Alpha (α) thalassemia is the most common inherited disorder of hemoglobin (Hb) worldwide and is caused by HBA1 and HBA2 gene variants. Decreased or absent synthesis of the hemoglobin (Hb) α chain results in clinical presentations ranging from asymptomatic silent carriers to severe anemia and fetal lethality. The two clinically significant forms of α thalassemia are Hb Bart hydrops fetalis syndrome and hemoglobin H (HbH) disease. α Thalassemia is found more often in certain ethnicities, including African, African American, Mediterranean, Middle Eastern, and Southeast Asian.

### 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 HbH disease are more frequent in Southeast Asian, Asian Indian, and Mediterranean populations than in African populations.

#### Symptoms

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Associated Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Thalassemia silent carrier</td>
<td>Typically asymptomatic, though borderline anemia or mild microcytosis may be present. Often misdiagnosed as iron deficiency. Normal Hb electrophoresis.</td>
</tr>
<tr>
<td>α-Thalassemia trait</td>
<td>Mild microcytic anemia may be present. Often misdiagnosed as iron deficiency. Normal Hb electrophoresis.</td>
</tr>
<tr>
<td>HbH disease</td>
<td>Moderate to severe form of α thalassemia. Moderate microcytic hypochromic anemia. Hemolysis with Heinz bodies. Splenomegaly. Rare extramedullary hematopoeisis. Propensity for acute hemolysis after oxidative stress, drug therapy, or infection.</td>
</tr>
</tbody>
</table>

### Tests to Consider

#### Alpha Thalassemia (HBA1 and HBA2)
- **Deletion/Duplication with reflex to Hb Constant Spring** 11/1/2021-003651
  - **Method**: Multiplex Ligation-dependent Probe Amplification with Sanger sequencing confirmation of HbCS
  - Preferred first-tier genetic test for confirmation of suspected α thalassemia or α-thalassemia trait.
  - Detect common, rare, and novel deletions or duplications in the α-globin gene cluster and its HS-40 regulatory region.
  - Multiplex ligation-dependent probe amplification (MLPA) used to detect Hb Constant Spring (HBA2 c.427T>C; p.Ter143Gln); targeted Sanger sequencing is performed to assess HbCS copy number in absence of a concurrent HBA2 deletion.

#### Alpha Globin (HBA1 and HBA2)
- **Deletion/Duplication** 2011622
  - **Method**: Multiplex Ligation-dependent Probe Amplification
  - First-tier genetic test for confirmation of suspected α-thalassemia or α-thalassemia trait.
  - Detect common, rare, and novel deletions or duplications in the α-globin gene cluster and its HS-40 regulatory region.

#### Alpha Globin (HBA1 and HBA2)
- **Sequencing and Deletion/Duplication** 2011708
  - **Method**: Polymerase Chain Reaction/Sequencing./Multiplex Ligation-dependent Probe Amplification.
  - Comprehensive genetic test for detection of α-thalassemia or α-thalassemia trait.
  - Detect deletional and nondeletional variants in HBA1 and HBA2.

#### Alpha Thalassemia (HBA1 and HBA2)
- **Deletion/Duplication with reflex to Hb Constant Spring, Fetal** 3003656
  - **Method**: Multiplex Ligation-dependent Probe Amplification with Sanger sequencing confirmation of HbCS
  - Diagnostic testing for α thalassemia in fetus with suggestive clinical findings or at risk for...
Pathophysiology

Typically, individuals have four functioning α-globin genes (αα/αα). Two genes, HBA1 and HBA2, are present on each copy of chromosome 16, and α-globin chains function as subunits of fetal Hb (HbF: α2γ2) and adult Hb (HbA: α2β2). The number of α-globin genes deleted or inactivated correlates with different α-thalassemia phenotypes.

Genotype/phenotype correlations in α-thalassemia are complex and may be influenced by coinheritance of other Hb variants or α-globin gene duplications.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Thalassemia silent carrier</td>
<td>-α/-α</td>
</tr>
<tr>
<td>α-Thalassemia trait</td>
<td>-α/-α</td>
</tr>
<tr>
<td></td>
<td>--/-α</td>
</tr>
<tr>
<td>HbH disease</td>
<td>--/-α</td>
</tr>
<tr>
<td>Hb Bart hydrops fetalis syndrome</td>
<td>--/--</td>
</tr>
</tbody>
</table>

