

## Hemoglobin Evaluation Reflexive Cascade

Hemoglobinopathies are a group of common, inherited disorders of hemoglobin (Hb), resulting in the synthesis of structurally abnormal globin subunits.<sup>1</sup> Some of these disorders may also cause a reduced synthesis of structurally normal globin subunits (thalassemias).<sup>1</sup> The hemoglobin evaluation reflexive cascade initially tests for abnormal hemoglobin. Additional testing, including genetic testing, is added if the results are suggestive of a hemoglobinopathy.

### Disease Overview

#### Prevalence/Incidence

~5% of the world's population carries clinically important Hb variants, and 300,000 individuals with a severe hemoglobinopathy are born annually.

The most common hemoglobinopathies are beta ( $\beta$ ) thalassemia, alpha ( $\alpha$ ) thalassemia, sickle cell Hb (HbS), HbC (common in West Africa), and HbE (common in Southeast Asia).

$\beta$  thalassemia is most commonly observed in individuals from southern Europe, northern Africa, and India. Sickle cell Hb is frequently observed in Southeast Asian, Indian, and Mediterranean populations and ~10% of African Americans have sickle cell trait.

The carrier frequency for  $\alpha$  thalassemia varies depending on ethnicity, as follows:

- African, African American: 1/3
- Middle Eastern, Southeast Asian: 1/20
- Mediterranean: 1/30-50

Hb Barts hydrops fetalis syndrome is more frequent in Southeast Asian, Indian, and Mediterranean populations than African populations.

#### Pathophysiology

- Hb is a tetrameric molecule that reversibly binds oxygen to red blood cells
- Major adult Hb (HbA) is composed of two  $\beta$ -globin chains and two  $\alpha$ -globin chains
- Defects in the formation of the Hb complex
  - Hemoglobinopathies: structurally abnormal Hb
    - Many Hb variants have no clinical effect unless paired with a second variant
    - Reduced oxygen affinity
      - Microcytic anemia
      - Hemolytic anemia
      - Cyanosis

### Tests to Consider

#### Hemoglobin Evaluation Reflexive Cascade 2005792

**Method:** High Performance Liquid Chromatography/Electrophoresis/RBC Solubility/Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer/Sequencing

Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy

#### Indications for Ordering

- Confirms diagnosis in individuals who have hematologic or clinical findings suggestive of a thalassemia or hemoglobinopathy
- Detects hemoglobin (Hb) variants
- Not recommended for routine carrier screening in healthy adults for purposes of reproductive decision making; for population screening for hemoglobinopathies, refer to The American College of Obstetricians and Gynecologists (ACOG) practice guideline<sup>2</sup>

#### Typical Testing Strategy

- Structural variants of Hb (eg, HbS, HbC, HbE, Hb Lepore, Hb Constant Spring, HbD Los Angeles, HbG Philadelphia) are often detectable by HPLC and electrophoresis
- Begin with HPLC analysis:
  - If abnormal Hb is detected, or if clinical data suggest a hemoglobinopathy, appropriate reflex testing is performed
  - A hematopathologist on the faculty of the University of Utah School of Medicine personally directs and interprets each stage of testing to completion
  - Reflex testing may include electrophoresis, solubility testing, and/or molecular analyses of globin genes

See [Related Tests](#) for tests that can be ordered individually or may be performed as part of Hb cascade testing

- Increased oxygen affinity: erythrocytosis
- $\alpha$  and  $\beta$  thalassemia: reduced synthesis of structurally normal globin subunits
  - Imbalance in the quantity of  $\alpha$  and  $\beta$  chains

