Genetic testing to confirm a diagnosis of α-thalassemia is considered not medically necessary.

Genetic testing of patients with hemoglobin H disease (α-thalassemia intermedia) to determine prognosis is considered investigational.

Preconception (carrier) testing for α-thalassemia in prospective parents may be considered medically necessary when both parents have evidence of possible α-thalassemia (including α-thalassemia minor, hemoglobin H disease [α-thalassemia intermedia], or α-thalassemia major) based on biochemical testing (see Policy Guidelines section).

Genetic testing for α-thalassemia in other clinical situations (recognizing that prenatal testing is not addressed in this policy) is considered investigational.

**POLICY GUIDELINES**

Biochemical testing to determine whether α-thalassemia is present should be the first step in evaluating the presence of the condition. Biochemical testing consists of complete blood count (CBC), microscopic examination of the peripheral blood smear, and hemoglobin electrophoresis. In silent carriers and in α-thalassemia trait, the hemoglobin electrophoresis will most likely be normal. However, there should be evidence of possible α-thalassemia minor on the CBC and peripheral smear.

The probability of a pregnancy with hemoglobin Bart’s (α-thalassemia major) depends on the specific genotype found in each parent. Table PG1 summarizes the risk according to each category of α-thalassemia.
Table PG1. Risk of α-Thalassemia

<table>
<thead>
<tr>
<th>Clinical Diagnosis in Parents</th>
<th>Genotype (Parent 1)</th>
<th>Genotype (Parent 2)</th>
<th>Probability of Hemoglobin Bart’s, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both parents silent carriers</td>
<td>αα/α-</td>
<td>αα/α-</td>
<td>0</td>
</tr>
<tr>
<td>One parent silent carrier, 1 parent trait</td>
<td>αα/α-</td>
<td>α/-/α-</td>
<td>0</td>
</tr>
<tr>
<td>Both parents trait</td>
<td>αα/--</td>
<td>αα/--</td>
<td>25</td>
</tr>
<tr>
<td>One parent HbH, 1 parent silent carrier</td>
<td>α/-/α-</td>
<td>αα/-/α-</td>
<td>0</td>
</tr>
<tr>
<td>One parent HbH, 1 parent trait</td>
<td>α/-/α-</td>
<td>αα/-/α-</td>
<td>25</td>
</tr>
<tr>
<td>Both parents HbH</td>
<td>α/-/α-</td>
<td>α/-/α-</td>
<td>25</td>
</tr>
</tbody>
</table>

HbH: hemoglobin H.

This policy does not address prenatal (in utero or preimplantation) genetic testing for α-thalassemia.

Genetics Nomenclature Update

Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG2). HGVS nomenclature is recommended by HGVS, the Human Variome Project, and the HUman Genome Organization (HUGO).

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from ACMG, AMP, and the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG3 shows the recommended standard terminology—“pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”—to describe variants identified that cause Mendelian disorders.

Table PG2. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA sequence</td>
<td></td>
</tr>
<tr>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
<td></td>
</tr>
</tbody>
</table>

Table PG3. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
</tbody>
</table>
**Genetic Testing for α-Thalassemia**

<table>
<thead>
<tr>
<th>Likely benign</th>
<th>Likely benign change in the DNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

**Genetic Counseling**

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual’s family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

**Coding**

There is a tier 1 molecular pathology CPT code for testing for common deletions or variants:

81257 - HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)

81258 Known familial variant (new codes effective 01/01/18)

81259 Full gene sequence (new code effective 01/01/18)

81269 Duplication/deletion variants (new code effective 01/01/18).

**BENEFIT APPLICATION**

**BLUECARD/NATIONAL ACCOUNT ISSUES**

Some Plans may have contract or benefit exclusions for genetic testing.

**BACKGROUND**

**Alpha-Thalassemia**

Alpha-thalassemia is a common genetic disorder, affecting approximately 5% of the world’s population.1 The frequency of variants is highly dependent on ethnicity, with the highest rates seen in Asians, and much lower rates in Northern Europeans. The carrier rate is estimated to be 1 in 20 in Southeast Asians, 1 in 30 for Africans, and between 1 in 30 and 1 in 50 for individuals of Mediterranean ancestry. By contrast, for individuals of northern European ancestry, the carrier rate is less than 1 in 1000.

