

# Hemoglobin Evaluation Reflexive Cascade

## IDENTIFICATION OF ABNORMAL HEMOGLOBIN VARIANTS

### Test Highlights

- Cascade testing always begins with high-performance liquid chromatography (HPLC) analysis. If an abnormal hemoglobin is detected, or if the clinical data suggest a hemoglobinopathy, appropriate reflex testing will be performed in order to provide a clinical interpretation. Multiple reflex tests may be required. Reflex testing performed may include electrophoresis, solubility testing, and/or molecular analyses of the globin genes.
- Cascade testing allows for detection of hemoglobin variants from a single sample in a cost-effective manner.
- Clinical and laboratory information, including a recent CBC, is required for interpretation.

### Clinical Background

- Hemoglobin (Hb) is a tetrameric molecule that reversibly binds oxygen in red blood cells. The major adult Hb (Hb A) is composed of two beta globin chains and two alpha globin chains.

**HPLC Testing: Age-Defined Normal Hemoglobin Reference Intervals**

Age	Hb A%	Hb A <sub>2</sub> %	Hb F%	Hb S%	Hb C%	Hb E%	Hb Other%
0–1 month	17.7-54.0	17.7-54.0	0.0-1.3	0.0	0.0	0.0	0.0
2 months	37.1-70.6	37.1-70.6	0.4-1.9	0.0	0.0	0.0	0.0
3 months	41.0-84.0	41.0-84.0	1.0-3.0	0.0	0.0	0.0	0.0
4 months	68.2-88.6	68.2-88.6	2.0-2.8	0.0	0.0	0.0	0.0
5 months	74.9-95.6	74.9-95.6	2.1-3.1	0.0	0.0	0.0	0.0
6–8 months	83.5-95.8	83.5-95.8	1.9-3.5	0.0	0.0	0.0	0.0
9–12 months	91.7-96.7	91.7-96.7	2.0-3.3	0.0	0.0	0.0	0.0
13–23 months	94.5-98.2	94.5-98.2	1.6-3.5	0.0	0.0	0.0	0.0
2 years and older	94.3-98.5	94.3-98.5	1.5-3.7	0.0	0.0	0.0	0.0

- Defects in the formation of the hemoglobin complex can lead to hemoglobinopathies (structurally abnormal hemoglobin) and alpha- and beta-thalassemia (imbalance in the quantity of alpha and beta chains). At least 800 hemoglobin variants have been described.
- Clinical symptoms are related to inadequate hemoglobin (Hb) production and accumulation of globin subunits.
- Interactions between globin chains with different mutations may either alleviate or exacerbate the effects of the individual variants.

### Disease Overview

- Hemoglobinopathies
  - Many structural Hb variants may have no clinical effect (unless paired with a second variant); however, some may result in microcytic anemia, hemolytic anemia, cyanosis due to reduced oxygen affinity, or erythrocytosis due to increased oxygen affinity.
  - Sickle cell anemia (HbSS), the most common significant hemoglobinopathy, is characterized by hemolysis and episodes of vascular occlusion affecting numerous organs. Pain and swelling of hands and feet is often the first indication of the disease, and infection is a frequent complication.
  - Many common structural variants, including Hb S, Hb C, Hb E, Hb Lepore, Hb-Constant Spring, Hb D-Los Angeles and Hb G-Philadelphia, are often detectable by HPLC and electrophoresis.
- Beta Thalassemia
  - Carriers of only one beta thalassemia mutation (beta thalassemia minor) are clinically asymptomatic but have minor hematologic anomalies that typically include reduced mean corpuscular volume (MCV) and elevated Hb A<sub>2</sub> (>3.7%) on HPLC.
  - Patients who are homozygous or compound heterozygous for beta thalassemia mutations may be variably affected. Beta thalassemia major, the most severe presentation, is associated with severe microcytic anemia and hepatosplenomegaly; affected individuals are transfusion-dependent. Beta thalassemia intermedia is associated with a milder presentation.
- Alpha thalassemia
  - Carriers may have mild microcytic anemia, which is often misdiagnosed as iron deficiency. The majority of alpha thalassemia carriers will have normal HPLC and electrophoresis test results.
  - Hb H disease generally occurs due to loss of function of three alpha globin genes and is characterized by the presence of Hb H (β<sub>4</sub>) or Hb Bart (γ<sub>4</sub>) in a neonate. Clinical findings include moderate microcytic hypochromic anemia, hemolysis with Heinz bodies, splenomegaly, rare extramedullary hematopoiesis, and propensity of acute hemolysis after oxidative stress, drug therapy, or infection.
- Hb Bart hydrops fetalis syndrome is the most severe form of alpha thalassemia. Symptoms include fetal generalized edema, ascites, pleural and pericardial effusions, and severe hypochromic anemia. This syndrome often results in fetal or perinatal death.

## Epidemiology

- Approximately 5% of the world's population carries clinically important hemoglobin mutations.
- Beta thalassemia is most commonly observed in individuals from southern Europe, northern Africa, and India.
- Alpha thalassemia carrier frequencies in high-risk populations: Mediterranean (1/30–50), Southeast Asian (1/20), African, Middle Eastern, and African-American (~1/3).
- Hb S is most common in sub-Saharan Africa, India, and the Middle East. Approximately 10% of African-Americans have sickle cell trait.
- Hb C and Hb E occur frequently in individuals of West African and Southeast Asian ancestry, respectively.

