MAYO CLINIC LABORATORIES

Test Definition: WASEQ

Alpha Globin Gene Sequencing, Varies

Overview

Useful For

Diagnosing nondeletional alpha thalassemia

Testing for nondeletional alpha thalassemia in a symptomatic individual

Follow-up testing to an abnormal hemoglobin electrophoresis that identified an alpha-globin chain variant

Genetics Test Information

A hemoglobin electrophoresis evaluation (HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood) is always indicated prior to alpha-globin gene sequencing because these conditions can be complex and protein data allows accurate and rapid classification of the patient phenotype.

Not the preferred first-tier molecular test for carrier screening or diagnosis of alpha thalassemia. This test is used to identify nondeletional alpha-thalassemia variants when there is a strong clinical suspicion and AGDD / Alpha Globin Cluster Locus Deletion/Duplication, Varies is negative. This test can also identify alpha-globin variants that can result in variable phenotypes, such as erythrocytosis, chronic hemolytic anemia, and many that are clinically benign.

Special Instructions

- Informed Consent for Genetic Testing
- Metabolic Hematology Patient Information
- Informed Consent for Genetic Testing (Spanish)

Highlights

This test is a second-tier test in the evaluation of alpha-thalassemia carrier determination, hemoglobin H disease confirmation, and alpha-globin variant identification.

Method Name

Polymerase Chain Reaction (PCR)/ Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

For first tier testing for alpha thalassemia detection, order THEV1 / Thalassemia and Hemoglobinopathy Evaluation,

Serum and Blood.

For first tier testing for an alpha-globin variant, order HBEL1 / Hemoglobin Electrophoresis Evaluation, Blood.

If genetic testing is desired, the first-tier genetic test assesses large deletional alpha-thalassemia alterations. Order AGDD / Alpha Globin Cluster Locus Deletion/Duplication, Varies.

Necessary Information

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

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD), green top (sodium heparin)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix whole blood.
2. Send whole blood specimen in the original tube. **Do not aliquot**
Specimen Stability Information: Refrigerate 30 days(preferred)/Ambient 14 days

Specimen Type: Extracted DNA from whole blood
Container/Tube: 1.5- to 2-mL tube
Specimen Volume: Entire specimen
Collection Instructions:
1. DNA must be extracted from whole blood within 7 days of collection.
2. Label specimen as extracted DNA and source of specimen.
3. Provide volume and concentration of the DNA.
Specimen Stability Information: Frozen (preferred) 1 year/Refrigerate/Ambient
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). We cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied.

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:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Metabolic Hematology Patient Information (T810)
3. If not ordering electronically, complete, print, and send Benign Hematology Test Request Form (T755) with the specimen

Specimen Minimum Volume

Whole blood: 1 mL; Extracted DNA: 50 mcL at 50 ng/mcL concentration

Reject Due To

Gross hemolysis	OK
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Alpha-globin gene sequencing detects alpha-globin variants and nondeletional alpha-thalassemia variants.

Alpha thalassemia is the most common monogenic condition in the world. It is estimated that up to 5% of the world's population carries at least one alpha-thalassemia variant and, in the United States, approximately 30% of African Americans are thought to carry an alpha-thalassemia variant. Alpha-thalassemia variations are most common in individuals of Southeastern Asian, African, Mediterranean, Indian, and Middle Eastern descent, but they can be found in persons from any ethnic group.

Four alpha-globin genes are normally present, 2 copies on each chromosome 16. Alpha-thalassemia variants result in decreased alpha-globin chain production. In general, alpha thalassemia is characterized by hypochromic, microcytic anemia and varies clinically from asymptomatic (alpha-thalassemia silent carrier and alpha-thalassemia trait) to lethal hemolytic anemia (hemoglobin [Hb] Barts hydrops fetalis).

Large deletions of the alpha-globin genes account for approximately 90% of alpha-thalassemia alterations, and these variations will not be detected by alpha-globin gene sequencing. Other variants, such as point alterations or small deletions within the alpha-globin genes, account for most of the remaining 10% of alpha-thalassemia variations. These nondeletional subtypes can be detected by alpha-globin gene sequencing. The most common nondeletional alpha-thalassemia variant is Hb Constant Spring.

The majority of alpha-globin chain variants are clinically and hematologically benign however, some cause erythrocytosis and chronic hemolytic anemia. Hemoglobin electrophoresis may not be able to confirm their identity. In these instances, alpha-globin gene sequencing can be useful.

Reference Values

An interpretive report will be provided.

Interpretation
An interpretive report will be provided.

Cautions
This assay will not detect large deletions or duplications within the alpha-globin genes. Therefore, test results should be interpreted in the context of hemoglobin electrophoresis, clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Rare genetic alterations (ie, 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. Harteveld CL, Higgs DR: Alpha-thalassemia. Orphanet J Rare Dis. 2010;5:13
2. Hoyer JD, Hoffman DR: The Thalassemia and hemoglobinopathy syndromes. In: McClatchey, KD, ed. Clinical Laboratory Medicine. 2nd ed. Lippincott Williams and Wilkins. 2002;866-895
3. Farashi S, Harteveld CL: Molecular basis of a-thalassemia. Blood Cells Mol Dis. 2018;70:43-53. doi:10.1016/j.bcmd.2017.09.004
4. Henderson SJ, Timbs AT, McCarthy J, et al: Ten years of routine a- and b-globin gene sequencing in UK hemoglobinopathy referrals reveals 60 novel mutations. Hemoglobin. 2016;40(2):75-84. doi:10.3109/03630269.2015.1113990

Performance

Method Description
Genomic DNA is extracted from whole blood. The *HBA1* and *HBA2* genes are amplified by polymerase chain reaction (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 the trace files analyzed for variations in all exons, introns, and the polyadenylation site. Results are correlated with routine studies to identify unusual alpha globin variants.(Reddy PL, Bowie LJ: Sequence-based diagnosis of hemoglobinopathies in the clinical laboratory. Clin Lab Med. 1997;17[1]:85-96; Traeger-Synodinos J, Harteveld CL: Advances in technologies for screening and diagnosis of hemoglobinopathies. Biomarkers Med. 2014;8[1]:119-131)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
10 days

Specimen Retention Time
Whole blood: Ambient; Extracted DNA: Frozen

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81259-HBA1/HBA2; full sequence

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WASEQ	Alpha Globin Gene Sequencing, B	87730-8

Result ID	Test Result Name	Result LOINC® Value
43921	Interpretation	69047-9
61362	Alpha Globin Gene Sequencing, B	87730-8