

Overview

Useful For

Diagnosis of beta thalassemia intermedia or major

Identification of a specific beta thalassemia mutation (ie, unusually severe beta thalassemia trait)

Evaluation of an abnormal hemoglobin electrophoresis identifying a rare beta globin variant

Evaluation of chronic hemolytic anemia of unknown etiology

Evaluation of hereditary erythrocytosis with left-shifted p50 oxygen dissociation results

Preconception screening when there is a concern for a beta hemoglobin disorder based on family history

Genetics Test Information

A hemoglobin electrophoresis evaluation (HBELC / Hemoglobin Electrophoresis Cascade, Blood) is always indicated prior to beta globin gene sequencing because these conditions can be complex and protein data allows accurate and rapid classification of the patient phenotype.

Beta globin gene (*HBB*) sequencing can be used to identify hemoglobin variants and the most common beta thalassemia mutations, including beta plus and beta zero thalassemias. It also identifies hyperunstable hemoglobin variants and dominant beta thalassemia mutations, as well as other hemoglobin variants that cannot be identified by protein methods. Some hemoglobin disorders will not be detected by beta globin gene sequencing, such as large deletional mutations and crossover events. As such, the results of this test should always be interpreted within the context of the protein studies and RBC indices.

Testing Algorithm

This is a second-tier evaluation of beta thalassemia minor, intermedia, and major, as well as beta globin variant identification.

First-tier testing for beta thalassemia or beta globin variant detection is THEVP / Thalassemia and Hemoglobinopathy Evaluation or HBELC / Hemoglobin Electrophoresis Cascade, Blood.

Special Instructions

- [Thalassemia/Hemoglobinopathy Patient Information](#)
- [Informed Consent for Genetic Testing](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)

Method Name

Polymerase Chain Reaction (PCR) followed by DNA Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Advisory Information

For information on thalassemias and appropriate test ordering, see Thalassemia Tests in Special Instructions.

Necessary Information

1. Patient's age is required.
2. Include recent transfusion information.

Specimen Required

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: ACD, sodium heparin

Specimen Volume: 3 mL

Collection Instructions: Do not transfer blood to other containers

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:

[-Informed Consent for Genetic Testing](#) (T576)

[-Informed Consent for Genetic Testing-Spanish](#) (T826)

2. [Thalassemia/Hemoglobinopathy Patient Information](#) (T358) in Special Instructions.

Specimen Minimum Volume

1 mL

Reject Due To

Other	Moderately to severely clotted
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Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated	14 days	

Clinical and Interpretive

Clinical Information

Beta globin gene sequencing is useful in the evaluation of beta globin chain variants and beta thalassemia. It detects almost all beta globin variants and the most common beta thalassemia mutations, although prevalence is ethnicity-dependent. Because these conditions are often complex, this test should always be interpreted in the context of

protein studies, such as hemoglobin electrophoresis and RBC indices.

The majority of beta globin chain variants are clinically and hematologically benign; however, some have important clinical consequences, such as erythrocytosis, cyanosis/hypoxia, chronic hemolysis, or unexplained microcytosis. Most of the common clinically significant hemoglobin (Hb) variants (ie, Hb S, Hb C, Hb E, and others) are easily distinguished by hemoglobin electrophoresis and do not require molecular analysis. In addition, they are frequently found in complex hemoglobin disorders due to multiple mutations, and accurate classification requires sequencing data within the context of protein data. In some instances, beta globin sequencing is necessary to identify or confirm the identity of rare variants, especially those associated with erythrocytosis and chronic hemolytic anemia. Rare hyperunstable variants (also termed dominant beta thalassemia mutations) result in hemolytic anemia and do not create protein stable enough to be detectable by protein methods, including stability studies. They are not always associated with elevated Hb A2 or microcytosis and, therefore, can be electrophoretically silent. These require a high degree of clinical suspicion as all electrophoretic testing as well as stability studies cannot exclude this condition.

Beta thalassemia is an autosomal recessive condition characterized by decreased or absent synthesis of beta globin chains due to mutations in the beta globin gene (*HBB*). No abnormal protein is present and diagnosis by electrophoresis relies on hemoglobin fraction percentage alterations (ie, Hb A2 or Hb F elevations).