**Physiology**

Hemoglobin, which is the major oxygen-carrying protein molecule of red blood cells (RBCs), consists of two α-globin chains and two β-globin chains. Alpha-thalassemia refers to a group of syndromes that arise from a deficient production of α-globin chains. Deficient α-globin production leads to an excess of β-globin chains, which results in anemia by a number of mechanisms2:

- Ineffective erythropoiesis in the bone marrow.
- Production of nonfunctional hemoglobin molecules.
• Shortened survival of RBCs due to intravascular hemolysis and increased uptake of the abnormal RBCs by the liver and spleen.

The physiologic basis of α-thalassemia is a genetic defect in the genes coding for α-globin production. Each individual carries 4 genes that code for α-globin (2 copies each of HBA1 and HBA2, located on chromosome 16), with the wild genotype (normal) being αα/αα. Genetic variants may occur in any or all of these four α-globin genes. The number of genetic variants determines the phenotype and severity of the α-thalassemia syndromes. There are four different syndromes, which are classified below.

Silent Carrier
Silent carrier (α-thalassemia minima) arises from one of four abnormal α genes (αα/α-) and is a silent carrier state. A small amount of abnormal hemoglobin can be detected in the peripheral blood, and there may be mild hypochromia and microcytosis present, but there is no anemia or other clinical manifestations.

Thalassemia Trait
Thalassemia trait (α-thalassemia minor), also called α-thalassemia trait, arises from the loss of two α-globin genes, resulting in one of two genotypes (αα/−−, or α−/α−). Mild anemia is present, and RBCs are hypochromic and microcytic. Clinical symptoms are usually absent and, in most cases, the hemoglobin electrophoresis is normal.

Hemoglobin H Disease
HbH disease (α-thalassemia intermedia) results from three abnormal α-globin genes (α−/−−), resulting in moderate-to-severe anemia. In HbH disease, there is an imbalance in α- and β-globin gene chain synthesis, resulting in the precipitation of excess β chains into the characteristic hemoglobin H, or β-tetramer. This condition has marked phenotypic variability but most individuals have mild disease and live a normal life without medical intervention.

A minority of individuals may develop clinical symptoms of chronic hemolytic anemia. They include neonatal jaundice, hepatosplenomegaly, hyperbilirubinemia, leg ulcers, and premature development of biliary tract disease. Splenomegaly can lead to the need for splenectomy, and transfusion support may be required by the third to fourth decade of life. It has been estimated that approximately 25% of patients with HbH disease will require transfusion support during their lifetime. In addition, increased iron deposition can lead to premature damage to the liver and heart. Inappropriate iron therapy and oxidant drugs should be avoided in patients with HbH disease.

There is an association between genotype and phenotype among patients with HbH disease. Individuals with a nondeletion variant typically have an earlier presentation, more severe anemia, jaundice, and bone changes, and more frequently require transfusions.

Hemoglobin Bart’s
Hemoglobin Bart’s (α-thalassemia major) results from variants in all four α-globin genes (−−/−−), which prevents the production of α-globin chains. This condition causes hydrops fetalis, which often leads to intrauterine death or death shortly after birth. There are also increased complications during pregnancy for a woman carrying a fetus with hydrops fetalis. They include hypertension, preeclampsia, antepartum hemorrhage, renal failure, premature labor, and abruption placenta.

Genetic Testing
A number of types of genetic abnormalities are associated with α-thalassemia. More than 100 genetic variants have been described. Deletion of one or more of the α-globin chains is the most common
genetic defect. This type of genetic defect is found in approximately 90% of cases.\textsuperscript{4} Large genetic rearrangements can also occur from defects in crossover and/or recombination of genetic material during reproduction. Single nucleotide variants in one or more of the α genes that impair transcription and/or translation of the α-globin chains.

Testing is commercially available through several genetic labs. Targeted variant analysis for known α-globin gene variants can be performed by polymerase chain reaction (PCR).\textsuperscript{4,5} PCR can also be used to identify large deletions or duplications. Newer testing methods have been developed to facilitate identification of α-thalassemia variants, such as multiplex amplification methods and real-time PCR analysis.\textsuperscript{5,6,7} In patients with suspected α-thalassemia and a negative PCR test for genetic deletions, direct sequence analysis of the α-globin locus is generally performed to detect single nucleotide variants.\textsuperscript{4}

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Genetic testing for α-thalassemia is available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**RATIONALE**

This evidence review was created in August 2013 and has been updated regularly with searches of the MEDLINE database. The most recent literature update was performed through January 11, 2019.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

The published literature on genetic testing for α-thalassemia consists primarily of reports describing the molecular genetics of testing, the types of variants encountered, and genotype-phenotype correlations.\textsuperscript{4,5,8,9,10,11}

**TESTING FOR PATIENTS WITH SUSPECTED A-THALASSEMA**

**Clinical Context and Test Purpose**

The purpose of genetic testing for α-thalassemia is to provide a diagnostic option that is an alternative to or an improvement on existing tests, such as standard diagnostic workup for α-thalassemia, in patients with suspected α-thalassemia based on clinical signs and symptoms. Additionally, genetic testing can be used to confirm a diagnosis and inform clinical decisions such as initiating treatment with iron supplementation, folic acid, or blood transfusion that improve the net health outcome.