Genetics

Genes

HBA1 and HBA2

Inheritance

Autosomal recessive

Variants

- HBA1 and HBA2 large gene deletions account for approximately 90% of pathogenic α-thalassemia variants.
  - -α3.7 and -α4.2 deletions result in the deletion of a single gene.
  - -(α)20.S, -SEα, -MED-I, -FIL, and -THAI deletions result in the deletion of the HBA1 and HBA2 genes from the same chromosome.
- Sequence variants and regulatory region variants occur mainly in HBA2 and account for up to 15% of causative variants.
  - Nondeletional variants include:
    - Sequence variants that inactivate the gene
    - Small insertions/deletions
    - Variants that result in unstable α-globin protein (e.g., Hb Constant Spring)
  - 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 duplication results in three or more active α-globin genes on a single chromosome.
  - Typically benign
  - May alter expected clinical phenotypes and hematological features when co-inherited with beta (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
  - Deletion: at least 90%, varies by ethnicity
  - Sequencing: up to 15%, varies by ethnicity

<table>
<thead>
<tr>
<th>Results and Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha Thalassemia (HBA1 and HBA2) 7 Deletions</td>
</tr>
</tbody>
</table>

1. ATR-16, alpha-thalassemia-intellectual disability, chromosome 16-related; ATR-X, alpha-thalassemia-X linked intellectual disability; n/a, not applicable
<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></td>
<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>
</tr>
<tr>
<td></td>
<td>• Risk for a thalassemia is reduced but not excluded</td>
<td>• Risk for a thalassemia is reduced but not excluded</td>
<td>• Risk for a thalassemia is reduced</td>
</tr>
<tr>
<td></td>
<td>• Large deletions of the α-globin genes, which account for the majority of variants, are not detected by sequencing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive result</td>
<td>Predicted genotype (-α/αα)</td>
<td>Predicted genotype (-α/αα)</td>
<td>1 pathogenic variant detected</td>
</tr>
<tr>
<td></td>
<td>• Individual is predicted to be a silent carrier</td>
<td>• Individual is predicted to be a silent carrier</td>
<td>• Individual is predicted to be a silent carrier or carrier of α-thalassemia</td>
</tr>
<tr>
<td></td>
<td>Predicted genotype (-α/-α) or (-/αα)</td>
<td>Predicted genotype (-α/-α) or (-/αα)</td>
<td>• A more severe disorder is possible if another undetected α-globin variant is present</td>
</tr>
<tr>
<td></td>
<td>• Individual is predicted to have α-thalassemia trait</td>
<td>• Individual is predicted to have α-thalassemia trait</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predicted genotype (-/α)</td>
<td>Predicted genotype (-/α)</td>
<td>2 pathogenic variants detected</td>
</tr>
<tr>
<td></td>
<td>• Individual is predicted to be affected with HbH disease</td>
<td>• Individual is predicted to be affected with HbH disease</td>
<td>• Individual is predicted to be a carrier of α-thalassemia; mild microcytic anemia often present</td>
</tr>
<tr>
<td></td>
<td>Predicted genotype (-/-)</td>
<td>Predicted genotype (-/-)</td>
<td>• Homozygosity or compound heterozygosity for nondeletional variants rarely results in HbH disease</td>
</tr>
<tr>
<td></td>
<td>• Result is consistent with Hb Bart hydrops fetalis syndrome</td>
<td>• Result is consistent with Hb Bart hydrops fetalis syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predicted genotype (ααα/αα)</td>
<td>Predicted genotype (ααα/αα)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• An extra functional α-globin gene present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive result</td>
<td>n/a</td>
<td>Deletion or duplication of unknown clinical significance detected</td>
<td>Variant of unknown clinical significance detected</td>
</tr>
</tbody>
</table>

**Limitations**

- Rare α-globin gene deletions, nondeletional variants, gene duplications and variants of the regulatory region will not be detected
- Diagnostic errors can occur due to rare sequence variations
- Rare syndromic or acquired forms of α-thalassemia will not be detected
- Breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish variants of similar size
- This assay does not assess for nondeletional variants within the coding or regulatory regions of the α-globin cluster genes
- 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
- Rare syndromic or acquired forms of α-thalassemia associated with ATRX variants will not be detected
- Diagnostic errors can occur due to rare sequence variations
- Large deletions/duplications and some variants of the regulatory regions will not be detected
- The phase of identified variants may not be determined
- Diagnostic errors can occur due to rare sequence variations
- Sequencing of both HBA1 and HBA2 may not be possible in individuals harboring large α-globin deletions on both alleles
- Rare syndromes associated with α-thalassemia, such as ATR-X and ATR-16, will not be detected

ATR-16, α-thalassemia-intellectual disability, chromosome 16-related; ATR-X, α-thalassemia X-linked intellectual disability; n/a, not applicable

**References**


**Additional Resources**


**Related Tests**
Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610

**Method:** High Performance Liquid Chromatography/Electrophoresis/RBC Solubility

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 2001961

**Method:** Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing, Fetal 2001980

**Method:** Polymerase Chain Reaction/Sequencing

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