MAYO CLINIC LABORATORIES

Test Definition: WGSEQ

Gamma-Globin Full Gene Sequencing, Varies

Overview

Useful For

An adjunct in the interpretation of hemoglobin electrophoresis results

Evaluation for suspected gamma variants or nondeletional hereditary persistence of fetal hemoglobin

Assessment of unstable gamma chain variants when other tests for causes of hemolysis are unrevealing

Genetics Test Information

The beta-like hemoglobins include the epsilon, gamma, beta, and delta globins, whose genes are present on chromosome 11 in a linked cluster (ie, the beta globin complex). The gamma genes, *HBG1* (Ay) and *HBG2* (Gy), contain 3 exonic coding regions and 2 intronic intervening sequences (IVS). The genes produce gamma globin chains that form tetramers with alpha globin chains to create fetal hemoglobin (Hb F). *HBG1* and *HBG2* differ only in which amino acid is located at position 136 (alanine or glycine). The resultant proteins are named A-gamma and G-gamma, respectively. Although G-gamma is predominant at birth, this gradually reverses during the first year of life to become the normal adult G-gamma/A-gamma ratio, which is 2:3. Some people maintain an increased G-gamma:A-gamma ratio throughout life, which has been linked to certain alterations in either gene. Additionally, some alterations in the promoter regions of the gamma globin genes are known to cause a form of hereditary persistence of fetal hemoglobin (HPFH), which is characterized by a significant but harmless elevation of Hb F into adulthood. If coinherited with sickle cell disease, HPFH has a strong modulating effect on the condition and appears to protect against some, but not all, of its complications. Some gamma genetic variations result in gamma chain hemoglobin variants, most of which are clinically insignificant; however, an incompletely studied subset causes neonatal disorders, such as hemolytic anemia, cyanosis, and methemoglobinemia.

Special Instructions

- Thalassemia/Hemoglobinopathy Patient Information
- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

Highlights

This test should be used as an adjunct to abnormal results detected by hemoglobin electrophoresis testing. It will assist with:
-Diagnosis of nondeletional hereditary persistence of fetal hemoglobin (HPFH)
-Identification of abnormal gamma chain variants (eg, unstable, high- or low-oxygen affinity, or M hemoglobins)
-Predicting the severity of a coinherited sickling disorder
-Evaluation of unexplained neonatal anemia, cyanosis, or hyperbilirubinemia

Method Name

Polymerase Chain Reaction (PCR) Amplification/Sanger Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type
Varies

Necessary Information
A complete patient history is strongly encouraged.

Specimen Required
Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix 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: Label specimen as extracted DNA from blood and provide indication of volume and concentration of the DNA
Specimen Stability Information: Frozen (preferred)/Refrigerate/Ambient

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. Thalassemia/Hemoglobinopathy Patient Information (T358)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request (T755) with the specimen.

Specimen Minimum Volume
Blood: 1 mL; Extracted DNA: 50 mcL at 50 ng/mcL concentration

Reject Due To

Gross hemolysis	OK
Bone marrow	Reject

Paraffin-embedded tissue Frozen tissue Paraffin-embedded bone marrow aspirate clot Methanol-acetic acid (MAA)-fixed pellets Moderately to severely clotted	