The question addressed in this evidence review is: Does testing for variants in the \textit{HBA1} and \textit{HBA2} genes improve the net health outcome in individuals with suspected α-thalassemia?
The following PICOTS were used to select literature to inform this review.

**Patients**

The relevant population of interest are individuals with suspected α-thalassemia based on clinical signs and symptoms.

**Interventions**

The test being considered is genetic testing for α-thalassemia.

Polymerase chain reaction (PCR) is performed to analyze known α-globin gene variants and to identify large deletions or duplications. Direct sequence analysis of the α-globin locus is generally performed to detect single nucleotide variants in patients with suspected α-thalassemia and a negative PCR test for genetic deletions. Testing is commercially available through several genetic labs.

**Comparators**

Comparators of interest include standard diagnostic workup for α-thalassemia. Biochemical testing, including complete blood count (CBC) and hemoglobin electrophoresis, is currently being used to make diagnostic decisions about individuals who are suspected to have α-thalassemia.

**Outcomes**

The general outcomes of interest are overall survival (OS), disease-specific survival, test accuracy, test validity, symptoms, and quality of life (QOL).

The general outcomes of interest are related to the requirement and frequency of interventions for the management of anemia such as iron supplementation, folic acid supplementation, chelation therapy, and blood transfusion.

The potentially beneficial outcomes of primary interest would be improvements in overall or disease-specific survival and reduction in morbid events as a result of the timely initiation of appropriate treatment.

The potentially harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to the unnecessary initiation of treatment. False-negative test results can lead to lack of initiation of appropriate treatment.

**Timing**

The existing literature evaluating genetic testing for α-thalassemia as a diagnosis for biochemical evidence of α-thalassemia has varying lengths of follow-up. While studies described below all reported at least one outcome of interest, longer follow-up was necessary to fully observe outcomes.

**Setting**

Patients with suspected α-thalassemia are actively managed by a genomic disease specialist and primary care provider in an outpatient clinical setting.

**Study Selection Criteria**

Below are selection criteria for studies to assess whether a test is clinically valid.

1. The study population represents the population of interest. Eligibility and selection are described.
2. The test is compared with a credible reference standard.
3. If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
4. Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g., receiver operating characteristic, area under receiver operating characteristic, c-statistic, likelihood ratios) may be included but are less informative.

Studies should also report reclassification of diagnostic or risk category.

Simplifying Test Terms
There are three core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Diagnostic tests detect the presence or absence of a condition. Surveillance and treatment monitoring are essentially diagnostic tests over a time frame. Surveillance to see whether a condition develops or progresses is a type of detection. Treatment monitoring is also a type of detection because the purpose is to see if treatment is associated with the disappearance, regression, or progression of the condition.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Clinical validity is expected to be high when the causative variant is a large deletion of one or more α-globin genes, as PCR testing is generally considered highly accurate for this purpose. When a single nucleotide variant is present, clinical validity is less certain.

Henderson et al (2016) reported on a retrospective study assessing genotype and phenotype correlations of the novel thalassemia and abnormal hemoglobin variants identified after the adoption of routine DNA sequencing of α- and β-globin genes for all U.K. samples referred for evaluation of hemoglobinopathy for the preceding 10 years. Of a total of approximately 12000 samples, 15 novel α⁺-thal variants, 19 novel β-thal variants, and 11 novel β-globin variants were detected.

Section Summary: Clinically Valid
The clinical validity of genetic testing for α-thalassemia is high, especially when the causative variant is a large deletion of one or more α-globin gene. When a single nucleotide variant is present, the clinical validity may be less certain.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

There are several potential areas for clinical usefulness. Genetic testing can be used to determine the genetic abnormalities underlying a clinical diagnosis of α-thalassemia. It can also be used to define the genetics of α-globin genes in relatives of patients with a clinical diagnosis of α-thalassemia. Preconception (carrier) testing can be performed to determine the likelihood of an offspring with an α-thalassemia syndrome. Prenatal (in utero) testing can also be performed to determine the presence and type of α-thalassemia of a fetus. Prenatal testing is not addressed in this evidence review.

