

# Comprehensive Hemoglobin Analysis

## *HBA1/2 ( $\alpha$ -globin) and HBB ( $\beta$ -globin) mutation and deletion/duplication analysis and HBD ( $\delta$ -globin) and HBG1/2 ( $\gamma$ -globin) mutation analysis*

**Description:** Hemoglobin (Hb) is the oxygen-carrying protein within red blood cells (RBCs). It is composed of four globular protein subunits, called globins or globin chains, and four oxygen-binding heme groups, which are attached to each globin. The two main types of globins are the  $\alpha$ -globins and the  $\beta$ -globins, which are made in essentially equivalent amount in precursors of RBCs. Normal adult Hb (Hb A) has two  $\alpha$ -globins and two  $\beta$ -globins ( $\alpha_2\beta_2$ ). Genes on chromosomes 16 and 11 encode the  $\alpha$ -like globins and  $\beta$ -like globins, respectively. There are distinct embryonic, fetal, and minor adult analogs of the  $\alpha$ -globins and  $\beta$ -globins, all of which are encoded by separate genes. Different symbols and abbreviations may be used for the same gene, which can lead to confusion.

### Names, symbols and abbreviations for the adult and fetal globin genes

Name of Gene	Common Symbol	HGNC Symbol
<b>Beta-like globin genes (11p15)</b>		
Beta-globin gene	$\beta$	<i>HBB</i>
Delta-globin gene	$\delta$	<i>HBD</i>
A-gamma-globin gene	$\gamma^A$	<i>HBG1</i>
G-gamma-globin gene	$\gamma^G$	<i>HBG2</i>
<b>Alpha-like globin genes (16p13)</b>		
Alpha-1-globin gene	$\alpha 1$	<i>HBA1</i>
Alpha-2-globin gene	$\alpha 2$	<i>HBA2</i>

Disorders of Hb can be classified as qualitative or quantitative disorders. Qualitative abnormalities of Hb arise from mutations that change the amino acid sequence of the globin chain, thereby producing structural and

functional changes in the Hb. Qualitative abnormalities include: (1) decreased solubility, e.g., Hb S; (2) instability, e.g., Hb Hasharon; (3) increased oxygen affinity, e.g., Hb Chesapeake; (4) decreased oxygen affinity, e.g., Hb Kansas; and (4) altered oxidation state of the heme-coordinated iron, e.g., Hb M Saskatoon. Qualitative Hb disorders are often referred to as hemoglobinopathies, even though the term can technically apply to both qualitative and quantitative disorders. Qualitative Hb disorders can also be referred to as “structural variants”. Quantitative Hb disorders, in contrast, result from the decreased and imbalanced production of generally structurally normal globins. For example, if  $\beta$ -globin production is diminished by a mutation, there will be a relative excess of  $\alpha$ -globins. Such imbalanced production of  $\alpha$ - and  $\beta$ -globins damages RBCs and their precursors in the bone marrow. These quantitative Hb disorders produce the thalassemia syndromes. Some Hb mutations result in both qualitative and quantitative defects, e.g., Hb E and Hb Constant Spring.

## Disorders:

### $\alpha$ -thalassemia

$\alpha$ -thalassemia refers to a group of genetic disorders caused by mutations that decrease the production of normal  $\alpha$ -globin protein from *HBA1* and/or *HBA2* genes. The inheritance of  $\alpha$ -thalassemias is complex because each individual has 4 separate  $\alpha$ -globin genes (two on each chromosome 16). *HBA1*, the gene encoding  $\alpha 1$ -globin, and *HBA2*, the gene encoding  $\alpha 2$ -globin, are the two genes associated with  $\alpha$ -thalassemia. Approximately 5% of the world's population, particularly individuals from the Mediterranean and North Africa, Middle East, India and Southeast Asia are estimated to be carriers of a mutation in the  $\alpha$ -globin genes.

Deletion of all four  $\alpha$ -globin genes result in a condition known as Hb Barts syndrome (homozygous alpha thalassemia major), which is associated with hydrops fetalis and is generally seen only in patients of Southeast Asian descent. Mutations in three of four  $\alpha$ -globin genes result in classical Hb H disease which has highly variable clinical manifestations from mild anemia to moderately severe anemia with hepatosplenomegaly, jaundice and bony changes. Hb H disease can also occur due to coinheritance of  $\alpha$ -globin gene deletions and thalassemic  $\alpha$ -globin point mutations (e.g., Hb H-Constant Spring disease). If two of the four genes are inactivated, individuals are asymptomatic but have a mild, microcytic anemia ( $\alpha$ -thalassemia minor or trait); whether the dual deletions occur in cis or trans does not affect phenotype, but has significant genetic counseling implications because it clarifies which types of  $\alpha$ -thalassemias may occur in a future child. Specifically, partners who are carriers of two deletions in cis have an increased risk for Hb Barts in a future child, while partners who are carriers of two deletions in trans have an increased risk only for a child with  $\alpha$ -thalassemia minor. Carriers of a single mutation in *HBA1* or *HBA2* are known as silent carriers and are typically asymptomatic and have normal blood counts.

