MAYO CLINIC LABORATORIES

Test Definition: CALR

CALR Mutation Analysis, Myeloproliferative Neoplasm (MPN), Varies

Overview

Useful For

Rapid and sensitive detection of insertion and deletion-type mutations in exon 9 of *CALR*

Aiding in distinguishing between reactive thrombocytosis and leukocytosis versus a myeloproliferative neoplasm (MPN), especially essential thrombocythemia (ET) and primary myelofibrosis (PMF), and is highly informative in cases in which *JAK2* and *MPL* testing are negative

Especially helpful to the pathologist in those bone marrow cases with ambiguous etiology of thrombocytosis, equivocal bone marrow morphologic findings of MPN, and unexplained reticulin fibrosis

Aiding in the prognostication of PMF and thrombosis risk assessment in ET

Testing Algorithm

For information see:
-Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation
-Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation

Special Instructions

- Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation
- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation
- Hematopathology Patient Information

Method Name

Polymerase Chain Reaction (PCR) and Fragment Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Specimen must arrive within 7 days of collection.

Necessary Information

Specimen source is required

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA) or 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.**
3. Label specimen as whole blood.
Specimen Stability Information: Ambient (preferred)/Refrigerate
Additional Information: To ensure minimum volume and concentration of DNA is met, the preferred volume of blood must be submitted. Testing may be canceled if DNA requirements are inadequate.

Specimen Type: Bone marrow
Container/Tube: Lavender top (EDTA) or yellow top (ACD
Specimen Volume: 2 mL
Collection Instructions:
1. Invert several times to mix bone marrow.
2. Send bone marrow specimen in original tube. **Do not aliquot.**
3. Label specimen as bone marrow.
Specimen Stability Information: Ambient (preferred)/Refrigerate

Specimen Type: Extracted DNA from blood or bone marrow
Container/Tube: 1.5- to 2-mL tube
Specimen Volume: Entire specimen
Collection Instructions:
1. DNA must be extracted from blood or bone marrow within 7 days of collection.
2. Label specimen as extracted DNA and source of specimen.
3. Provide volume and concentration of the DNA.
Specimen Stability Information: Frozen (preferred) 1 year/Refrigerate/Ambient
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). We cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied.

Forms

1. Hematopathology Patient Information (T676)
2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Whole blood/bone marrow: 1 mL; Extracted DNA: 50 mcl at 20 ng/mcL concentration

Reject Due To

Gross hemolysis	Reject
Paraffin-embedded bone marrow aspirate clot	Reject
Bone marrow biopsies, slides, or paraffin shavings	Reject
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies	7 days	

Clinical & Interpretive

Clinical Information

The most frequent genetic mutation in *BCR-ABL1*-negative myeloproliferative neoplasm (MPN), essential thrombocythemia (ET), and primary myelofibrosis (PMF) is the *JAK2* V617F alteration, which is present in approximately 50% to 60% of patients. It serves as a confirmatory molecular marker of these diseases. Mutations in the *MPL* gene are found in an additional 5% to 10% of ET and PMF cases. It was recently discovered that somatic mutation (insertions and deletions) in exon 9 of the *CALR* gene is the second most frequent somatic mutation after *JAK2* in ET and PMF patients, and it is mutually exclusive of *JAK2* and *MPL* mutations.(1,2) It has a frequency of approximately 49% to 88% in *JAK2* and *MPL*-wild type (WT) ET and PMF and is not found in polycythemia vera (PV) patients.(1-4) Therefore, the *CALR* mutation serves as an important diagnostic molecular marker in ET and PMF.

The *CALR* gene encodes for calreticulin, a multifunctional protein with a C-terminus rich in acidic amino acids and a KDEL endoplasmic reticulum (ER)-retention motif. All the disease-causing *CALR* mutations reported to date are out-of-frame insertion and/or deletions in exon 9, generating a 1 base pair (bp) frame shift and an altered protein with a novel C-terminus rich in basic amino acids and loss of the KDEL ER-retention signal. The most common mutation types are 52 bp-deletion (c.1092_1143del, L367fs*46) and 5-bp insertion (c.1154_1155insTTGCC, K385fs*47), and they comprise approximately 85% of *CALR* mutations in MPN.(1,2) *CALR* mutations have been found in hematopoietic stem and progenitor cells in MPN patients(2) and may activate the STAT5 signaling pathway.(1) They are associated with decreased risk of thrombosis in ET (1,3-5), and better survival in PMF compared to *JAK2* mutations.(5)

Reference Values

An interpretive report will be provided

Interpretation

An interpretive report will be issued.

The results will be reported as 1 of the 3 states if DNA amplification is successful (see Cautions):
-Positive. A deletion-insertion-type mutation was detected in *CALR*, exon 9.
-Negative. No deletion or insertion was detected in *CALR*, exon 9.
-Equivocal. A small amplicon suspicious for a deletion-insertion type mutation was detected in *CALR*, exon 9.

Positive mutation status is highly suggestive of a myeloid neoplasm but must be correlated with clinical and other laboratory and morphologic features for definitive diagnosis.

Negative mutation status does not exclude the presence of a myeloproliferative neoplasm or other neoplastic disorders.

Cautions

A positive result is not specific for a particular myeloproliferative neoplasm (MPN) diagnosis, and clinicopathologic correlation is necessary in all cases.

A negative result does not exclude the presence of an MPN or other neoplastic process.

This test is a fragment analysis assay and only detects deletions-insertions (delins). It will not detect point mutations. However, all reported disease-causing mutations in MPN described to date are insertions and/or deletions.

This test may not differentiate between out-of-frame and in-frame delins in rare cases. However, in-frame delin mutations are very rare (<0.5%) and have only been reported in a few healthy individuals and myeloproliferative neoplasm patients with *JAK2*V617F mutation or out-of-frame *CALR* mutation. Most of the rare in-frame delins are considered germline variants and represent benign alterations (ie, polymorphisms).

Infrequently, amplification failure can be encountered in a given sample due to inadequate DNA, poor DNA quality, or a polymerase chain reaction inhibitor. In these circumstances, the assay will be reattempted, and if persistently unsuccessful, the report will be issued with an "Invalid" result.

Clinical Reference

1. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-2390
2. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25):2391-2405
3. Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544-1551
4. Rotunno G, Mannarelli C, Guglielmelli P, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. Blood. 2014;123(10):1552-1555
5. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472-1477

6. Greenfield G, McMullin MF, Mills K. Molecular pathogenesis of the myeloproliferative neoplasms. J Hematol Oncol. 2021;14(1):103

Performance

Method Description
Polymerase chain reaction (PCR) amplification of CALR exon 9 is performed on DNA isolated from the patient sample. The PCR product is then run on an ABI 3130xl Genetic Analyzer for fragment analysis to detect insertions and deletions. An unmutated CALR will show an amplicon at 266 base pairs (bp), a mutated CALR with insertion will show an amplicon greater than 266 bp, and a mutated CALR with deletion will show an amplicon smaller than 266 bp. This assay has an analytical sensitivity of approximately 6% (ie, 6 mutation-containing cells in 100 total cells) in most mutation types, except for the rare type of 1-bp deletion, which has a sensitivity of approximately 20%. This is a laboratory developed test.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 5 days

Specimen Retention Time
Whole blood/Bone marrow: 2 weeks; 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
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

81219-*CALR* (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CALR	MPN, CALR Gene Mutation, Exon 9	77174-1

Result ID	Test Result Name	Result LOINC® Value
36301	Final Diagnosis	22637-3
MP020	Specimen	31208-2