Beta thalassemia can be split into 3 broad classes (categorized by clinical features)

1. Beta thalassemia trait (also called beta thalassemia minor and beta thalassemia carrier) (B[A]B[+] or B[A]B[0]).
2. Beta thalassemia intermedia (B[+]B[+] or B[+]B[0])
3. Beta thalassemia major (B[+]B[0] or B[0]B[0])

Beta thalassemia trait is typically a harmless condition with varying degrees of microcytosis and hypochromia and sometimes mild anemia. Transfusions are not required. Beta thalassemia intermedia is a clinical distinction and is characterized by a more severe degree of anemia than beta thalassemia trait with few or intermittent transfusions required. Later in life, these individuals are at risk for iron overload even in the absence of chronic transfusion due to increased intestinal absorption of iron. Beta thalassemia major typically comes to medical attention early in life due to severe anemia, hepatosplenomegaly, and failure to thrive. Skeletal changes are also common due to expansion of the bone marrow. Without appropriate treatment these patients have a shortened lifespan.

The majority of beta thalassemia mutations (>90%) are point mutations, small deletions, or insertions, which are detected by beta globin gene sequencing. The remaining beta thalassemia mutations are either due to large genomic deletions of *HBB* or, very rarely, trans-acting beta thalassemia mutations located outside of the beta globin gene cluster. Some rare beta chain variants can be clinically or electrophoretically indistinguishable from beta thalassemia and cannot be confirmed without molecular analysis.

Reference Values

An interpretive report will be provided.

Interpretation

The alteration will be provided with the classification, if known. Further interpretation requires correlation with protein studies and RBC indices.

Cautions

This assay will not detect large deletions or duplications within the beta globin gene. In addition, hybrid beta globin variants (ie, Hb Lepore) will not be detected by this method. This method cannot distinguish between homozygous mutations and compound heterozygous mutations associated with large deletions. This method cannot distinguish between double substitution on single chromosome and a compound heterozygous state. Beta globin sequencing

alone is not able to distinguish between mutations that are found in the same copy of the *HBB* gene (ie, mutations that are "linked" or "in cis") and mutations found on different *HBB* gene copies (ie, are "in trans"). This limitation of sequencing may complicate diagnosis and has implications for inheritance and proper genetic counseling. To resolve these cases, molecular results must be correlated with electrophoretic and protein data, other laboratory findings, clinical findings, and family studies. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Rare polymorphisms exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.

Clinical Reference

1. Hoyer JD, Hoffman DR: The thalassemia and hemoglobinopathy syndromes. In Clinical Laboratory Medicine. Second edition. Edited by KD McClatchey. Philadelphia, Lippincott Williams and Wilkins, 2002, pp 866-895
2. Thein SL: The Molecular Basis of Beta-Thalassemia. Cold Spring Harb Perspect Med 2013;1;3(5):a011700
3. Hoyer JD, Kroft, SH: Color Atlas of Hemoglobin Disorders: A Compendium Based on Proficiency Testing. Northfield, IL. CAP, 2003
4. Merchant S, Oliveira JL, Hoyer JD, Viswanatha DS: Molecular diagnosis in hematopathology. In Hematopathology: A Volume in Foundations in Diagnostic Pathology Series. Second edition Edited by J Goldblum. Volume Editor E Hsi. Churchill Livingstone, 2012

Performance

Method Description

Genomic DNA is extracted from whole blood. The *HBB* gene is amplified by PCR. The PCR product is then purified and sequenced in both directions using fluorescent dye-terminator chemistry. Sequencing products are separated on an automated sequencer and trace files analyzed for variations in all exons, introns with the exception of IVS-II-82 through IVS-II-650, the 5'UTR, the 3'UTR, and the promoter region. Results are correlated with routine studies to identify unusual beta globin variants.(Reddy PL, Bowie LJ: Sequence-based diagnosis of hemoglobinopathies in the clinical laboratory. Clin Lab Med 1997;17[1]:85-96)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday, Wednesday, Friday

Analytic Time

2 days

Maximum Laboratory Time

10 days

Specimen Retention Time

DNA 3 months

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

81364-HBB (hemoglobin, beta) full sequence

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
WBSEQ	Beta Globin Gene Sequencing, B	79401-6

Result ID	Test Result Name	Result LOINC Value
62128	Beta Globin Gene Sequencing, B	82939-0
43922	Interpretation	69047-9