Deletions of either one or both *HBA* genes account for the majority of pathogenic mutations, while small nucleotide changes or other mutations occur less frequently. Seven specific deletions account for the majority of  $\alpha$ -thalassemias. The -3.7 and -4.2 deletions are due to recombination events which delete *HBA2*, while the larger deletions including the Southeast Asia (SEA), Filipino (FIL), Thai, Mediterranean (MED) and -20.5 remove both *HBA1* and *HBA2* genes in cis. Many other deletions are known to occur with lesser frequency. Point mutations in coding or regulatory regions can also cause  $\alpha$ -thalassemia, such as the Hb Constant Spring mutation, the poly(A) site mutation, and several others.

Co-segregating thalassemic mutations in the  $\beta$ -globin, *HBB*, may modify the  $\alpha$ -thalassemia phenotype. Conversely, co-segregating increases in the copy number (triplication or quadruplication) of the normally paired (duplicated)  $\alpha$ -globin genes, can modify a  $\beta$ -thalassemia phenotype.

### $\alpha$ -globin structural variants (hemoglobinopathies)

These variants arise from genetic defects that change the structure of the  $\alpha$ -globin chain of the Hb molecule. Many hemoglobinopathies are named but have no clinical significance ("benign variants"). Some can be clinically significant and cause potentially severe conditions, such as thalassemia (e.g., Hb Constant Spring). Other  $\alpha$ -globin variants may be unstable causing hemolytic anemia (e.g., Hb Evans), prone to methemoglobin formation (e.g., Hb M-Boston), have increased oxygen affinity causing polycythemia (e.g., Hb Chesapeake), and decreased oxygen affinity causing anemia or cyanosis (e.g., Hb Lyon-Bron).

## **$\beta$ -thalassemia**

$\beta$ -thalassemia refers to a group of genetic disorders caused by mutations that decrease the production of normal  $\beta$ -globin protein from the *HBB* genes.  $\beta$ -thalassemia mutations can be classified by decreased synthesis ( $\beta^+$ -thalassemia) or complete absence of synthesis ( $\beta^0$ -thalassemia) of the  $\beta$ -globin protein. Small nucleotide changes account for the majority of pathogenic mutations in *HBB*, while larger deletions or fusion genes are less common. Approximately 4% of the world's population, particularly individuals from the Mediterranean, Middle East, Southeast Asia and China, are estimated to be carriers of  $\beta$ -thalassemia.  $\beta^+$ -thalassemia has many genetic causes, but the most common types of mutations occur in the promoter, 5' or 3' untranslated region, splice sites, or polyadenylation signal, while  $\beta^0$ -thalassemia is typically caused by nonsense, frameshift, or splicing mutations.

Patients with  $\beta$ -thalassemia major have a severe or complete loss of  $\beta$ -globin synthesis, and become severely anemic as fetal Hb (Hb F) expression decreases during the first several months of life; repeated transfusions are necessary to sustain life. Patients with  $\beta$ -thalassemia major typically present in early childhood with severe anemia, hepatosplenomegaly, and failure to thrive and are at risk for a shortened life expectancy. In contrast, individuals with thalassemia intermedia present later in life and have milder anemia that requires transfusions only intermittently; associated genetic mutations are usually biallelic  $\beta^+$ -thalassemia mutations or compound heterozygous  $\beta^+/\beta^0$  mutations that result in diminished  $\beta$ -globin production. Patients with beta thalassemia trait or minor are asymptomatic and have a mild, hypochromic, microcytic anemia. In  $\beta$ -thalassemia, the clinical severity is a function of the number and type ( $\beta^0$  or  $\beta^+$ ) of genetic defects. Also, co-segregating increases or decreases in the copy number of the  $\alpha$ -globin genes, can modify a  $\beta$ -thalassemia phenotype.

