Beta Globin (HBB) Sequencing and Deletion/Duplication

Variants in the beta (β)-globin gene (HBB) can result in anemia, β thalassemia or sickling disorders of varying severity. Typical testing strategy is as follows:

- **Initial testing:** screen for abnormal hemoglobin (Hb) variants using high-performance liquid chromatography (HPLC) and electrophoresis
- **Secondary testing:** molecular analysis to identify or confirm abnormal Hb variant(s) detected by HPLC or Hb electrophoresis

### Prevalence

- ~5% of the world’s population carries clinically important Hb variants
- 300,000 individuals with a severe hemoglobinopathy are born annually
- β thalassemias are most commonly observed in individuals from southern Europe, northern Africa, and India

### Symptoms

<table>
<thead>
<tr>
<th>Phenotypes caused by HBB variants</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td><strong>Thalassemia: decrease in protein produced</strong></td>
<td>β thalassemia minor (trait)</td>
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<td>Usually clinically asymptomatic, mild anemia may be present</td>
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<td>Minor hematologic anomalies, including reduced MCV and elevated HbA2</td>
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<td>β thalassemia major</td>
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<td>Associated with severe microcytic anemia and hepatosplenomegaly</td>
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<td>Affected individuals are transfusion dependent</td>
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<td></td>
<td>β thalassemia intermedia</td>
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<td>Milder clinical presentation than β thalassemia major</td>
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<td><strong>Hemoglobinopathy: structurally abnormal protein</strong></td>
<td>Sickle disorders</td>
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<td>Sickle cell anemia (HbSS)</td>
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<td></td>
<td>Hb S-C disease</td>
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<td></td>
<td>Microcytic or hemolytic anemia</td>
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<td>Cyanosis</td>
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<td>Reduced oxygen-affinity Hbs</td>
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<td>Erythrocytosis</td>
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<td>Increased oxygen-affinity Hbs</td>
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<td>No clinical effect</td>
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<td><strong>Hereditary persistence of fetal Hb (HPFH)</strong></td>
<td>Persistent HbF production resulting from variants of β-globin gene cluster that alter normal Hb switching</td>
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<td>Clinically benign condition</td>
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### Genetics

**Gene**  
HBB

**Inheritance**  
Autosomal recessive (typically)
Detects large deletions of the β-globin gene cluster associated with β thalassemia or HPFH

Indications for Ordering
- Confirm carrier status or diagnosis of β thalassemia or β globinopathy in individual with clinical findings or family history of β thalassemia or hemoglobinopathy
- Assess for deletional HPFH in individuals with elevated Hb F

Test Description
Multiplex ligation-dependent probe amplification (MLPA) of the β-globin gene cluster (HBB, HBD, HBG1, HBG2, HBE1) and its locus control region

Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility
0050610
Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility
Effective test for screening and follow up of individuals with known hemoglobinopathies

Structure/Function
- Major adult Hb (HbA): composed of two β-globin chains and two α-globin chains
- Normal adults have two functional β-globin genes (HBB) and four functional α-globin genes (two copies each of HBA1 and HBA2)
- β-globin chains with different variants may interact to alleviate or exacerbate the effects of the individual variants
  - Variants in HBB gene can result in formation of a structurally abnormal protein or decrease the amount of protein produced
  - Certain HBB deletions impair the developmental switch from fetal to adult Hb, resulting in hereditary persistence of fetal Hb

Variants
>500 β-globin variants

TEST INTERPRETATION

Sensitivity/Specificity
- Clinical sensitivity: 99% (~97% by sequencing and ~2% by deletion analysis) for β thalassemia and hemoglobinopathies associated with the HBB gene
- Analytical sensitivity: 99%

Results
- Pathogenic HBB gene variant(s) detected
  - Heterozygous
    - Carrier of a structurally abnormal Hb or β thalassemia, depending on the specific variant identified
  - Homozygous or compound heterozygous
    - VARially affected, depending on the specific variant(s) identified
- No pathogenic HBB gene variants detected
  - Significantly decreases possibility of β thalassemia or β globinopathy
  - Clinically benign structural variants predicted to produce an abnormal electrophoresis/HPLC result will be reported

Limitations
- Diagnostic errors can occur due to rare sequence variations
- Breakpoints of large deletions and duplications will not be determined
- Precise clinical phenotype associated with a particular deletion may not be known (eg, HPFH vs. delta-beta [δ-β] thalassemia)
- Intragenic deletions in the β-globin cluster genes, other than HBB, may not be detected
- Does not assess for sequence variants within the coding or regulatory regions of the HBD, HBG1, HBG2, and HBE1 genes

RELATED INFORMATION
Hemoglobinopathies
Hemoglobinopathies Testing Algorithm
Thalassemias
Unstable Hemoglobinopathies

RELATED TESTS
Hemoglobin Evaluation Reflexive Cascade 2005792
Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility/Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer/Sequencing

Beta Globin (HBB) Sequencing, Fetal 0050388
Method: Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing 2001961
Method: Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing, Fetal 2001980
Method: Polymerase Chain Reaction/Sequencing