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

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
- Confirm a specific *HBB* variant in parents prior to prenatal testing
- Prenatal diagnosis of β thalassemia or hemoglobinopathy

Test Description

- Polymerase chain reaction (PCR) amplification and bidirectional sequencing of *HBB* coding region, intron/exon boundaries, proximal promoter and untranslated regions, and deep intronic variants (IVS-II-654, IVS-II-705, IVS-II-745)
- Multiplex ligation-dependent probe amplification (MLPA) of the β-globin gene cluster (*HBB, HBD, HBG1, HBG2, HBE1*) and its locus control region

Tests to Consider

Typical testing strategy

- 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 hemoglobin electrophoresis

Primary tests

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*) Gene Sequencing 0050578
- Molecular confirmation of a suspected structural hemoglobinopathy or β thalassemia

Beta Globin (*HBB*) Deletion/Duplication 2010113
- Detects large deletions of the β-globin gene cluster associated with β thalassemia or hereditary persistence of fetal hemoglobin (HPFH)

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

Related tests

Beta Globin (*HBB*) Sequencing, Fetal 0050388
- Confirmatory genetic test on fetal specimens for prenatal detection of structural hemoglobinopathies and β thalassemia

Beta Globin (*HBB*) HbS, HbC, and HbE Mutations 0051421
- Confirm suspected HbS, HbC, or HbE variants

Beta Globin (*HBB*) HbS, HbC, and HbE Mutations, Fetal 0051422
- Genetic test on fetal specimens for prenatal detection of HbS, HbC, and HbE variants

Hemoglobin Evaluation Reflexive Cascade 2005792
- Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy
- Cascade reflex testing may include electrophoresis, solubility testing, and/or molecular analyses of the globin genes

Familial Mutation, Targeted Sequencing 2001961
- Useful when a pathogenic familial variant identifiable by sequencing is known

Familial Mutation, Targeted Sequencing, Fetal 2001980
- Fetal test to detect a previously characterized variant in a family member

Disease Overview

Prevalence

- ~5% of the world's population carries clinically important hemoglobin 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

Thalassemia – decrease in protein produced
- β thalassemia minor (trait)
  - Clinically asymptomatic
  - Minor hematologic anomalies, including reduced mean corpuscular volume (MCV) and elevated HbA_2

- 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

Hemoglobinopathy – structurally abnormal protein
- Sickling disorders
  - Sickle cell anemia (HbSS)
  - Hemoglobin S/C disease
- Microcytic or hemolytic anemia
- Cyanosis
  - Reduced oxygen-affinity hemoglobins
- Erythrocytosis
  - Increased oxygen-affinity hemoglobins
- No clinical effect

HPFH
- Variants of β-globin gene cluster that alter normal Hb switching at birth and result in persistent HbF production
- Clinically benign condition

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

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
- Positive − one or more *HBB* gene variant(s) detected
  - Heterozygous
    - Carrier of a structurally abnormal hemoglobin, β thalassemia, or a benign variant, depending on the specific variant identified
  - Homozygous or compound heterozygous
    - Variably affected, depending on the specific variants identified
- Negative – *HBB* gene variant not detected
  - Decreases possibility of β thalassemia, but does not exclude disease

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