Confirming a Diagnosis

The diagnosis of α-thalassemia can be made without genetic testing. This is first done by analyzing the CBC and peripheral blood smear, in conjunction with testing for other forms of anemia. Patients with a CBC demonstrating microcytic, hypochromic red blood cell indices who are not found to have an iron deficiency, have a high likelihood of thalassemia. On peripheral blood smear, the presence of inclusion bodies and target cells is consistent with the diagnosis of α-thalassemia.

Hemoglobin electrophoresis can distinguish between the asymptomatic carrier states and αHbH disease (α-thalassemia intermedia) by identifying the types and amounts of abnormal hemoglobin present. In the carrier states, greater than 95% of the hemoglobin molecules are normal (hemoglobin A), with a small minority of hemoglobin A₂ present (1%-3%). In α-thalassemia intermedia is diagnosed by finding a substantial portion of hemoglobin H (1%-30%) on electrophoresis. In α-thalassemia major, the majority of the hemoglobin is abnormal, in the form of hemoglobin Bart’s (85%-90%).

However, biochemical testing, including CBC and hemoglobin electrophoresis, cannot always reliably distinguish between the asymptomatic carrier state and α-thalassemia trait, because the hemoglobin electrophoresis is typically normal in both conditions. Genetic testing can differentiate between the asymptomatic carrier state (α-thalassemia minima) and α-thalassemia trait (α-thalassemia minor) by elucidating the number of abnormal genes present. This distinction is not important clinically because both the carrier state and α-thalassemia trait are asymptomatic conditions that do not require specific medical care treatment. Alpha-thalassemia trait may overlap in red blood cell indices values with iron deficiency states, so it is important that iron supplementation not be continued unnecessarily in patients with α-thalassemia trait. However, it would be reasonable to make a diagnosis of α-thalassemia trait in a patient with microcytic, hypochromic red blood cell indices without evidence of iron deficiency, either before or after a trial of iron supplementation. Because the diagnosis of clinically relevant α-thalassemia conditions can usually be made without genetic testing, there is little utility to genetic testing of a patient with a clinical diagnosis of thalassemia to determine the underlying genetic abnormalities.

Section Summary: Clinically Useful

The clinical usefulness of genetic testing for α-thalassemia either for confirming a diagnosis in individuals who are suspected to have α-thalassemia based on clinical signs and symptoms is low. Confirmation of a diagnosis of α-thalassemia that is clinically actionable can generally be made by nongenetic testing, and therefore there is little utility to genetic testing.
Section Summary: Testing for Patients with Suspected α-Thalassemia

For patients with suspected α-thalassemia, one retrospective study was available for clinical validity, in which several novel variants were detected. The clinical usefulness of genetic testing for α-thalassemia either for confirming a diagnosis in individuals who are suspected to have α-thalassemia based on clinical signs and symptoms is low.

testing for PATIENTS WITH HEMOGLOBIN H DISEASE

Clinical Context and Test Purpose

The purpose of genetic testing for those who have been diagnosed with HbH disease based on clinical signs and symptoms is to provide a prognostic option that is an alternative to or an improvement on existing tests, such as standard diagnostic workup for α-thalassemia. Additionally, genetic testing can be used to confirm a diagnosis and inform clinical decisions such as initiating treatment with iron supplementation, folic acid, or blood transfusion that improve the net health outcome.

The question addressed in this evidence review is: Does testing for variants in the HBA1 and HBA2 genes improve the net health outcome in individuals with HbH disease?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest are individuals with HbH disease based on clinical signs and symptoms.

Interventions

The test being considered is genetic testing for α-thalassemia.

PCR is performed to analyze known α-globin gene variants and to identify large deletions or duplications. Testing is commercially available through several genetic labs.

Comparators

Comparators of interest include standard diagnostic workup for α-thalassemia. Biochemical testing, including CBC and hemoglobin electrophoresis, is currently being used to make diagnostic decisions about individuals who have been diagnosed with HbH disease.

Outcomes

The general outcomes of interest are OS, disease-specific survival, test accuracy, test validity, symptoms, and QOL.

The general outcomes of interest are related to the requirement and frequency of interventions for the management of anemia such as iron supplementation, folic acid supplementation, chelation therapy, and blood transfusion.