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Hemoglobin F (HbF) is the dominant hemoglobin at birth but is gradually replaced by adult hemoglobin (HbA) during the year after birth (normal value < or =1% of total hemoglobin after age 2 years). Increased HbF levels may continue after the neonatal period and into adulthood for various reasons. Genetic causes include deletional and nondeletional forms of hereditary persistence of fetal hemoglobin (HPFH) and delta-beta thalassemia variants. Over 100 genetic variants have been described in the gamma genes and, if detectable, the protein expression will vary over time according to the overall HbF expression. Gamma globin variants can manifest either as a quantitative (gamma thalassemia or nondeletional HPFH) or a qualitative (gamma variant) abnormality. Nondeletional HPFH alterations frequently modulate the expected severity of sickling disorders due to the inhibitory properties of HbF on sickle formation. Many gamma chain variants are benign, although some, such as unstable, high- and low-oxygen affinity, or M hemoglobin variants, cause hemolytic anemia/hyperbilirubinemia, erythrocytosis, cyanosis, and methemoglobinemia, respectively. The percentages of gamma variants will vary according to if they are present on the *HBG1* or *HBG2* genes, as these genes are differentially expressed depending on the age of the patient. Symptoms due to gamma variants are expected to decrease along with the normal decrease in HbF and therefore, most resolve after the first 6 months of life.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided and will include specimen information, assay information, and whether the specimen was positive for any variants in the gene. If positive, the alteration will be correlated with clinical significance if known.

Cautions

This test cannot be used in isolation to confirm or exclude hemoglobin conditions. Large deletions, crossover events, as well as other variants may not be detected. This test is used in conjunction with adequately studied protein analysis results.

If multiple alterations are identified, gamma globin gene sequencing is not able to distinguish between variants that are found in the same allele (in cis) and variants found on different alleles (in trans). This limitation of sequencing may complicate diagnosis or classification and has implications for inheritance and genetic counseling. To resolve these cases, molecular results must be correlated with electrophoretic and protein data and/or family studies.

Clinical Reference

1. Crowley MA, Mollan TL, Abdulmalik OY, et al. A hemoglobin variant associated with neonatal cyanosis and anemia. N Engl J Med. 2011;364(19):1837-1843
2. Cui J, Baysdorfer C, Azimi M, et al. Identification of three novel Hb F variants: Hb F-Hayward [(G)gamma1(NA1)Gly>Asp, GGT>GAT], Hb F-Chori-I [(A)gammaT16(A13)Gly>Asp, GGC>GAC] and Hb F-Chori-II [(A)gammaI29(B11)Gly>Glu, GGA>GAA]. Hemoglobin. 2012;36:305-309
3. Akinsheye I, Alsultan A, Solovieff N, et al. Fetal hemoglobin in sickle cell anemia. Blood. 2011;118(1):19-27
4. Steinberg M, Forget B, Higgs D, Weatherall D, eds. Disorders of Hemoglobin Genetics, Pathophysiology, and Clinical Management. 2nd ed. Cambridge University Press; 2009
5. Provan D, Gribben J, eds. Molecular Hematology. 3rd ed. Blackwell Publishing; 2010
6. Hoyer JD, Kroft SH, eds. Color Atlas of Hemoglobin Disorders: A Compendium Based on Proficiency Testing. College of American Pathologists; 2003
7. Merchant S, Oliveira JL, Hoyer JD, Viswanatha DS. Molecular diagnosis in hematopathology. In: Goldblum J. Hsi E, eds. Hematopathology: A Volume in the Series: Foundations in Diagnostic Pathology. 2nd ed. Churchill Livingstone; 2012:chap 24
8. Semkiu KM, Oliveira JL, Nguyen PL, Porter TR, Wilson DB. Hb F-Wentzville [(G)gamma24(B6)Gly>Glu; *HBG2*: c.74G>A, p.Gly25Glu]: An unstable (G)gamma-globin variant associated with neonatal hemolytic anemia. Hemoglobin. 2020;44(1):67-69. doi:10.1080/03630269.2020.1716002

Performance

Method Description

Total genomic DNA is extracted from the sample, and the full gamma globin genes are amplified by polymerase chain reaction in separate reactions followed by Sanger sequencing. Review of the sequence data is performed using a combination of automated calls and manual inspection.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

10 days

Specimen Retention Time

Blood: 2 weeks; DNA: 3 months

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

81479-Unlisted molecular

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
WGSEQ	Gamma Globin Full Gene Sequencing	95795-1

Result ID	Test Result Name	Result LOINC® Value
46952	Gamma Globin Gene Sequencing Result	50397-9
46953	Gamma Globin Interpretation	59466-3