Hemoglobin Evaluation Reflexive Cascade

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Hemoglobinopathies are a group of common, inherited disorders of hemoglobin (Hb), resulting in the synthesis of structurally abnormal globin subunits.[1] Some of these disorders may also cause a reduced synthesis of structurally normal globin subunits (thalassemias).[1] 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.

For typical testing strategy, refer to the Hemoglobinopathies Testing algorithm.

Featured ARUP Testing

Hemoglobin Evaluation Reflexive Cascade 2005792

Method: High Performance Liquid Chromatography (HPLC) / Capillary Electrophoresis / RBC Solubility / Polymerase Chain Reaction (PCR) / Fluorescence Resonance Energy Transfer (FRET) / Sequencing / Massively Parallel Sequencing

- Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy 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[2]

Reflex Pattern

- Begins 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

Disease Overview

Prevalence/Incidence

Approximately 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 (β) thalassemia, alpha (α) thalassemia, sickle cell Hb (HbS), HbC (common in West Africa), and HbE (common in Southeast Asia).

β 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 approximately 10% of African Americans have sickle cell trait.

The carrier frequency for α 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 β-globin chains and two α-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
        - Increased oxygen affinity: erythrocytosis
    - α and β thalassemia: reduced synthesis of structurally normal globin subunits
        - Imbalance in the quantity of α and β chains

Symptoms

Clinical Symptoms and Laboratory Test Findings for Common Hemoglobinopathies		
Hemoglobinopathy	**Laboratory Test Results**	**Clinical Symptoms[a]**
β Globin		
Sickle cell anemia (HbS) • Homozygous for HbS	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
β thalassemia minor (trait) • Heterozygous for β thalassemia variant	HPLC pattern in individuals ≥12 months • HbA is decreased: 92-95% • HbA2 is increased: >3.7% • HbF may be slightly elevated: 1-4% MCV: reduced	Clinically asymptomatic
β thalassemia major • Homozygous β0 variant • Compound heterozygote for 2 different β0 variants	HPLC: no HbA present, HbF 95-100%	Affected individuals are transfusion dependent Microcytic anemia, hepatosplenomegaly Infants • Symptoms typically appear at 6-24 months ◦ Growth retardation, failure to thrive, pallor, jaundice • HbF is protective in early infancy Older individuals: leg ulcers, extramedullary hematopoiesis, thrombophilia, pulmonary arterial hypertension, endocrine dysfunction, osteoporosis
β thalassemia intermedia • β+ homozygote or β0/β+ compound heterozygote	HPLC pattern in individuals ≥12 months • HbA: 10-30% • HbA2: 2-5% • HbF: 70-90%	Milder presentation than β 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
α Globin		
Silent carrier • Loss of function of a single α-globin gene (-α/αα)	HPLC: normal Possible mild microcytic anemia	Often clinically asymptomatic If anemia present, may be misdiagnosed as iron deficiency
Carrier: α thalassemia trait • Loss of function of α-globin genes in trans (-α/-α) or in cis (--/αα)	HPLC: normal for most Mild microcytic anemia May have normal red cell indices	May be misdiagnosed as iron deficiency

Hemoglobinopathy	Laboratory Test Results	Clinical Symptoms[a]
HbH disease • Loss of function of 3 α-globin genes	HPLC • Adult: presence of HbH (β4) • Neonate: presence of Hb Barts (γ4) 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 • Loss of function of all 4 α-globin genes (--/--)	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

[a]Related to inadequate Hb production and accumulation of globin subunits

MCV, mean corpuscular volume

Genetics

Genes

HBB (β globin), *HBA1*, *HBA2* (α globin)

Inheritance

Primarily autosomal recessive, though some β-globin variants have dominant effects

Structure/Function

- Normal adults have two functional β-globin genes (*HBB*) and four functional α-globin genes (two copies each of *HBA1* and *HBA2*)
- 90% of α thalassemia is caused by large deletions in the *HBA1* and *HBA2* genes
- -α3.7 and -α4.2 α-globin gene deletions result in deletion of a single gene
- -(α)20.5, --SEA, --MED, --FIL, or --THAI deletions result in deletion of *HBA1* and *HBA2* genes from the same chromosome
- β-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

- Please refer to individual test components for their background and 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
- Single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement.
- Individuals carrying both a deletion and a duplication within the α-globin gene cluster may appear to have a normal number of α-globin gene copies
- Sequencing of both *HBA1* and *HBA2* genes may not be possible in individuals harboring large α-globin deletions on both alleles
- Rare syndromic or acquired forms of α thalassemia associated with *ATRX* gene variants will not be detected
- Diagnostic errors can occur due to rare sequence variations
- Certain gene therapies may impact the performance of this test and interpretation of results; the presence or absence of variants, zygosity, and *HBB* gene copy number may not be determined in such cases.

References

1. 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 2024.

2. ACOG Committee on Obstetrics. ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. *Obstet Gynecol*. 2007;109(1):229-237.

Related Information

Hemoglobinopathies
Hemoglobinopathies Testing Algorithm
Thalassemias
Unstable Hemoglobinopathies