## Genetics

- Inheritance is typically autosomal recessive; however, some beta globin variants have dominant effects.
- Normal adults have two functional beta globin genes (*HBB*) and four functional alpha globin genes (two copies each of *HBA1* and *HBA2*).
- Approximately 97% of beta globin gene mutations are identified by *HBB* sequence analysis.
- Ninety-five percent of alpha thalassemia is caused by large *HBA1* and *HBA2* gene deletions. The  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$  alpha globin gene deletions result in the deletion of a single gene; the  $-(\alpha)^{20.5}$ ,  $-\text{SEA}$ ,  $-\text{MED}$ ,  $-\text{FIL}$ , or  $-\text{THAI}$  deletions result in the deletion of the *HBA1* and the *HBA2* genes from the same chromosome.

## Indication for Ordering

Diagnostic testing in individuals with hematological or clinical findings suggestive of a thalassemia or hemoglobinopathy.

## Contraindication for Ordering

This test is not recommended for routine carrier screening in healthy adults for purposes of reproductive decision-making. Please see The American Congress of Obstetricians and Gynecologists (ACOG) practice guidelines for population screening for hemoglobinopathies.

## Interpretation

Patient History for Hemoglobinopathy/Thalassemia Testing Form and/or a recent CBC is required for interpretation.

## Limitations

This cascade may not detect all hemoglobin variants, including large *HBB* deletions, rare *HBA1/2* deletions, regulatory region mutations, or mutations involving the delta or gamma genes.

## Methodology of Available Cascade Test Components

- Cascade testing always begins with HPLC analysis. If an abnormal hemoglobin is detected, or if the clinical data suggest a hemoglobinopathy, appropriate reflex testing will be performed. Reflex testing may include electrophoresis, solubility testing, and/or molecular analyses of the globin genes.
- HPLC
  - Bio-Rad Variant II automated cation-exchange instrument is used to quantify Hb A, Hb A2, Hb F, Hb S, Hb C, and other variants by HPLC. As the ionic strength of the eluting solution is increased, hemoglobin variants will separate from the exchange column at a particular retention time based on charge.
- Sickle Cell Solubility
  - Performed as a screening test for Hb S or other sickling hemoglobins. Red blood cell lysate is placed in a high-

phosphate buffer solution; sodium hydrosulfite is then added to lower the oxygen tension. Hemoglobins with sickling properties will precipitate and give a positive result.

- Hemoglobin, Alkaline Electrophoresis
  - Electrophoresis is performed at a pH of 8.6, at which point the Hb A molecule is negatively charged. Hb variants with differing charges will separate based on mobility.
- Hemoglobin, Acid Electrophoresis
  - Electrophoresis is performed at a pH of 6.2 using a citrate buffer. Hb variants with differing charges will separate by mobility. Useful for identification of Hb S and distinguishing Hb C from Hb E and Hb O-Arab.
- Beta Globin (*HBB*) HbS, HbC, & HbE Mutations (0051421)
  - Polymerase chain reaction (PCR) and fluorescence resonance energy transfer for detection of c.19G>A (Hb C), c.20A>T (Hb S), and c.79G>A (Hb E). Multiplex loci-spanning probe interrogates the three loci simultaneously.
- Beta Globin (*HBB*) Sequencing (0050578)
  - PCR amplification, followed by bidirectional sequencing of the *HBB* coding region, intron/exon borders, proximal promoter, 5' and 3' untranslated regions, and IVS-II-654, IVS-II-705, and IVS-II-745 deep intronic mutations; PCR followed by gel electrophoresis for the 619del mutation.
- Alpha Thalassemia (*HBA1* & *HBA2*) 7 Deletions (0051495)
  - Common deletions of *HBA1* and *HBA2* ( $-\alpha 3.7$ ,  $-\alpha 4.2$ ,  $-(\alpha)20.5$ ,  $-\text{SEA}$ ,  $-\text{MED}$ ,  $-\text{FIL}$ , and  $-\text{THAI}$ ) are assayed by PCR and gel electrophoresis.
- Alpha Globin (*HBA1* & *HBA2*) Sequencing (2001582)
  - PCR amplification, followed by bidirectional sequencing of the complete protein coding sequence with exon/intron boundaries, proximal promoter region, 5' and 3' untranslated regions, and polyadenylation signal.
- Hemoglobin Lepore (*HBD/HBB* Fusion) 3 Mutations (2004686)
  - Multiplex PCR and gel electrophoresis to detect the three common Hb Lepore mutations: Hb Lepore-Washington-Boston (g.63632\_71046del), Hb Lepore-Baltimore (g.63564\_70978del), and Hb Lepore-Hollandia (g.63290\_70702del).
- Hereditary Persistence of Fetal Hemoglobin (HPFH) 8 Mutations (2005408)
  - Multiplex PCR and gel electrophoresis to detect eight common deletions associated with HPFH: HPFH-1 (g.5174452\_5259368del84917), HPFH-2 (g.5180404\_5263982del83579), HPFH-3 (g.5215683\_5265453del49771), HPFH-4 (g.5217940\_5260078del42139), HPFH-5 (g.5246023\_5258951del12929), HPFH-6 (g.5193975\_5273259del79278), HPFH-7 (g.5247860\_5270651del22792) and SEA-HPFH (g.5222878\_5250288del27411).

## References

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## Test Information

**2005792 Hemoglobin Evaluation Reflexive Cascade**

For specific collection, transport, and testing information, refer to the ARUP website at [www.aruplab.com](http://www.aruplab.com).

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at [www.arupconsult.com](http://www.arupconsult.com).

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