Comprehensive Hemoglobin Analysis
HBA1/HBA2 and *HBB* Mutation Analysis

Disorders:

α-thalassemia is a genetic disorder which is caused by mutations in the *HBA1* or *HBA2* genes. α-thalassemia mutations decrease the production of the α-globin chain of hemoglobin, which in conjunction with β-globin, is essential to the formation of red blood cells and delivery of oxygen throughout the body. The inheritance of α-thalassemias is complex because each individual has 4 separate α-globin genes (two on each chromosome 16). *HBA1*, the gene encoding α1-globin, and *HBA2*, the gene encoding α2-globin, are the two genes associated with α-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 α- globin genes.

Deletion of all four α-globin genes result in a condition known as Hb Bart 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 α-globin genes result in Hemoglobin H disease which has highly variable clinical manifestations from mild anemia to severe anemia with hepatosplenomegaly, jaundice and bony changes. If two of the four genes are inactivated, individuals are asymptomatic but have a mild, microcytic anemia (α-thalassemia minor or trait); whether the dual deletions occur in cis or trans does not affect phenotype, but has significant genetic counseling implications. Carriers of a single mutation in *HBA1* or *HBA2* are known as silent carriers and are typically asymptomatic and have normal blood counts.

Large deletions of either one or both *HBA* genes account for the majority of pathogenic mutations in these genes while small nucleotide changes or other mutations are relatively rare. α-globin genes may also be triplicated or quadruplicated. Seven specific deletions account for the majority of α-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. Large deletions result in a compensatory mechanism which upregulates protein production from the normal remaining cis allele. Co-segregating mutations in the gene for β-thalassemia, *HBB*, or triplication of the α-thalassemia genes may influence the predicted phenotype.

α-hemoglobinopathies are genetic defects which result in abnormal structure of the α-globin chain of the hemoglobin molecule. Many hemoglobinopathies are not clinically significant while a very few such as HbH-Constant Spring, cause serious sequalae, especially when coinherited with deletional α- thalassemia mutations.

β-thalassemia is a genetic disorder which is caused by mutations in the *HBB* gene. β-thalassemia mutations decrease the production of the β-globin chain of hemoglobin, which in conjunction with α-globin, is essential to the formation of red blood cells and delivery of oxygen throughout the body. β-thalassemia is characterized by decreased synthesis (β+-thalassemia) or complete absence (β°-thalassemia) of the hemoglobin beta chain, resulting in an accumulation of the α-subunit. This overabundance of α-globin results in ineffective erythropoiesis, microcytic hypochromic anemia, and an abnormal peripheral blood smear. Small nucleotide changes account for the majority of pathogenic mutations in *HBB*, while larger deletions of fusions genes are much rarer. These mutations change the structure of the β-globin itself but do not necessarily decrease its synthesis. Approximately 4% of the world's population, particularly individuals from the Mediterranean, Middle East, Southeast Asia and China,

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Disorders continued:

are estimated to be carriers of a mutation in *HBB* which causes β-thalassemia. β+-thalassemia arises largely from mutations in the promoter, 5' or 3' untranslated region splice sites, or polyadenylation signal, while β°-thalassemia is associated with nonsense, frameshift, or splicing mutations.

Patients with β-thalassemia major have a severe or complete loss of β-globin, and become severely anemic as fetal globin expression decreases during the first several months of life; repeated transfusions are necessary to sustain life. Patients with β-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 β+-thalassemia mutation that result in diminished β-globin production. Compound heterozygotes who carry one mutation associated with β-thalassemia major and one mutation associated with β-thalassemia intermedia are likely to experience symptoms similar to β–thalassemia major, but there is great variability in the clinical expression of these mutations.

β-hemoglobinopathies result from mutations in *HBB* which cause abnormal structure of the β-globin chain. These mutations cause several clinically significant hemoglobinopathies, including HbS (sickle Hb) and HbC which are particularly common in individuals of African descent, and HbE which is common in Southeast Asians. Some large deletions that encompass the β-globin gene greatly increase the production of fetal Hb (HbF) from the upstream γ-globin genes. Many β-hemoglobinopathies are not clinically significant, producing a Hb variant without any abnormal function. Coexisting copy number variations in α-globin genes (inherited independently) may affect the phenotype associated with *HBB* mutations.

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

Additional information and test requisitions are available at: www.cchmc.org/molecular-genetics

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
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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.

Indications:

- 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 suggestive of thalassemia or hemoglobinopathy

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:

At least 3 mL whole blood in lavender top (EDTA) tube for each test ordered. Label 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:

HBA1/2 deletion/duplication analysis	81404
HBA1/2 full gene sequencing	81405
HBB deletion/duplication analysis	81403
HBB full gene sequencing	81404

* Comprehensive analysis includes deletion/duplication and full gene sequencing of both *HBA1/2* and *HBB* and will be billed using the CPT codes as specified above.

Targeted analysis (family study)	81403

Results:

Results will be reported to the referring physician or health care provider as specified on the requisition form.