The potentially beneficial outcomes of primary interest would be improvements in overall or disease-specific survival and reduction in morbid events as a result of the timely initiation of appropriate treatment.

The potentially harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to the unnecessary initiation of treatment. False-negative test results can lead to lack of initiation of appropriate treatment.

Timing
Genetic Testing for α-Thalassemia

The existing literature evaluating genetic testing for α-thalassemia as a diagnosis for biochemical evidence has varying lengths of follow-up. While studies described below all reported at least one outcome of interest, longer follow-up was necessary to fully observe outcomes.

Setting

Patients with HbH disease are actively managed by a genomic disease specialist and primary care provider in an outpatient clinical setting or in specialized thalassemia clinics by a multidisciplinary team of physicians.

Study Selection Criteria

Below are selection criteria for studies to assess whether a test is clinically valid.

1. The study population represents the population of interest. Eligibility and selection are described.
2. The test is compared with a credible reference standard.
3. If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
4. Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (eg, receiver operating characteristic, area under receiver operating characteristic, c-statistic, likelihood ratios) may be included but are less informative.

Studies should also report reclassification of diagnostic or risk category.

Simplifying Test Terms

There are three core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful

Because different specialties may use different terms for the same concept, we are highlighting the core characteristics. The core characteristics also apply to different uses of tests, such as diagnosis, prognosis, and monitoring treatment.

Prognostic tests predict the risk of developing a condition in the future. Tests to predict response to therapy are also prognostic. Response to therapy is a type of condition and can be either a beneficial response or adverse response. The term predictive test is often used to refer to the response to therapy. To simplify terms, we use prognostic to refer both to predicting a future condition or predicting response to therapy.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).
Clinical validity is expected to be high when the causative variant is a large deletion of one or more \( \alpha \)-globin genes, as PCR testing is generally considered highly accurate for this purpose. When a single nucleotide variant is present, clinical validity is less certain.

**Section Summary: Clinically Valid**

The clinical validity of genetic testing for \( \alpha \)-thalassemia is high, especially when the causative variant is a large deletion of one or more \( \alpha \)-globin gene. When a single nucleotide variant is present, the clinical validity may be less certain.

**Clinically Useful**

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

**Direct Evidence**

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

There are several potential areas for clinical usefulness. Genetic testing can be used to determine the genetic abnormalities underlying a clinical diagnosis of \( \alpha \)-thalassemia. It can also be used to define the genetics of \( \alpha \)-globin genes in relatives of patients with a clinical diagnosis of \( \alpha \)-thalassemia. Preconception (carrier) testing can be performed to determine the likelihood of an offspring with an \( \alpha \)-thalassemia syndrome. Prenatal (in utero) testing can also be performed to determine the presence and type of \( \alpha \)-thalassemia of a fetus. Prenatal testing is not addressed in this evidence review.

**Prognostic Testing in Patients with HbH Disease**

Among patients with HbH disease, there is heterogeneity in the nature of the variant (ie, deletional vs nondeletional), with differences across geographic areas and ethnic groups.\(^{14}\) Patients with deletional variants may have a less severe course of illness than those with nondeletional variants.\(^{14}\) In a 2009 cohort of 147 Thai pediatric patients with HbH disease, those with nondeletional variants were more likely to have pallor after fever, hepatomegaly, splenomegaly, jaundice, short stature, need for transfusions, and gallstones.\(^{15}\)

The evidence suggests that different genetic variants leading to \( \alpha \)-thalassemia are associated with different prognoses. New treatments for some complications of HbH disease that result from ineffective erythropoiesis and iron overload and may differ for genotypes are under development.\(^{16}\) However, no evidence was identified to indicate that patient management or outcomes would be changed by prognostic testing.

**Section Summary: Clinically Useful**

The clinical usefulness of genetic testing for \( \alpha \)-thalassemia for prognostic testing of individuals who have been diagnosed with HbH disease based on clinical signs and symptoms is low.

**Section Summary: Testing for Patients with HbH Disease**

For patients with HbH disease, no evidence was available for clinical validity. The clinical usefulness of genetic testing for \( \alpha \)-thalassemia who have HbH disease based on clinical signs and symptoms is low.

**Testing for Patients Diagnosed with \( \alpha \)-thalassemia Who Are Considering Conception**
Clinical Context and Test Purpose

The purpose of genetic testing for α-thalassemia is to provide a diagnostic option that is an alternative to or an improvement on existing therapies, such as standard diagnostic workup for α-thalassemia, in patients with HbH disease who are considering conception to define the likelihood of α-thalassemia major in a prospective pregnancy.

