## Alpha Thalassemia

### Indications for Ordering

- Carrier screening
  - Healthy individuals of African, Mediterranean, Middle Eastern, and Southeast Asian descent
  - Individuals with a family history of alpha (α) thalassemia
  - Individuals with reproductive partners who are affected with, or carriers of, α thalassemia
  - Individuals with microcytosis and no identified iron deficiency

- Diagnostic testing
  - Confirm a clinical diagnosis of hemoglobin (Hb) Bart hydrops fetalis syndrome or Hb H disease
  - Confirm the identity of a variant detected by Hb evaluation that may be pathogenic or benign

### Test Description

- Targeted testing for common deletions
  - PCR/gel electrophoresis
  - 7 common deletions of HBA1 and HBA2 genes (-α3.7, -α4.2, -(α)20.5, --SEA, --MED-I, --FIL, and --THAI)

- Deletion/duplication analysis by multiplex ligation-dependent probe amplification of the α-globin gene cluster (HBZ, HBM, HBA1, HBA2, HBQ1) and its HS-40 regulatory region

- Sequencing by PCR amplification followed by bidirectional sequencing of
  - HBA1 and HBA2 coding regions
  - Intronic/exonic boundaries
  - Proximal promoter regions
  - 5’ and 3’ untranslated regions
  - Polyadenylation signals

### Tests to Consider

**Typical testing strategy**

- Molecular analysis for large deletions in the α-globin genes
- Consider α-globin gene sequencing if
  - Deletion testing does not explain the clinical phenotype OR
  - Hb evaluation suggests the presence of an abnormal α-chain variant

### Primary tests

- **Alpha Globin (HBA1 and HBA2) Deletion/Duplication 2011622**
  - Preferred first-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait
  - Detects common, rare, and novel deletions or duplications in the α-globin gene cluster

- **Alpha Thalassemia (HBA1 and HBA2) 7 Deletions 0051495**
  - Acceptable first-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait

- **Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication 2011708**
  - Comprehensive genetic test for detection of α thalassemia or α thalassemia trait
  - Detects deletional and nondeletional variants in HBA1 and HBA2

### Related tests

- **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 analysis of the globin genes

- **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 Hemoglobin Evaluation Reflexive Cascade

- **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 sequence variant in a family member

### Disease Overview

**Prevalence and/or incidence**

- Most common inherited disorder of Hb worldwide
- Carrier frequencies in high-risk populations
  - African, African American (1/3)
  - Middle Eastern, Southeast Asian (1/20)
  - Mediterranean (1/30-50)
- Hb Bart hydrops fetalis syndrome and Hb H disease
  - More frequent in Southeast Asian, Asian Indian, and Mediterranean populations than in African populations
Symptoms
- α thalassemia silent carrier
  o Typically asymptomatic
    - Borderline anemia or mild microcytosis may be present
    - Often misdiagnosed as iron deficiency
  o Normal Hb electrophoresis
- α thalassemia trait
  o Mild microcytic anemia may be present
    - Often misdiagnosed as iron deficiency
  o Normal Hb electrophoresis
- Hb H disease
  o Moderate to severe form of α thalassemia
  o Moderate microcytic hypochromic anemia
  o Hemolysis with Heinz bodies
  o Splenomegaly
  o Rare extramedullary hematopoiesis
  o Propensity for acute hemolysis after oxidative stress, drug therapy, or infection
- Hb Bart hydrops fetalis syndrome
  o Most severe form of α thalassemia
  o Fetus
    - Lethal in fetal or early neonatal period
    - Generalized edema, ascites, pleural and pericardial effusions
    - Severe hypochromic anemia
    - Usually detected on ultrasound at 22-28 weeks gestation
  o Maternal complications during pregnancy
    - Preeclampsia
    - Polyhydramnios or oligohydramnios
    - Antepartum hemorrhage
    - Premature delivery

Physiology
- Typically, individuals have four functioning α-globin genes (αα/αα)
  o Two genes, HBA1 and HBA2, are present on each copy of chromosome 16
  o α-globin chains function as subunits of fetal Hb (Hb F – α2γ2) and adult Hb (Hb A – α2β2)
- Genotype/phenotype correlations in α thalassemia are complex and may be influenced by coinheritance of other Hb variants or α-globin gene duplications
- α thalassemia silent carrier
  o One nonfunctional α-globin gene (−α/αα)
- α thalassemia trait
  o Two nonfunctional α-globin genes in trans (−α/−α) or cis (−/−αα)
- Hb H disease
  o Three nonfunctional α-globin genes (−/−α)
  o Hb Bart hydrops fetalis syndrome
  o Four nonfunctional α-globin genes (−/−−/−)

