Test Definition: PKLRZ

PKLR Full Gene Analysis, 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

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in *PKLR*. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for pyruvate kinase deficiency.

Special Instructions

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

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

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

Targeted testing (also called site-specific or known variants testing) is available for variants identified in the *PKLR* gene.

See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Necessary Information

1. *PKLR* Gene Sequencing Patient Information is strongly recommended but not required. Testing may proceed without the patient information; however, it aids in providing a more thorough interpretation. Ordering healthcare professionals are strongly encouraged to complete the form and send it with the specimen.
2. If form not provided, include the following information with the test request: clinical diagnosis, pertinent clinical history (ie, complete blood cell count results and relevant clinical notes) and differentials based on any previous bone marrow studies, clinical or morphologic presentation.

Specimen Required

Specimen Type: Whole blood
Patient Preparation: A previous bone marrow transplant from an allogeneic donor will interfere with testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.
Container/Tube:
Preferred: Lavender top (EDTA)
Acceptable: Yellow top (ACD)
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: Ambient (preferred) 4 days/Refrigerated 4 days
Additional Information: To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.

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. *PKLR* Gene Sequencing Patient Information (T766)
3. If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume

1 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 (PK), the rate-limiting glycolytic enzyme that catalyzes the transphosphorylation from phosphoenolpyruvate to adenosine diphosphate, creating pyruvate and ATP.

Pyruvate kinase 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 expected to be symptomatic.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(3) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

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.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multiexon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

Deletion/duplication events that extend past the genes included on the panel may occur. In these instances, genes included in the ordered test are provided on the report and interpreted, and genomic breakpoints are reported if they are confirmed. However, copy number variants for genes not listed in the Method Description are typically not reported or interpreted for haploinsufficiency/triplosensitivity. CMACB / Chromosomal Microarray, Congenital, Blood; WESPR / Panel to Whole Exome Sequencing Reflex Test, Varies; or WGSDX / Whole Genome Sequencing for Hereditary Disorders, Varies is recommended for a full interpretation of deletions/duplications predicted to extend past the genes included on the panel.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

Reclassification of Variants:

Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(3) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign or intronic variants without known or suspected pathogenicity are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

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;21(4):217-231
3. Richards S, Aziz N, Bale S, et al. 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;17(5):405-424
4. OMIM: 609712 Pyruvate Kinase, Liver and Red Blood Cell; PKLR. Johns Hopkins University; 2005. Updated October 2022. Accessed September 18, 2025. 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;95(4):1702-1709
6. Bianchi P, Zanella A. Hematologically important mutations: red cell pyruvate kinase (Third update). Blood Cells Mol Dis. 2000;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;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;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;101(4):1596-1062

Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *PKLR* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 20X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp, and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the *PKLR* gene.

There may be regions of this gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

The reference transcript for *PKLR* gene is NM_000298.6. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

The following additional noncoding variants are being analyzed by this test:
+/-30 bp flanking exons, c.-98 to c.-1, c.1269+43T>C, NM_181871.3 Exon 1

PDF Report

No

Day(s) Performed

Varies

Report Available

28 to 42 days

Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted 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

Test Definition: PKLRZ

PKLR Full Gene Analysis, Varies

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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PKLRZ	PKLR Full Gene Analysis	94212-8

Result ID	Test Result Name	Result LOINC® Value
618935	Test Description	62364-5
618936	Specimen	31208-2
618937	Source	31208-2
618938	Result Summary	50397-9
618939	Result	82939-0
618940	Interpretation	59465-5
618941	Additional Results	82939-0
621816	Resources	99622-3
621817	Additional Information	48767-8
621818	Method	85069-3
621819	Genes Analyzed	82939-0
621820	Disclaimer	62364-5
621821	Released By	18771-6