The question addressed in this evidence review is: Does genetic testing avoids a prospective α-thalassemia major pregnancy in individuals diagnosed with α-thalassemia based on clinical signs and symptoms who are considering conception?

The following PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest are individuals who have been diagnosed with α-thalassemia based on clinical signs and symptoms who are considering conception.

Interventions

The test being considered is genetic testing for α-thalassemia for determination of the number or pattern of abnormal alpha genes

Comparators

Comparators of interest include standard diagnostic workup for α-thalassemia. Biochemical testing, including CBC and hemoglobin electrophoresis, is being used to make diagnostic decisions about individuals with α-thalassemia.

Outcomes

The general outcomes of interest are OS, disease-specific survival, symptoms, and QOL.

The potential major beneficial outcome is avoiding pregnancy with α-thalassemia major, which is of benefit to a prospective mother or a couple who can make reproductive decisions about the possibility of a nonviable pregnancy and avoid increased obstetrical complications associated with a fetus with α-thalassemia major.

The potentially harmful outcomes are those resulting from false-positive or false-negative test results. False-positive test results can lead to unnecessary termination of an otherwise normal pregnancy. False-negative test results can lead to a full-term carriage of an otherwise nonviable pregnancy and the increased obstetrical complications associated with a fetus with α-thalassemia major.

Timing

The timing of avoidance of a nonviable pregnancy would be anytime during the reproductive age of the individuals with b-thalassemia.

Setting

The timing of avoidance of a nonviable pregnancy would be anytime during the reproductive age of the individuals with b-thalassemia.

Study Selection Criteria

Below are selection criteria for studies to assess whether a test is clinically valid.
1. The study population represents the population of interest. Eligibility and selection are described.
2. The test is compared with a credible reference standard.
3. If the test is intended to replace or be an adjunct to an existing test; it should also be compared with that test.
4. Studies should report sensitivity, specificity, and predictive values. Studies that completely report true- and false-positive results are ideal. Studies reporting other measures (e.g., receiver operating characteristic, area under receiver operating characteristic, c-statistic, likelihood ratios) may be included but are less informative.

Studies should also report reclassification of diagnostic or risk category.

Simplifying Test Terms

There are three core characteristics for assessing a medical test. Whether imaging, laboratory, or other, all medical tests must be:

- Technically reliable
- Clinically valid
- Clinically useful

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse). See the preceding discussion of clinical validity.

See the discussion in the previous section on Clinically Valid.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Carrier screening with biochemical testing is recommended for all patients who are from ethnic groups with a high incidence of α-thalassemia. Biochemical screening consists of a CBC with peripheral smear analysis. If their abnormalities are noted (e.g., anemia, microcytosis, hypochromia), hemoglobin electrophoresis is then performed to identify the specific types of hemoglobin present. As noted, the hemoglobin electrophoresis may be normal in the asymptomatic carrier and α-thalassemia trait states, but the states may be suspected based on CBC and peripheral smear analysis.

Unlike clinical diagnosis, for carrier testing, it is important to distinguish between α-thalassemia carrier (one abnormal gene) and α-thalassemia trait (two abnormal genes), and important to distinguish between the two variants of α-thalassemia trait, i.e., the αα/-- (cis variant) and the α-α/α- (trans variant). This is important because only when both parents have the αα/-- cis variant is there a risk for a fetus with α-thalassemia major.12 When both parents are α-thalassemia carriers (αα/--), there is a one in four likelihood that an offspring will have α-thalassemia major and hydrops fetalis. These parents may decide to pursue preimplantation genetic diagnosis in conjunction with in vitro fertilization to avoid a pregnancy with hydrops fetalis.
In this situation, genetic testing has incremental utility over biochemical testing. Whereas biochemical testing can determine whether a silent carrier/trait syndrome is present, and can distinguish those syndromes from HbH disease, it cannot provide a precise determination of the number or pattern of abnormal alpha genes. As a result, using biochemical screening alone, the probability of developing a hemoglobin Bart’s fetus cannot be accurately assessed. By contrast, genetic testing can delineate the number of abnormal genes with certainty. Also, genetic testing can determine whether an α-thalassemia trait exists as the *cis* (αα/-- variant or the *trans* (α/-α-) variant. Using this information from genetic testing, the probability of hemoglobin Bart’s can be determined according to Table 1.

