### Overview

#### Useful For

Aid in the diagnosis of pyruvate kinase (PK) deficiency

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

Ascertain 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

#### Advisory Information

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 EEEVP / Red Blood Cell [RBC] Enzyme Evaluation) should be run before ordering this evaluation.

#### Necessary Information

1. [PKLR Gene Sequencing Patient Information](#) is **required**, see Special Instructions. 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.

#### Specimen Required

Submit only 1 of the following specimens:

- **Specimen Type:** Whole blood
- **Container/Tube:** Yellow top (ACD solution B) or Purple top (EDTA)
- **Specimen Volume:** 3 mL
**Collection Instructions:**

1. Invert several times to mix blood.
2. Send specimen in original tube.

**Specimen Stability Information:** Refrigerated

**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 acceptable

**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 in Special Instructions:
   - [Informed Consent for Genetic Testing](#) (T576)
   - [Informed Consent for Genetic Testing-Spanish](#) (T826)

2. [PKLR Gene Sequencing Patient Information](#) in Special Instructions

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**

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
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Clinical and Interpretive

Clinical Information

The glycolytic pathway is used by all tissues for energy production through the formation of 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 (PEP) to adenosine diphosphate (ADP) creating pyruvate and adenosine triphosphate (ATP). Pyruvate kinase (PK) deficiency is a relatively common cause of hereditary nonspherocytic hemolytic anemia, 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. Over 200 different variants have been reported in the PKLR gene. Most are single nucleotide substitutions although rarer large deletions have also been identified. Pyruvate kinase deficiency is inherited in an autosomal recessive manner and genetic results should be correlated with enzyme levels performed remote from transfusion when possible. Pyruvate kinase 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. Comparison to other RBC 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 ACMG recommendations. 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 samples may contain donor DNA if obtained from patients who received heterologous blood transfusions or allogeneic blood or marrow transplantation. Results from samples 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 (PK / Pyruvate Kinase, Erythrocytes) 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 genotype may be seen on genetic analysis. The enzyme assay can be run after transplantation; order PK / Pyruvate Kinase, Erythrocytes.

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. OMIN 609712 Pyruvate Kinase, Liver and Red Blood Cell; PKLR. Accessed November 2016. Available at OMIM.org

**Performance**

**Method Description**
Genomic DNA is extracted from whole blood. The *PKLR* gene is amplified by 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 trace files analyzed for variations in the exons and intron/exon boundaries of all exons using mutation 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) and Time(s) Test Performed**
Varies
**Test Definition: PKLRG**

**PKLR Full Gene and Deletion**

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**Analytic Time**
3 days (Not reported Saturday or Sunday)

**Maximum Laboratory Time**
7 days

**Specimen Retention Time**
Whole Blood: 2 weeks; Extracted DNA: 2 months

**Performing Laboratory Location**
Rochester

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**Fees and 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 Regional Manager. For assistance, contact [Customer Service](#).

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**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

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**CPT Code Information**

81405-PKLR

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**LOINC® Information**

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