Hemoglobin Evaluation Reflexive Cascade

Indications for Ordering

- Confirm diagnosis in individual with hematologic or clinical findings suggestive of a thalassemia or a hemoglobinopathy
- Detect hemoglobin (Hb) variants
- Not recommended for routine carrier screening in healthy adults for purposes of reproductive decision making
  - Refer to The American Congress of Obstetricians and Gynecologists (ACOG) practice guidelines for population screening for hemoglobinopathies (2006)

Test Description

High-performance liquid chromatography (HPLC)/ electrophoresis/RBC solubility/polymerase chain reaction/ fluorescence resonance energy transfer/gene sequencing

Tests to Consider

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 the globin genes

Primary test

Hemoglobin Evaluation Reflexive Cascade 2005792

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

Related tests

Can be ordered individually or may be performed as part of Hb cascade testing

Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610

- Effective test for screening and follow up of individuals with known hemoglobinopathies
- Optimal test for the initial diagnosis of a suspected hemoglobinopathy is the Hemoglobin Evaluation Reflexive Cascade

Hemoglobin S, Sickle Solubility 2013399

- Effective test for secondary confirmation of HbS
- Not recommended for newborns <6 months due to high concentration of HbF
- Confirm results with Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility

Beta Globin (*HBB*) HbS, HbC, and HbE Mutations 0051421

- Confirm suspected HbS, HbC, or HbE variants

Beta Globin (*HBB*) Sequencing and Deletion/Duplication 2010117

- Preferred test for molecular confirmation of β thalassemia or a hemoglobinopathy involving the β-globin gene

Beta Globin (*HBB*) Deletion/Duplication 2010113

- Second-tier test
- Preferred initial test is the combined sequencing and deletion/duplication test

Alpha Globin (*HBA1* and *HBA2*) Sequencing and Deletion/Duplication 2011708

- Comprehensive genetic test for detection of α thalassemia or α thalassemia trait
- Detects deletional and nondeletional variants in *HBA1* and *HBA2*

Alpha Globin (*HBA1* and *HBA2*) Deletion/Duplication 2011622

- Preferred first-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait
- Detects common, rare, and novel deletions or duplications of the α-globin gene cluster

Hemoglobin Lepore (*HBD*/*HBB* Fusion) 3 Mutations 2004686

- Molecular confirmation of a suspected Hb Lepore variant identified by Hb evaluation
- Carrier screening for individuals with a family history of Hb Lepore
- Detects 3 common Hb Lepore variants
  - Hb Lepore-Washington-Boston (g.63632_71046del)
  - Hb Lepore-Baltimore (g.63564_70978del)
  - Hb Lepore-Hollandia (g.63290_70702del)

Disease Overview

Prevalence/incidence

- ~5% of the world's population carries clinically important Hb variants
- 300,000 individuals with a severe hemoglobinopathy are born annually
- Most common hemoglobinopathies
  - β thalassemia
    - Most commonly observed in individuals from southern Europe, northern Africa, and India
  - α thalassemia
    - Carrier frequencies in high-risk populations
      - African, African American (1/3)
      - Middle Eastern, Southeast Asian (1/20)
      - Mediterranean (1/30-50)
    - Hb Bart hydrops fetalis syndrome
      - More frequent in Southeast Asian, Indian, and Mediterranean populations than in African populations
  - Sickle cell Hb (HbS)
    - Most common in sub-Saharan Africa, India, and the Middle East
    - ~10% of African Americans have sickle cell trait
  - HbC – common in West Africa
  - HbE – common in Southeast Asia

Symptoms – see table

Pathophysiology

- Hb is a tetrameric molecule that reversibly binds oxygen to red blood cells
- Major adult Hb (HbA) is composed of 2 β-globin chains and 2 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

Genetics

Genes – *HBB* (β-globin), *HBA1, HBA2* (α-globins)

Inheritance – primarily autosomal recessive

- Some β-globin variants have dominant effects

Structure/function

- Normal adults have 2 functional β-globin genes (*HBB*) and 4 functional α-globin genes (2 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
  - Results in hereditary persistence of fetal Hb (HbF)

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
  - It may not be possible to distinguish variants of similar size
- 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

References

- The American Congress of Obstetricians and Gynecologists (ACOG) practice guidelines for population screening for hemoglobinopathies (2006 – subscription required)
- Bender MA, Douthitt Seibel G. Sickle Cell Disease. 2003 Sep 15 [Updated 2014 Oct 23]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. (www.ncbi.nlm.nih.gov/books/NBK1377/)

Clinical Symptoms and Laboratory Test Findings for Common Hemoglobinopathies		
β thalassemia		
Hemoglobinopathy	**Laboratory test results**	**Clinical symptoms** Related to inadequate Hb production and accumulation of globin subunits
Sickle cell anemia (HbS) • Homozygous for HbS	HPLC – HbS present and no HbA hemolysis	• 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 o HbA is decreased – 92-95% o HbA_2 is increased – >3.7% o HbF may be slightly elevated – 1-4% • Mean corpuscular volume (MCV) – reduced	Clinically asymptomatic
β thalassemia major • Homozygous β^0 variant • Compound heterozygote for 2 different β^0 variants	HPLC – no HbA present, HbF 95-100%	• Affected individuals are transfusion dependent • Microcytic anemia, hepatosplenomegaly • Infants o Symptoms typically appear at 6-24 months ▪ Growth retardation, failure to thrive, pallor, jaundice o 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 β^0/β^+ compound heterozygote	HPLC pattern in individuals ≥12 months • HbA – 10-30% • HbA_2 – 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 thalassemia		
Hemoglobinopathy	**Laboratory test results**	**Clinical symptoms** Related to inadequate Hb production and accumulation of globin subunits
Silent carrier • Deletion 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
HbH disease • Loss of function of 3 α-globin genes	• HPLC o Adult – presence of HbH (β4) o 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