**Table 1. Probability of Hemoglobin Bart’s**

<table>
<thead>
<tr>
<th>Clinical Diagnosis in Parents</th>
<th>Genotype (Parent 1)</th>
<th>Genotype (Parent 2)</th>
<th>Probability of Hemoglobin Bart’s, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both parents silent carriers</td>
<td>αα/α-</td>
<td>αα/α-</td>
<td>0</td>
</tr>
<tr>
<td>One parent silent carrier, 1 parent trait</td>
<td>αα/α-</td>
<td>α/-α-</td>
<td>0</td>
</tr>
<tr>
<td>Both parents trait</td>
<td>αα/--</td>
<td>αα/--</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>α/-α-</td>
<td>αα/--</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>α/-α-</td>
<td>αα/--</td>
<td>0</td>
</tr>
<tr>
<td>One parent HbH, 1 parent silent carrier</td>
<td>α/-α-</td>
<td>αα/α-</td>
<td>0</td>
</tr>
<tr>
<td>One parent HbH, 1 parent trait</td>
<td>α/-α-</td>
<td>αα/--</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>α/-α-</td>
<td>α/-α-</td>
<td>0</td>
</tr>
<tr>
<td>Both parents HbH</td>
<td>α/-α-</td>
<td>α/-α-</td>
<td>25</td>
</tr>
</tbody>
</table>

HbH: hemoglobin H.

Parents can also determine the likelihood of HbH disease in an offspring through genetic testing. However, because this is, in most cases, a mild condition, it is less likely to be considered information that is actionable in terms of altering reproductive decision making.

**Section Summary: Clinically Useful**

Preconception (carrier) testing is likely to have clinical usefulness by providing incremental diagnostic information over biochemical testing. Genetic testing can identify the pattern of abnormal α genes and estimate more precisely the risk of hydrops fetalis. While these tests cannot completely determine the risk of a pregnancy with hemoglobin Bart’s and hydrops fetalis, genetic testing can determine with certainty the number of abnormal genes present, and therefore can more precisely determine the risk of hydrops fetalis.

**Section Summary: Testing for Patients Diagnosed with α-thalassemia Who Are Considering Conception**

For individuals who have biochemical evidence of α-thalassemia who are considering conception who receive genetic testing for α-thalassemia, the evidence includes case reports and case series that correlate pathogenic variants with clinical disease.

**Summary of Evidence**

For individuals who have suspected α-thalassemia who receive genetic testing for α-thalassemia, the evidence includes case reports and case series documenting the association between pathogenic
variants and clinical syndromes. The relevant outcomes are OS, disease-specific survival, test accuracy and validity, symptoms, and QOL. For the α-thalassemia syndromes that have clinical implications, diagnosis can be made based on biochemical testing without genetic testing. The evidence is sufficient to determine that the technology is unlikely to improve the net health outcome.

For individuals who have HbH disease who receive genetic testing for α-thalassemia, the evidence includes case series that correlate specific variants with a prognosis of the disease. The relevant outcomes are OS, disease-specific survival, symptoms, and QOL. There is some evidence for a genotype-phenotype correlation with disease severity but no current evidence indicates that patient management or outcomes would be altered by genetic testing. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have biochemical evidence of α-thalassemia who are considering conception who receive genetic testing for α-thalassemia, the evidence includes case reports and case series that correlate pathogenic variants with clinical disease. The relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. Preconception carrier testing is intended to avoid the most serious form of α-thalassemia, hemoglobin Bart’s. This condition leads to intrauterine death or death shortly after birth and is associated with increased obstetrical risks for the mother. Screening of populations at risk is first done by biochemical tests, including hemoglobin electrophoresis and CBC and peripheral smear, but these tests cannot reliably distinguish between the carrier and trait syndromes, and cannot determine which configuration of variants is present in α-thalassemia trait. Therefore, these tests cannot completely determine the risk of a pregnancy with hemoglobin Bart’s and hydrops fetalis. Genetic testing can determine with certainty the number of abnormal genes present, and therefore can more precisely determine the risk of hydrops fetalis. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

Society of Obstetricians and Gynecologists of Canada

The Society of Obstetricians and Gynecologists of Canada (2008) published guidelines on carrier testing for thalassemia.17 These guidelines included the following recommendations:

1. Carrier screening for α-thalassemia should be offered to all woman from ethnic groups with an increased prevalence of α-thalassemia. Initial screening should consist of “complete blood count, hemoglobin electrophoresis or hemoglobin high performance liquid chromatography....” ferritin testing [and examination of peripheral] blood smear to identify H bodies.”

2. If a woman’s screening is abnormal ..., then screening the partner should be performed [using the same battery of tests].”