## **$\beta$ -globin structural variants (hemoglobinopathies)**

These Hb variants result from mutations in *HBB* that cause abnormal structure of the  $\beta$ -globin chain. These mutations cause several clinically significant hemoglobinopathies, including Hb S (sickle Hb) and Hb C which are particularly common in individuals of African descent, and Hb E which is common in Southeast Asians. Sickle cell disease (SCD) is the name for a group of genetic blood disorders caused by Hb S. The most common and severe form of SCD is the homozygous state for the Hb S mutation ( $\beta^S$ ) called sickle cell anemia (Hb SS). Other forms of SCD are compound heterozygous states due to coinheritance of the  $\beta^S$  mutation with one of several other abnormal  $\beta$ -globin genes, including  $\beta$ -thalassemia mutations, Hb C, Hb D, Hb E, and Hb O. Coexisting copy number variations in  $\alpha$ -globin genes (inherited independently) may affect the phenotype of SCD, so testing for  $\alpha$ -thalassemia may be prognostic in patients with SCD. For example, two-gene deletion  $\alpha$ -thalassemia can decrease hemolytic rate and risk of stroke; however, it may also increase the frequency of painful episodes and avascular necrosis of bone. Homozygosity for Hb C (or Hb C /  $\beta$ -thalassemia) causes a mild hemolytic anemia. Homozygosity for Hb E (or Hb E /  $\beta$ -thalassemia) causes a thalassemia syndrome.

Other  $\beta$ -globin variants may be unstable causing hemolytic anemia (e.g., Hb Köln), prone to methemoglobin formation (e.g., Hb M-Saskatoon), have increased oxygen affinity causing polycythemia (e.g., Hb Montefiore), and decreased oxygen affinity causing anemia or cyanosis (e.g., Hb Kansas). Some  $\beta$ -globin variants may have multiple abnormalities, such as instability and altered oxygen affinity. Hyper-unstable  $\beta$ -globin variants can cause an autosomal dominant  $\beta$ -thalassemia syndrome (ineffective erythropoiesis and hemolysis).

### **Hereditary persistence of fetal hemoglobin (HPFH)**

HPFH is characterized by persistent elevation of fetal hemoglobin (Hb F) in adult red blood cells. In healthy individuals, elevated Hb F levels have no clinical significance. However, HPFH can be beneficial in patients with sickle cell anemia or  $\beta$ -thalassemia, as elevated Hb F can ameliorate the disease. HPFH is a genetically heterogeneous group of disorders with a number of different systems of classification. Very broadly, HPFH can be caused by large deletions in the  $\beta$ -globin gene cluster involving *HBB* (gene-deletion HPFH) or point mutations in the promoter regions of the  $\gamma$ -globin genes (non-gene-deletion HPFH). Gene-deletion HPFH is tested for by MLPA analysis of the  $\beta$ -globin gene cluster, whereas non-gene-deletion HPFH is tested for by sequence analysis of the  $\gamma$ -globin genes (*HBG1* and *HBG2*). Sickle cell anemia (Hb SS) with co-inherited non-gene-deletion HPFH is not the same condition as compound heterozygosity for Hb S and gene-deletion HPFH, the latter being a very mild or asymptomatic condition. Sequence analysis only of the  $\beta$ -globin genes will not differentiate these two conditions; both *HBB* sequence analysis and MLPA analysis are necessary. HPFH can also result from polymorphisms in genes outside the  $\beta$ -globin gene cluster (e.g., *BCL11A*), which are not tested in this assay.

### **Fetal or gamma-globin (*HBG1/2*) disorders**

Hb F structural variants arise from mutations that change the amino acid sequence of the  $\gamma$ -globin chain of the fetal Hb molecule ( $\alpha_2\gamma_2$ ). Mutations may occur in either of the two linked  $\gamma$ -globin genes (*HBG1*, *HBG2*). Many fetal variants are named yet have no clinical significance (e.g., Hb F-Hull), but they are detected on newborn screening as an abnormal finding that necessitates further work-up. There are a few clinically significant  $\gamma$ -globin variants. These may be unstable causing hemolytic anemia (e.g., Hb F-Poole), prone to methemoglobin formation (e.g., Hb F-M

Osaka), have increased oxygen affinity (e.g., Hb F-La Grange), or decreased oxygen affinity causing cyanosis (e.g., Hb F-Cincinnati). These variants can all be identified by sequence analysis of the *HBG1/2* genes.

Some Hb F disorders can cause a transient neonatal thalassemia syndrome with prominent hemolysis, especially large deletions such as  $\gamma\beta$ -thalassemia or  $\epsilon\gamma\delta\beta$ -thalassemia. Such large deletions are tested for by MLPA (deletion/duplication analysis) of the  $\beta$ -globin gene cluster, rather than sequencing of the *HBG1/2* genes alone.

