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

Disease Overview

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

Phenotypes caused by *HBB* variants

Phenotype	Characteristics
Thalassemia: decrease in protein produced	β thalassemia minor (trait) • Usually clinically asymptomatic, mild anemia may be present • Minor hematologic anomalies, including reduced MCV and elevated HbA2 β thalassemia major • Associated with severe microcytic anemia and hepatosplenomegaly • Affected individuals are transfusion dependent β thalassemia intermedia • Milder clinical presentation than β thalassemia major

MCV, mean corpuscular volume

Tests to Consider

Beta Globin (HBB) Sequencing and Deletion/Duplication 2010117

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

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

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
- Identify or confirm abnormal Hb variant(s) detected by HPLC or Hb electrophoresis

Test Description

Polymerase chain reaction (PCR) amplification and bidirectional sequencing of HBB coding regions, intron/exon boundaries, 5' proximal promoter and untranslated region, 3' polyadenylation signal, and intronic positions c.93-21 (IVS-I-110), c.316-197 (IVS-II-654), c.316-146 (IVS-II-705), c.316-106 (IVS-II-745), and c.316-86_316-85 (IVS-II-765 L1)

- Includes analysis of the following pathogenic intronic variants: c.93-21G>A, c.316-197C>T, c.316-146T>G, c.316-106C>G, and c.316-86_316-85insCTGCTTTTATTT

Beta Globin (HBB) Gene Sequencing 0050578

Method: Polymerase Chain Reaction/Sequencing

Molecular confirmation of a suspected structural hemoglobinopathy or β thalassemia

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

Phenotype	Characteristics
Hemoglobinopathy: structurally abnormal protein	Sickling disorders • Sickle cell anemia (HbSS) • Hb S-C disease Microcytic or hemolytic anemia Cyanosis • Reduced oxygen-affinity Hbs Erythrocytosis • Increased oxygen-affinity Hbs No clinical effect
Hereditary persistence of fetal Hb (HPFH)	Persistent HbF production resulting from variants of β-globin gene cluster that alter normal Hb switching Clinically benign condition

MCV, mean corpuscular volume

Genetics

Gene

HBB

Inheritance

Autosomal recessive (typically)

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

- Identify or confirm abnormal Hb variant(s) detected by HPLC or Hb electrophoresis

Test Description

Polymerase chain reaction (PCR) amplification and bidirectional sequencing of HBB coding regions, intron/exon boundaries, 5' proximal promoter and untranslated region, 3' polyadenylation signal, and intronic positions c.93-21 (IVS-I-110), c.316-197 (IVS-II-654), c.316-146 (IVS-II-705), c.316-106 (IVS-II-745), and c.316-86_316-85 (IVS-II-765 L1)

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Beta Globin (HBB) Deletion/Duplication 2010113

Method: Multiplex Ligation-dependent Probe Amplification

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

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
    - Variably 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 Tests

Hemoglobin Evaluation Reflexive Cascade 2005792

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

Familial Mutation, Targeted Sequencing, Fetal 2001980

Method: Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing 2001961

Method: Polymerase Chain Reaction/Sequencing

Beta Globin (HBB) Sequencing, Fetal 0050388

Method: Polymerase Chain Reaction/Sequencing

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