MAYO CLINIC LABORATORIES

Test Definition: KLF1

KLF1 Full Gene Sequencing, Varies

Overview

Useful For

Aiding in the diagnosis and carrier detection of *KLF1* sequence alterations that are reported to be responsible for neonatal anemia or jaundice, hydrops fetalis, increased fetal hemoglobin and hemoglobin A2.

Assessing patients with sickle cell disease with unexpected phenotypes, individuals with unexplained decreased pyruvate kinase activity levels, or unexplained microcytic hypochromic complete blood cell count parameters.

Genetics Test Information

This test is intended to aid in the diagnosis and carrier detection of *KLF1* sequence alterations that are reported to be responsible for increased fetal hemoglobin (HbF) and hemoglobin A2 (HbA2). Variants in *KLF1* have also been associated with severe neonatal anemia and congenital dyserythropoietic anemia.

Special Instructions

- Informed Consent for Genetic Testing
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR) Amplification followed by Sanger Sequencing

Specimen

Specimen Type

Varies

Necessary Information

The following information is required on patient information or test request form:

1. Clinical diagnosis
2. Pertinent clinical history (submit complete blood cell count and hemoglobin electrophoresis results and relevant clinical notes)
3. Date of collection
4. Specimen type, whole blood or extracted DNA

Specimen Required

Submit only 1 of the following specimens:

Preferred:
Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD) or green top (heparin)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
3. Label specimen as blood.
Specimen Stability Information: Ambient 14 days (preferred)/ Refrigerated 30 days

Acceptable:
Specimen Type: Extracted DNA from whole blood
Container/Tube: 1.5- to 2-mL tube with indication of volume and concentration of DNA
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/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. Metabolic Hematology Patient Information (T810)
3. If not ordering electronically, complete, print, and send an 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 biopsies Paraffin-embedded tissue Frozen tissue Paraffin-embedded bone marrow aspirate clot Methanol-acetic acid (MAA)-fixed pellets	Reject

Moderately to severely clotted	

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Kruppel-like factor 1 (KLF1) is an essential erythroid transcription factor. Genetic alterations of the gene have been reported to have different phenotypic outcomes ranging from a persistent increase in fetal hemoglobin (HbF) to chronic nonspherocytic hemolytic anemia. Some *KLF1* variants have been associated with increased HbF and a milder phenotype in patients with sickle cell disease. *KLF1* variants have been categorized into four functional classes that affect the phenotype and inheritance pattern. Class 2 and 3 variants result in an autosomal recessive congenital microcytic hypochromic anemia, which can be associated with neonatal jaundice. Some *KLF1* variants are associated with decreased pyruvate kinase enzyme activity. Type IV congenital dyserythropoietic anemia is associated with a commonly *de novo* autosomal dominant *KLF1* E325K variant and is associated with a variable phenotype that ranges from hydrops fetalis, sex reversal/hypospadias, severe transfusion dependent anemia to a milder anemic course that resolves after infancy. Very small quantities of embryonic hemoglobins (Hb Portland or zeta chains) may be evident in sensitive hemoglobin studies.

Reference Values

An interpretive report will be provided

Interpretation

A negative result means no variants were detected by Sanger sequencing of this gene.

A positive result means the DNA sequencing detected an alteration in the *KLF1* gene. *KLF1* encodes for Kruppel-like factor 1 (erythroid), a transcription factor essential for erythropoiesis and the expression of adult hemoglobin. Clinically significant variants in *KLF1* cause a wide variety of red cell phenotypes, including elevated HbF or HbA2 levels, microcytosis, nonspherocytic hemolytic anemia, or congenital dyserythropoietic anemia.(1) Some *KLF1* altered cases have decreased pyruvate kinase activity. Phenotype and inheritance pattern is dependent on the specific underlying causative variant.

More specific interpretations will depend on what variants are found.

Cautions

Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Individuals may have a variant, deletion, or duplication in the gene tested that is not identifiable by the described testing methodology. Rare variants (polymorphisms) exist and could lead to false negative results. In addition, the phenotype observed in the

individual tested here may be due to a variant in a gene not analyzed by this test. This assay will not detect deep intronic or large deletion-insertion (delins) sequence alterations.

