosis/hypoxia, anemia (chronic, compensated, or episodic), and increased methemoglobin or sulfhemoglobin results (M-hemoglobins).

For many common Hb variants (eg, HbS, HbC, HbD and HbE, among many others), protein studies will be sufficient for definitive identification. However, some Hb conditions may be difficult to identify by protein methods alone and may require molecular methods for confirmation. Hb disorders commonly occur as compound disorders (2 or more genetic variants) that can have complex interactions and variable phenotypes. In these situations, molecular testing may be necessary for accurate classification. It is important to note that although powerful as an adjunct for a complete and accurate diagnosis, molecular methods without protein data can give incomplete and possibly misleading information due to limitations of the methods. Accurate classification of hemoglobin disorders and interpretation of genetic data requires the incorporation of protein analysis results. This profile is well-suited for the classification of hemoglobin disorders.

Mayo Clinic Laboratories receives specimens from a wide geographic area and nearly one-half of all specimens tested exhibit abnormalities. The most common abnormality is an increase in HbA2 to about 4% to 8%, which indicates beta-thalassemia minor when present in the correct clinical context. A wide variety of other hemoglobinopathies are also frequently encountered. Ranked in order of relative frequency, these are: Hb S (sickle cell disease and trait), C, E, Lepore, G-Philadelphia, HbH disease, D-Los Angeles, Koln, Constant Spring, O-Arab. Other variants associated with hemolysis, erythrocytosis/polycythemia, microcytosis, cyanosis/hypoxia are routinely identified; however, some will not be detected by routine screening methods and require communication of clinical findings to prompt indicated reflex testing options. Alpha-thalassemia genetic variants are 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-thalassemia conditions (eg, HbH, Barts) can be identified in the hemoglobin electrophoresis protocol, although Hb Constant Spring may or may not be evident by protein methods alone dependent upon the percentage present. It is important to note, alpha thalassemias that are from only 1 or 2 alpha-globin gene deletions are not recognized by protein studies alone and alpha-gene deletion and duplication testing is required.

Reference Values

Hemoglobin Electrophoresis Interpretation
Definitive results and an interpretative report will be provided.

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

Interpretation

The hemoglobin fractions, including hemoglobin variants are identified and quantitated. An interpretive report that summarizes all testing, including the significance of the findings, is issued.

Cautions

Some hemoglobin disorders and variants are not detected by the screening methods including, common alpha-thalassemia conditions and require further reflex testing to identify. If a family history of a known hemoglobin disorder, prior therapy for a hemoglobin disorder, or otherwise unexplained lifelong/familial symptoms, such as hemolysis, microcytosis, erythrocytosis/polycythemia, cyanosis, or hypoxia are present, this should be clearly communicated to the laboratory so appropriate reflex testing can be added, see Metabolic Hematology Patient Information.

Recent transfusion may mask protein results including hemoglobin electrophoresis, hereditary persistence of hemoglobin F by flow cytometry, stability studies, and sickle solubility studies depending on percentage of transfused cells present.

Some hemoglobin variants can originate from the donor blood product and not from the tested recipient. These are typically found in low percentage.

If the patient has undergone a bone marrow transplant, the results may show atypical results and should be interpreted in the context of clinical information.

Some therapies cause artefactual effects in protein studies, including hydroxyurea and decitabine (increased hemoglobin F levels), voxelotor (artefactual peaks) and gene therapy (alternate protein detection, beta T87Q, by mass spectrometry). Clear communication of prior therapy is strongly recommended.

Clinical Reference

1. Hoyer JD, Hoffman DR. The thalassemia and hemoglobinopathy syndromes. In: McClatchey KD, Amin HM, Curry JL, eds. Clinical Laboratory Medicine. 2nd ed. Lippincott Williams and Wilkins; 2002:866-895
2. Oliveira JL. Diagnostic strategies in hemoglobinopathy testing, the role of a reference laboratory in the USA. Thalassemia Reports. 2018;8(1):7476. doi:10.4081/thal.2018.7476
3. Brancaleonai V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol. 2016;38 Suppl 1:32-40. doi:10.1111/ijlh.12527
4. Hartveld CI. State of the art and new developments in molecular diagnostics for hemoglobinopathies in multiethnic societies. Int J Lab Haematol. 2014;36(1):1-12. doi:10.1111/ijlh.12108
5. Riou J, Szuberski J, Godart C, et al. Precision of CAPILLARYS 2 for the detection of hemoglobin variants based on their migration positions. Am J Clin Pathol. 2018;149(2):172-180. doi:10.1093/ajcp/aqx148

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 hemoglobin. 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: HbA2', C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Barts, 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. 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; 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)

PDF Report
No

Day(s) Performed
Monday through Thursday

Report Available
2 to 25 days

Specimen Retention Time
Whole blood: 7 days; Abnormal samples: 14 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 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 US Food and Drug Administration.

CPT Code Information
83020
83021
82664 (if appropriate)
83068 (if appropriate)

83789 (if appropriate)
88184 (if appropriate)
83020-26 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HBEL1	Hb Electrophoresis Evaluation	94538-6

Result ID	Test Result Name	Result LOINC® Value
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
608088	Hb Electrophoresis Interpretation	49316-3
609421	Hb Electrophoresis Interp Cancel	No LOINC Needed