Test Definition: CALFM

CALR Mutation Analysis, Myeloproliferative Neoplasm (MPN), Bone Marrow

Overview

Useful For

Diagnosis or differential diagnosis of myeloproliferative disorders by *CALR* gene sequencing using bone marrow specimens

Method Name

Polymerase Chain Reaction (PCR) and Fragment Analysis

NY State Available

No

Specimen

Specimen Type

Bone Marrow

Shipping Instructions

Specimen must arrive within 7 days of collection.

Necessary Information

The following information is required:

1. Pertinent clinical history
2. Clinical or morphologic suspicion
3. Date of collection
4. Specimen source

Specimen Required

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 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 Minimum Volume

1 mL

Reject Due To

Gross	Reject

hemolysis	
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Bone Marrow	Ambient (preferred)	7 days	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Mutations in the *JAK2, CALR* and *MPL* genes are considered driver events in the BCR-ABL1 negative myeloproliferative neoplasms (MPN) including polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET). The *JAK2 V617F* mutation occurs in 95% to 98% of patients with PV, 50% to 60% of patients with PMF and 50% to 60% of patients with ET respectively at diagnosis. Other *JAK2* mutations in exon 12 to 15 occur in the remaining patients with PV. Mutations in the *CALR* gene occur in 20% to 30% of patients with PMF and 20% to 30% of patients with ET at diagnosis. A 52 base pairs (bp) deletion (53%) and a 5 bp deletion (32%) are the most common mutations in the *CALR* gene while other types of mutations may occur in the remaining cases. MPL exon 10 mutations occur in 5% to 10% of patients with PMF and 5% to 10% of patients with ET. Mutations in JAK2, CALR and MPL are mutually exclusive. The *JAK2 V617F* mutation is detected by quantitative polymerase chain reaction (qPCR). The *CALR* mutations are detected by PCR targeting the exon 9. The MPL mutations in exon 10 are detected by Sanger sequencing. All mutations in *JAK2, CALR* and *MPL* can also be detected by next generation sequencing (NGS). In addition to the mutations in *JAK2, CALR* and *MPL*, mutations in many other genes *including ASXL1, TET2, DNMT3A, SRSF2, SF3B1, U2AF1, ZRSR2, EZH2, IDH1, IDH2, CBL, KRAS, NRAS, STAG2*, and *TP53* can occur in MPN. These additional mutations are more frequent in PMF and advanced disease, as compared to PV and ET. It is known that mutations in the *ASXL1, SRSF2, U2AF1, EZH2, IDH1* and *IDH2* are correlated with a poor prognostic risk. While a single gene test on *JAK2, CALR* and *MPL* can be clinically useful, all above mentioned gene mutations can be detected by NGS.

Reference Values

An interpretive report will be provided.

Interpretation

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 MPN or other neoplastic processes.

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 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 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. 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
3. Greenfield G, McMullin MF, Mills K. Molecular pathogenesis of the myeloproliferative neoplasms. J Hematol Oncol. 2021;14(1):103
4. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719. doi:10.1038/s41375-022-01613-1

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%.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed
Monday through Friday

Report Available
1 to 8 days

Specimen Retention Time
Bone marrow: 2 weeks; Extracted DNA: 1 year

Performing Laboratory Location
Mayo Clinic Jacksonville Clinical Lab

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

LOINC® Information

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

Result ID	Test Result Name	Result LOINC® Value
618655	Final Diagnosis	69047-9
618656	Method	85069-3
618657	Signing Pathologist	18771-6
618659	Additional Information	48767-8
618660	Disclaimer	62364-5