3. “If both partners are found to be carriers of thalassemia ... or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counseling.... Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus.”

American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (2017) published an opinion document that includes multiple general recommendations about carrier screening of genetic conditions.18 They are not summarized in this evidence review. Specific descriptions of genetic testing for α-thalassemia include the following: DNA-based genetic testing should be used to detect α-globin gene characteristics
of suspected cases of thalassemia “[i]f the mean corpuscular volume is below normal, iron deficiency anemia has been excluded, and the hemoglobin [Hb] electrophoresis is not consistent with b-thalassemia trait (ie, there is no elevation of Hb A2 or Hb F).”

**U.S. Preventive Services Task Force Recommendations**

Not applicable.

**Medicare National Coverage**

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

**Ongoing and Unpublished Clinical Trials**

A search of ClinicalTrials.gov in January 2019 did not identify any ongoing or unpublished trials that would likely influence this review.

**ESSENTIAL HEALTH BENEFITS**

The Affordable Care Act (ACA) requires fully insured non-grandfathered individual and small group benefit plans to provide coverage for ten categories of Essential Health Benefits (“EHBs”), whether the benefit plans are offered through an Exchange or not. States can define EHBs for their respective state.

States vary on how they define the term small group. In Idaho, a small group employer is defined as an employer with at least two but no more than fifty eligible employees on the first day of the plan or contract year, the majority of whom are employed in Idaho. Large group employers, whether they are self-funded or fully insured, are not required to offer EHBs, but may voluntary offer them.

The Affordable Care Act requires any benefit plan offering EHBs to remove all dollar limits for EHBs.

**REFERENCES**


### CODES

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>CPT</td>
<td>81257</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)</td>
</tr>
<tr>
<td></td>
<td>81258</td>
<td>known familial variant (new codes effective 01/01/18)</td>
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<tr>
<td></td>
<td>81259</td>
<td>full gene sequence</td>
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<tr>
<td></td>
<td>81269</td>
<td>duplication/deletion variant</td>
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<tr>
<td>ICD-10-CM</td>
<td>Z31.430</td>
<td>Encounter of female for testing for genetic disease carrier status for procreative management</td>
</tr>
<tr>
<td></td>
<td>Z31.440</td>
<td>Encounter of male for testing for genetic disease carrier status for procreative management</td>
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<td>ICD-10-PCS</td>
<td>Not applicable. ICD-10-PCS codes are only used for inpatient services. There are no ICD procedure codes for laboratory tests.</td>
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## POLICY HISTORY

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>08/14/14</td>
<td>Replace policy</td>
<td>Policy updated with literature review through July 1, 2014. References 4 and 14-16 added. Changes made to policy statement and policy guidelines to clarify biochemical testing algorithm, but policy statement otherwise unchanged.</td>
</tr>
<tr>
<td>06/11/15</td>
<td>Replace policy – correction only</td>
<td>In Rationale, the statement “In the carrier states, less than 95% of the Hb molecules are normal…” was corrected to state “…greater than 95%…”</td>
</tr>
<tr>
<td>08/13/15</td>
<td>Replace policy</td>
<td>Policy updated with literature review through July 1, 2015; no references added. New policy statement added stating that testing of patients with hemoglobin H disease to determine prognosis is considered investigational; no other change to policy statements.</td>
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<tr>
<td>09/01/16</td>
<td>Replace policy</td>
<td>Blue Cross of Idaho annual review; no change to policy.</td>
</tr>
<tr>
<td>02/24/17</td>
<td>Replace policy</td>
<td>Policy updated with literature review through December 20, 2016; references 13-14 added. The policy is revised with updated genetics nomenclature; the intent of the policy statements is unchanged.</td>
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<tr>
<td>02/26/18</td>
<td>Replace policy</td>
<td>Blue Cross of Idaho adopted changes as noted. Policy updated with literature review through December 11, 2017; reference 18 was added. The policy is revised with updated format; Policy statements unchanged.</td>
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<tr>
<td>02/21/19</td>
<td>Replace policy</td>
<td>Blue Cross of Idaho annual review; no change to policy.</td>
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<tr>
<td>06/20/19</td>
<td>Replace policy</td>
<td>Blue Cross of Idaho adopted changes as noted, effective 06/20/2019. Policy updated with literature review through January 3, 2019; no references added. This policy was tabled in February 2019 to obtain clinical input regarding specified indications. No clinical input responses were received as of June 2019. Policy statements unchanged.</td>
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