## Symptoms



### Clinical Symptoms and Laboratory Test Findings for Common Hemoglobinopathies

Hemoglobinopathy	Laboratory Test Results	Clinical Symptoms <sup>a</sup>
<b><math>\beta</math> Globin</b>		
Sickle cell anemia (HbS) <ul style="list-style-type: none"> <li>• Homozygous for HbS</li> </ul>	HPLC: HbS present and no HbA normocytic hemolytic anemia	Asymptomatic at birth Episodes of vascular occlusion affecting numerous organs Pain and swelling of hands and feet: often the first indication of the disease Infection: frequent complication
$\beta$ thalassemia minor (trait) <ul style="list-style-type: none"> <li>• Heterozygous for <math>\beta</math> thalassemia variant</li> </ul>	HPLC pattern in individuals $\geq 12$ months <ul style="list-style-type: none"> <li>• HbA is decreased: 92-95%</li> <li>• HbA2 is increased: <math>&gt;3.7\%</math></li> <li>• HbF may be slightly elevated: 1-4%</li> </ul> MCV: reduced	Clinically asymptomatic
$\beta$ thalassemia major <ul style="list-style-type: none"> <li>• Homozygous <math>\beta 0</math> variant</li> <li>• Compound heterozygote for 2 different <math>\beta 0</math> variants</li> </ul>	HPLC: no HbA present, HbF 95-100%	Affected individuals are transfusion dependent Microcytic anemia, hepatosplenomegaly Infants <ul style="list-style-type: none"> <li>• Symptoms typically appear at 6-24 months               <ul style="list-style-type: none"> <li>○ Growth retardation, failure to thrive, pallor, jaundice</li> </ul> </li> <li>• HbF is protective in early infancy</li> </ul> Older individuals: leg ulcers, extramedullary hematopoiesis, thrombophilia, pulmonary arterial hypertension, endocrine dysfunction, osteoporosis
$\beta$ thalassemia intermedia <ul style="list-style-type: none"> <li>• <math>\beta+</math> homozygote or <math>\beta 0/\beta+</math> compound heterozygote</li> </ul>	HPLC pattern in individuals $\geq 12$ months <ul style="list-style-type: none"> <li>• HbA: 10-30%</li> <li>• HbA2: 2-5%</li> <li>• HbF: 70-90%</li> </ul>	Milder presentation than $\beta$ thalassemia major: individuals may require transfusions occasionally Pallor Jaundice Cholelithiasis Liver and spleen enlargement Moderate/severe skeletal changes Leg ulcers Extramedullary masses of hyperplastic erythroid marrow
<b><math>\alpha</math> Globin</b>		
Silent carrier <ul style="list-style-type: none"> <li>• Loss of function of a single <math>\alpha</math>-globin gene (<math>-\alpha/\alpha</math>)</li> </ul>	HPLC: normal Possible mild microcytic anemia	Often clinically asymptomatic If anemia present, may be misdiagnosed as iron deficiency
Carrier: $\alpha$ thalassemia trait	HPLC: normal for most	May be misdiagnosed as iron deficiency

- Loss of function of  $\alpha$ -globin genes in trans ( $-\alpha/\alpha$ ) or in cis ( $-/\alpha\alpha$ )
- Mild microcytic anemia  
May have normal red cell indices



<sup>a</sup>Related to inadequate Hb production and accumulation of globin subunits  
MCV, mean corpuscular volume

Hemoglobinopathy	Laboratory Test Results	Clinical Symptoms <sup>a</sup>
HbH disease <ul style="list-style-type: none"> <li>• Loss of function of 3 <math>\alpha</math>-globin genes</li> </ul>	HPLC <ul style="list-style-type: none"> <li>• Adult: presence of HbH (<math>\beta 4</math>)</li> <li>• Neonate: presence of Hb Barts (<math>\gamma 4</math>)</li> </ul> Hemolysis with Heinz bodies  Moderate microcytic hypochromic anemia	Splenomegaly  Rare extramedullary hematopoiesis  Propensity of acute hemolysis after oxidative stress, drug therapy, or infection
Hb Barts hydrops fetalis syndrome <ul style="list-style-type: none"> <li>• Loss of function of all 4 <math>\alpha</math>-globin genes (<math>-/-</math>)</li> </ul>	HPLC: Hb Barts near 100% Significant hemolysis	Fetal generalized edema Ascites Pleural and pericardial effusions Severe hypochromic anemia Often results in fetal or perinatal death

<sup>a</sup>Related to inadequate Hb production and accumulation of globin subunits  
MCV, mean corpuscular volume

## Genetics

### Genes

*HBB* ( $\beta$  globin), *HBA1*, *HBA2* ( $\alpha$  globin)

### Inheritance

Primarily autosomal recessive, though some  $\beta$ -globin variants have dominant effects