### **Delta-globin (HBD) disorders**

Mutations in the delta-globin gene (*HBD*) alone are not pathologically relevant. However, there are a number of scenarios in which HBD mutations may obscure the diagnosis of an underlying blood disorder. Most importantly,  $\delta$ -thalassemia can mask the diagnosis of  $\beta$ -thalassemia. In  $\beta$ -thalassemia, there is usually an increase in hemoglobin A<sub>2</sub> levels, while in  $\delta$ -thalassemia (thalassemic HBD mutations) there is usually a decrease in hemoglobin A<sub>2</sub> levels. The co-existence of  $\delta$ -thalassemia and  $\beta$ -thalassemia mutations can result in hemoglobin A<sub>2</sub> levels in the normal range, thereby obscuring the diagnosis of  $\beta$ -thalassemia.  $\delta$ -thalassemia can also lead to a mistaken diagnosis of  $\alpha$ -thalassemia or iron deficiency anemia, because all three conditions are associated with low levels of hemoglobin A<sub>2</sub>.

HBD structural variants may produce abnormalities on hemoglobin electrophoresis that might be unidentified by standard methods or mistaken for other low-abundance pathologic hemoglobin variants. Finally, the *HBD* gene may be involved in large deletions (e.g.,  $\delta\beta$ -thalassemia, gene-deletion hereditary persistence of fetal hemoglobin) or complex rearrangements (e.g., Lepore hemoglobins). Such large deletions and rearrangements are best tested for by MLPA (duplication/deletion analysis) of the  $\beta$ -globin gene cluster, rather than sequencing of the *HBD* gene alone.

## Additional Resources:

The HbVar database <http://globin.cse.psu.edu/globin/hbvar/> is a resource that can help correlate specific *HBA1/2*, *HBB*, *HBD* and *HBG1/2* mutations with clinical expression.

## Indications:

- Anemia
- Hemolysis
- Cyanosis
- Hemoglobin desaturation
- Methemoglobinemia
- Polycythemia
- Confirmation of diagnosis in a symptomatic individual
- Carrier/heterozygote detection in individuals with a family history of thalassemia or hemoglobinopathy
- Prenatal diagnosis of an at-risk fetus after confirmation of mutations in the parents (by prior arrangement only)
- Abnormal hemoglobin electrophoresis or newborn screen suggestive of thalassemia or hemoglobinopathy

## Testing Methodology:

Large deletions, which account for approximately 90% of mutations in *HBA* and less than 10% of mutations in *HBB*, are detected by multiple ligation-dependent probe amplification (MLPA) analysis.

Point mutations, small deletions and small insertions which account for approximately 10% of *HBA* and 90% of *HBB* mutations are detected by PCR-based sequencing of the entire coding regions and intron/exon boundaries of the *HBA1/2* and/or *HBB* genes and/or the promoters, entire coding regions and intron/exon boundaries of the *HBD* and/or *HBG1/2* genes.

## Test Sensitivity:


The sensitivity of deletion analysis by MLPA is over 90% for large deletions while the sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Large exonic deletions, large insertions and genetic recombination events may not be identified by this test. Rare primer site variants may lead to erroneous results with either methodology. Parental studies are sometimes necessary to determine the phase of identified sequence variants and their clinical significance.

**Specimen:** Collect two tubes of whole blood, 5 mL in lavender top (EDTA) tube and 2 mL in lavender top (EDTA) tube, for hemoglobin disorder testing. Label each tube with patient's name, birth date, and date of collection. Buccal swabs may be accepted when a blood sample is not easily obtained. Please call for a free buccal (cytobrush) collection kit.

**Turn-Around Time:** 28 days; when needed for interpretation, parental analyses may extend the turn-around time.

## CPT Codes:

<i>HBA1/2</i> locus deletion/duplication analysis	81404
<i>HBA1/2</i> full gene sequencing	81405
<i>HBB</i> locus deletion/duplication analysis	81403
<i>HBB</i> full gene sequencing	81404
*Comprehensive Alpha ( <i>HBA1/2</i> ) and Beta ( <i>HBB</i> ) globin gene locus analysis includes deletion/duplication and full gene sequencing of both <i>HBA1/2</i> and <i>HBB</i> and will be billed using the CPT codes as specified above.	
<i>HBD</i> full gene sequencing	81479
<i>HBG1/2</i> gene sequencing	81479x2
Targeted analysis (family study)	81403



**Results:** Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for clinical management and additional testing, if warranted. Biochemical and functional test results will be incorporated, when available. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

### **Shipping Instructions**

Please enclose **test requisition** with sample. **All information must be completed before sample can be processed.** Place samples in Styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

### **Ship to:**

Cytogenetics and Molecular Genetics Laboratories  
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