Rare, undocumented variants (ie, polymorphisms) under the primers can cause polymerase chain reaction failure.

Patients who have received an allogenic blood transfusion within the preceding 6 weeks, or who have received an allogenic blood or marrow transplant can have inaccurate genetic test results due to presence of donor DNA.

Clinical Reference

1. Perkins A, Xu S, Higgs DR, et al: Kruppeling erythropoiesis: an unexpected broad spectrum of human red blood cell disorders due to KLF1 variants. Blood. 2016 Apr;127(15):1856-1862. doi: 10.1182/blood-2016-01-694331
2. Liu D, Zhang X, Xu X, et al: KLF1 mutations are relatively more common in a thalassemia endemic region and ameliorate the severity of b-thalassemia. Blood. 2014 Jul;124(5): 803-811. doi: 10.1182/blood-2014-03-561779
3. Tallack MR, Perkins AC: Three fingers on the switch: Kruppel-like factor 1 regulation of gamma-globin to beta-globin gene switching. Curr Opin Hematol. 2013 May;20(3):193-200. doi: 10.1097/MOH.0b013e32835f59ba.
4. Tepakhan W, Yamsri S, Sanchaisuriya K, Fucharoen G, Xu X, Fucharoen S: Nine known and five novel mutations in the erythroid transcription factor KLF1 gene and phenotypic expression of fetal hemoglobin in hemoglobin E disorder. Blood Cells Mol Dis. 2016 Jul;59:85-91. doi: 10.1016/j.bcmd.2016.04.010 5. Planutis A, Xue L, Trainor CD, et al: Neomorphic effects of the neonatal anemia (Nan-Eklf) mutation contribute to deficits throughout development. Development. 2017 Feb 1;144(3):430-440. doi: 10.1242/dev.145656
6. Gillinder KR, Ilsley MD, Nebor D, et al: Promiscuous DNA-binding of a mutant zinc finger protein corrupts the transcriptome and diminishes cell viability. Nucleic Acids Res. 2017 Feb;45(3):1130-1143. doi: 10.1093/nar/gkw1014
7. Satta S, Paglietti ME, Sollaino MC, et al: Changes in HbA2 and HbF in alpha thalassemia carriers with KLF1 mutation. Blood Cells Mol Dis. 2017 May;64:30-32. doi: 10.1016/j.bcmd.2017.03.007
8. Wienert B, Martyn GE, Kurita R, Nakamura Y, Quinlan KGR, Crossley M: KLF1 drives the expression of fetal hemoglobin in British HPFH. Blood. 2017 Aug;130(6):803-807. doi: 10.1182/blood-2017-02-767400
9. Gallienne AE, Dreau HM, Schuh A, Old JM, Henderson S: Ten novel mutations in the erythroid transcription factor KLF1 gene associated with increased fetal hemoglobin levels in adults. Haematologica. 2012 Mar;97(3):340-343. doi: 10.3324/haematol.2011.055442
10. Viprakasit V, Ekwattanakit S, Riolueang S, et al: Mutations in Kruppel-like factor 1 cause transfusion-dependent hemolytic anemia and persistence of embryonic globin gene expression. Blood 2014;123(10):1586-1595. doi:10.1182/blood-2013-09-526087
11. Ravindranath Y, Johnson RM, Goyette G, Buck S, Gadgeel M, Gallagher PG: KLF1 E325K-associated congenital dyserythropoietic anemia type IV: Insights into the variable clinical severity. J Pediatr Hematol Oncol. 2018 Aug;40(6):e405-e409. doi: 10.1097/MPH.0000000000001056

Performance

Method Description

Genomic DNA is extracted from whole blood. The *KLF1* gene is 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 trace files analyzed for variations in the promoter region (cover to c.-183), 5'UTR (untranslated region), exons 1, 2 and 3, +/- 10 base pairs (bp), and the last 100 bp of the 3'UTR covering

the polyadenylation site.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
28 to 42 days

Specimen Retention Time
DNA: 3 months; Peripheral blood: 2 weeks

Performing Laboratory Location
Rochester

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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
KLF1	KLF1 Full Gene Sequencing, V	41103-3

Result ID	Test Result Name	Result LOINC® Value
618219	Interpretation	69047-9
618220	Signing Pathologist	18771-6