### Structure/Function

- Normal adults have two functional  $\beta$ -globin genes (*HBB*) and four functional  $\alpha$ -globin genes (two copies each of *HBA1* and *HBA2*)
- 90% of  $\alpha$  thalassemia is caused by large deletions in the *HBA1* and *HBA2* genes
- $-\alpha 3.7$  and  $-\alpha 4.2$   $\alpha$ -globin gene deletions result in deletion of a single gene
- $-(\alpha)20.5$ ,  $-\text{SEA}$ ,  $-\text{MED}$ ,  $-\text{FIL}$ , or  $-\text{THAI}$  deletions result in deletion of *HBA1* and *HBA2* genes from the same chromosome
- $\beta$ -globin chains with different variants may interact to alleviate or exacerbate effects of the individual variants
- Certain deletions in the *HBB* gene impair the developmental switch from fetal to adult Hb, resulting in hereditary persistence of fetal Hb (HPFH)

### Variants

>800 variants of Hb have been described

## Test Interpretation

## Sensitivity/Specificity

Varies, depending on test components



## Results

Optimal interpretation requires submission of recent CBC test results

- Positive: one or more Hb variants detected
- Negative: no Hb variants detected

## Limitations

- May not detect all Hb variants
- Regulatory region variants and sequence variants in genes other than *HBB*, *HBA1*, and *HBA2* will not be detected
- The phase of identified variants may not be determined
- Specific breakpoints of large deletions/duplications will not be determined
  - May not be possible to distinguish variants of similar size
- Individuals carrying both a deletion and a duplication within the  $\alpha$ -globin gene cluster may appear to have a normal number of  $\alpha$ -globin gene copies
- Sequencing of both *HBA1* and *HBA2* genes may not be possible in individuals harboring large  $\alpha$ -globin deletions on both alleles
- Rare syndromic or acquired forms of  $\alpha$  thalassemia associated with *ATRX* gene variants will not be detected
- Diagnostic errors can occur due to rare sequence variations

## References

1. U.S. Health and Human Services, Centers for Disease Control and Prevention. [Hemoglobinopathies - current practices for screening, confirmation and follow-up](#). Association of Public Health Laboratories. [Published: Dec 2015; Accessed: Jul 2020]
2. ACOG Committee on Obstetrics. [ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy](#). *Obstet Gynecol.* 2007;109(1):229-237. PubMed

## Related Information

[Hemoglobinopathies](#)  
[Hemoglobinopathies Testing Algorithm](#)  
[Thalassemias](#)  
[Unstable Hemoglobinopathies](#)

## Related Tests

[Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610](#)

**Method:** High Performance Liquid Chromatography/Electrophoresis/RBC Solubility

[Hemoglobin S, Sickle Solubility 2013399](#)

**Method:** RBC Solubility

[Beta Globin \(HBB\) Sequencing and Deletion/Duplication \(Temporary Referral as of 12/07/20\) 2010117](#)

**Method:** Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

[Deletion/Duplication Analysis by MLPA 3003144](#)

**Method:** Multiplex Ligation-dependent Probe Amplification

[Alpha Globin \(HBA1 and HBA2\) Sequencing and Deletion/Duplication \(Extended TAT as of 1/11/21-no referral available\) 2011708](#)

**Method:** Polymerase Chain Reaction/Sequencing./Multiplex Ligation-dependent Probe Amplification.

[Alpha Globin \(HBA1 and HBA2\) Deletion/Duplication \(Extended TAT as of 11/20/20-no referral available\) 2011622](#)

**Method:** Multiplex Ligation-dependent Probe Amplification

[Gamma Globin \(HBG1 and HBG2\) Sequencing 3001957](#)

**Method:** Polymerase Chain Reaction/Sequencing

ARUP Laboratories is a nonprofit enterprise of the University of Utah and its Department of Pathology. 500 Chipeta Way, Salt Lake City, UT 84108  
(800) 522-2787 | (801) 583-2787 | aruplab.com | arupconsult.com  
Content Review July 2020 | Last Update February 2021



© 2021 ARUP Laboratories. All Rights Reserved.

Client Services - (800) 522-2787