MAYO CLINIC LABORATORIES

Test Definition: PKLRG

Pyruvate Kinase Liver and Red Blood Cell (PKLR), Full Gene Sequencing and Large Deletion Detection, Varies

Overview

Useful For

Aiding in the diagnosis of pyruvate kinase (PK) deficiency

Ascertaining a causative variant in the *PKLR* gene of patients with low or relatively low levels of erythrocytic PK enzymatic activity

Ascertaining carrier status of family members of individuals diagnosed with PK deficiency for genetic counseling purposes

Special Instructions

- Informed Consent for Genetic Testing
- PKLR Gene Sequencing Patient Information
- Informed Consent for Genetic Testing (Spanish)

Method Name

Polymerase Chain Reaction (PCR) followed by DNA Sequence Analysis/Large Deletion Detection by PCR followed by Fragment Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Preliminary screening tests, such as complete blood count with peripheral smear, direct Coombs test, and pyruvate kinase (PK) enzyme activity assays (preferably as a part of EEEV1 / Red Blood Cell [RBC] Enzyme Evaluation, Blood) should be run before ordering this evaluation.

Necessary Information

1. **PKLR Gene Sequencing Patient Information is required**. Testing may proceed without the patient information however it aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to complete the form and send it with the specimen.
2. Include physician name and phone number with specimen.

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Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Yellow top (ACD solution B) or lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Refrigerated 30 days

Specimen Type: DNA
Container/Tube: 2 mL screw top tube
Specimen Volume: 100 microliters
Collection Instructions:
1. The preferred volume is 100 microliters at a concentration of 250 ng/mcL
2. Include concentration and volume on tube
Specimen Stability Information: Frozen (preferred)/Ambient/Refrigerate

Forms

1. ***PKLR* Gene Sequencing Patient Information is required**
2. **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)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume

Whole blood: 0.5 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

The glycolytic pathway is used by all tissues for energy production through the formation of adenosine triphosphate (ATP). It is particularly important in red blood cells, which are dependent upon this pathway for energy due to their lack of mitochondria. The *PKLR* gene encodes for pyruvate kinase, the rate-limiting glycolytic enzyme that catalyzes the transphosphorylation from phosphoenolpyruvate to adenosine diphosphate, creating pyruvate and ATP. Pyruvate kinase (PK) deficiency is a relatively common cause of hereditary nonspherocytic hemolytic anemia,(1) with an estimated prevalence of 1:20,000 among people of European descent. The severity of hemolysis varies from fully compensated forms to life-threatening neonatal anemia requiring transfusions.(2) Over 200 different variants have been reported in the *PKLR* gene. Most are single nucleotide substitutions, although rarer large deletions have also been identified. PK deficiency is inherited in an autosomal recessive manner, and genetic results should be correlated with enzyme levels performed as remote from transfusion when possible. PK deficiency can be difficult to interpret based on enzyme level alone and may be only mildly decreased or normal in those with the most severe symptoms or after splenectomy due to reticulocytosis.(2) Comparison to other erythrocyte enzyme levels is usually very helpful in this regard. Heterozygous carriers of *PKLR* variants have intermediate enzyme levels and are not symptomatic.

Reference Values

An interpretive report will be provided.

Interpretation

All detected alterations will be evaluated according to current American College of Genetics and Genomics recommendations.(3) Variants will be classified based on known, predicted, or possible effect on gene pathogenicity and reported with interpretive comments detailing their potential or known clinical significance.

Cautions

Blood specimens may contain donor DNA if obtained from patients who received blood transfusions or allogeneic blood or marrow transplantation. Results from specimens obtained under these circumstances may not accurately reflect the recipient's genotype.

For individuals who have received blood transfusions, the genotype usually reverts to that of the recipient within 6 weeks. For individuals who have received allogeneic blood or marrow transplantation, a pretransplant DNA specimen is recommended for testing. For patients who have been transfused within the preceding 6 weeks, the enzyme assay (PK1 / Pyruvate Kinase Enzyme Activity, Blood) will also be affected, so it is not an appropriate alternative test.

Patients who have received an allogeneic blood or marrow transplant would be expected to convert to the *PKLR* status of the donor. However, if the patient's transplant was partially successful or if there is a relapse of an underlying hematologic malignancy, a mixture of donor and recipient genotypes may be seen on genetic analysis. The enzyme assay can be performed after transplantation; order PK1 / Pyruvate Kinase Enzyme Activity, Blood.

Rare variants exist that could lead to false-negative or false-positive results. Other variants in the primer binding regions can affect the testing and, ultimately, the genotype assessment made.

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Large deletions or rearrangements that are not within the intron 2 through exon 11 region are not detected by this assay.

Sometimes a genetic alteration of unknown significance may be identified. In this case, testing of appropriate family members may be useful to determine pathogenicity of the alteration.

This test is not designed to provide specific dosing or drug selection recommendations and is to be used as an aid to clinical decision making only. Drug-label guidance should be used when dosing patients with medications regardless of the predicted phenotype.

Clinical Reference

1. van Wijk R, Huizinga E, van Wesel AC, et al: Fifteen novel mutations in PKLR associated with pyruvate (PK) deficiency: structural implications of amino acid substitutions in PK. Hum Mutat. 2009;30(3):446-453
2. Zanella A, Fermo E, Bianchi P, et al: Pyruvate kinase deficiency: the genotype-phenotype association. Blood Rev. 2007 Jul;21(4):217-231
3. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-424
4. OMIM: 609712 Pyruvate Kinase, Liver and Red Blood Cell; PKLR. Johns Hopkins University; 2005. Updated November 2022. Accessed January 6, 2023. Available at www.omim.org/entry/609712
5. Baronciani L, Beutler E: Molecular study of pyruvate deficient patients with hereditary nonspherocytic hemolytic anemia. J Clin Invest. 1995 April;95(4):1702-1709
6. Bianchi P, Zanella A: Hematologically important mutations: red cell pyruvate kinase (Third update). Blood Cells Mol Dis. 2000 Feb;26(3):47-53
7. Costa C, Albuisson J, Le TH, et al: Severe hemolytic anemia in a Vietnamese family, associated with novel mutations in the gene encoding for pyruvate kinase. Haematologica. 2005 Jan;90(1):25-30
8. So CC, Tang M, Li CH, et al: First reported case of prenatal diagnosis for pyruvate kinase deficiency in a Chinese family. Hematology. 2011 Nov;16(6):377-379
9. van Wijk R, van Solinge WW, Nerlov C, et al: Disruption of a novel regulatory element in the erythroid-specific promoter of the human *PKLR* gene causes severe pyruvate kinase deficiency. Blood. 2003 Feb;101(4):1596-1062

Performance

Method Description

Genomic DNA is extracted from whole blood. The *PKLR* gene is amplified by polymerase chain reaction (PCR). The PCR products are 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 the exons and intron/exon boundaries of all exons using variant detection software and visual inspection.(Unpublished Mayo method)

Large Deletion Detection:
A single PCR product is amplified and separated by gel electrophoresis for fragment size detection.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed
Varies

Report Available
10 to 21 days

Specimen Retention Time
Whole blood: 2 weeks; Extracted DNA: 2 months

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
81405
81479

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PKLRG	PKLR Full Gene and Deletion	94212-8

Result ID	Test Result Name	Result LOINC® Value
37858	Interpretation	69047-9
37860	Reviewed by	18771-6
48398	Result Details	82939-0
48396	Disclaimer	62364-5
48397	Method	85069-3
37857	Result Summary	50397-9
91971	Additional Information	48767-8