Genetics

Genes – HBA1 and HBA2

Inheritance – autosomal recessive

Variants
- HBA1 and HBA2 large gene deletions account for up to 90% of α thalassemia
  o −α3.7 and −α4.2 deletions result in the deletion of a single gene
  o −(α−)20.5, −SEA, −MED-I, −FL, and −THAI deletions result in the deletion of the HBA1 and HBA2 genes from the same chromosome
- Point variants and regulatory region variants occur mainly in HBA2 and account for up to 10% of causative variants
  o Nondeletional variants include
    - Point variants that inactivate the gene
    - Small insertions/deletions
    - Variants that result in unstable α-globin protein (eg, Hb Constant Spring)
  o Nondeletional α-globin variants may be pathogenic or benign
    - Both may result in an abnormal protein detectable by Hb evaluation
    - Pathogenic nondeletional variants often have a more severe effect than single gene deletions
- α-globin gene triplications result in three active α-globin genes on a single chromosome
  o Typically benign
  o May alter expected clinical phenotypes and hematological features when co-inherited with β thalassemia

Test Interpretation

Sensitivity/specificity
- Analytical sensitivity/specificity – 99% for both duplication/deletion analysis and sequencing
- Clinical sensitivity – most pathogenic HBA1 and/or HBA2 gene variants are large deletions not detectable by sequencing
  o Deletion ≤90%, depending on ethnicity (Origa, 2013)
  o Sequencing ≤9%, depending on ethnicity (Origa, 2013)

Results and limitations – see table

References
<table>
<thead>
<tr>
<th>Negative result</th>
<th>Alpha Thalassemia (HBA1 and HBA2) 7 Deletions</th>
<th>Alpha Globin (HBA1 and HBA2) Deletion/Duplication</th>
<th>Alpha Globin (HBA1 and HBA2) Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>● No common α-globin gene deletions were detected</td>
<td>● No large α-globin deletions or duplications were detected</td>
<td>● No pathogenic variants were detected</td>
<td></td>
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<tr>
<td>○ Risk for α thalassemia is reduced but not excluded</td>
<td>○ Risk for α thalassemia is reduced but not excluded</td>
<td>○ Large deletions of the α-globin genes, which account for the majority of variants, are not detected by sequencing</td>
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</table>

| Positive result | | | |
|-----------------|---------|----------------|
| ● Predicted genotype (-α/αα) | ● Predicted genotype (-α/αα) | ● One pathogenic variant detected |
| ○ Individual is predicted to be a silent carrier | ○ Individual is predicted to have α thalassemia trait | ○ Individual is predicted to be a carrier of α thalassemia |
| ● Predicted genotype (-α/-α) or (-/-αα) | ● Predicted genotype (-/-αα) | ○ A more severe disorder is possible if another undetected α-globin variant is present |
| ○ Individual is predicted to have α thalassemia trait | ○ Individual is predicted to have α thalassemia trait | ○ Two pathogenic variants detected |
| ● Predicted genotype (-/-α) | ● Predicted genotype (-/-α) | ○ Individual is predicted to be a carrier of α thalassemia; mild microcytic anemia often present |
| ○ Individual is predicted to be affected with Hb H disease | ○ Individual is predicted to be affected with Hb H disease | ○ Homozygosity or compound heterozygosity for nondeletional variants results rarely in Hb H disease |
| ● Predicted genotype (-/-/-) | ● Predicted genotype (-/-/-) | | |
| ○ Result is consistent with Hb Bart hydrops fetalis syndrome | ○ Result is consistent with Hb Bart hydrops fetalis syndrome | | |
| ● Predicted genotype (ααα/ααα) | | | |
| ○ An extra functional α-globin gene present | | | |

<table>
<thead>
<tr>
<th>Inconclusive result</th>
<th>Deletion or duplication of unknown clinical significance detected</th>
<th>Variant of unknown clinical significance detected</th>
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<table>
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<tr>
<th>Limitations</th>
<th>Alpha Thalassemia (HBA1 and HBA2) 7 Deletions</th>
<th>Alpha Globin (HBA1 and HBA2) Deletion/Duplication</th>
<th>Alpha Globin (HBA1 and HBA2) Sequencing</th>
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<tbody>
<tr>
<td>● Rare α-globin gene deletions, nondeletional variants, gene duplications and variants of the regulatory region will not be detected</td>
<td>● Breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish variants of similar size</td>
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<tr>
<td>● Diagnostic errors can occur due to rare sequence variations</td>
<td>● This assay does not assess for nondeletional variants within the coding or regulatory regions of the α-globin cluster genes</td>
<td>● Large deletions/duplications and some variants of the regulatory regions will not be detected</td>
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<tr>
<td>● Rare syndromic or acquired forms of α thalassemia will not be detected</td>
<td>● Individuals carrying both a deletion and duplication within the α-globin gene cluster may appear to have a normal number of α-globin gene copies</td>
<td>● The phase of identified variants may not be determined</td>
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<tr>
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<td>● Rare syndromic or acquired forms of α thalassemia associated with ATRX variants will not be detected</td>
<td>● Diagnostic errors can occur due to rare sequence variations</td>
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<td>● Sequencing of both HBA1 and HBA2 may not be possible in individuals harboring large α-globin deletions on both alleles</td>
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<td>● Rare syndromes associated with α thalassemia, such as ATR-X and ATR-16, will not be detected</td>
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