MAYO CLINIC LABORATORIES

Test Definition: MPNCM

Myeloproliferative Neoplasm, CALR with Reflex to MPL, Varies

Overview

Useful For

Aiding in the distinction between a reactive cytosis and a myeloproliferative neoplasm when *JAK2V617F* testing result is negative

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
MPNML	MPL Exon 10 Sequencing, Reflex	No, (Bill Only)	No

Testing Algorithm

This test reflexively evaluates for variants in the *CALR* and *MPL* genes commonly associated with *BCR*::*ABL1*-negative myeloproliferative neoplasms. The testing sequence is based on the reported frequency of gene variants in this disease group. It is usually ordered when a *JAK2* V617F result is known to be negative. Initial testing evaluates for the presence of the *CALR* insertions and deletions. If out-of-frame *CALR* insertions or deletions are detected, the testing algorithm ends. If the *CALR* result is negative or an in-frame *CALR* insertion or deletion is identified, then testing proceeds, at an additional charge, to evaluate for variants in exon 10 of the *MPL* gene by Sanger sequencing. An integrated report is issued with the summary of test results.

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

Special Instructions

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

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

The following information is required:

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

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Whole Blood
Container/Tube: Lavender top (EDTA) or yellow top (ACD-B)
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

Specimen Type: Bone marrow
Container/Tube: Lavender top (EDTA) or yellow top (ACD-B)
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

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

Specimen Minimum Volume

Whole blood, bone marrow: 0.5 mL; Extracted DNA: 50 mcL at 20 ng/mcL concentration

Reject Due To

Gross hemolysis	Reject
Paraffin-embedded bone marrow aspirate clot or biopsy blocks	Reject
Slides	Reject
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 *JAK2* V617F variant is present in 95% to 98% of patients with polycythemia vera, 50% to 60% of patients with primary myelofibrosis (PMF), and 50% to 60% of patients with essential thrombocythemia (ET) patients. Detection of the *JAK2* V617F variant helps establish the diagnosis of a myeloproliferative neoplasm (MPN). However, a negative *JAK2* V617F result does not indicate the absence of MPN. Other important molecular markers in *BCR::ABL1*-negative MPN include *CALR* exon 9 variants (20%-30% of PMF and ET) and *MPL* exon 10 variants (5%-10% of PMF and 3%-5% of ET). Variants in *JAK2*, *CALR*, and *MPL* are essentially mutually exclusive. A *CALR* variant is associated with decreased risk of thrombosis in both ET and PMF and confers a favorable clinical outcome in patients with PMF. A triple negative (*JAK2* V617F, *CALR*, and *MPL*-negative) genotype is considered a high-risk molecular signature in PMF.

Reference Values

An interpretive report will be provided.

Interpretation

The results will be reported as 1 of the 3 following states:
-Positive for *CALR* variant
-Positive for *MPL* variant
-Negative for *CALR* and *MPL* variants

Positive variants status is highly suggestive of a myeloid neoplasm and clinicopathologic correlation is necessary in all cases.

Negative variant status does not exclude the presence of a myeloproliferative neoplasm or other neoplasms.

Cautions

A positive result is not specific for a particular subtype of myeloproliferative neoplasm and clinicopathologic correlation is necessary in all cases.

A negative result does not exclude the presence of a myeloproliferative neoplasm or other neoplastic process.

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. 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
4. 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
5. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3(7):e270
6. Pardanani AD, Levine RL, Lasho T, et al. *MPL*515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108(10):3472-3476
7. Defour JP, Chachoua I, Pecquet C, Constantinescu SN. Oncogenic activation of MPL/thrombopoietin receptor by 17 mutations at W515: implications for myeloproliferative neoplasms. Leukemia. 2016;30(5):1214-1216. doi:10.1038/leu.2015.271
8. 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 Genetic Analyzer for fragment analysis to detect insertions and deletions. An unaltered *CALR* will show an amplicon at 266 base pairs (bp), an altered *CALR* with insertion will show an amplicon greater than 266 bp, and an altered *CALR* with deletion will show an amplicon smaller than 266 bp. This assay has an

analytical sensitivity of approximately 6% (ie, 6 variant-containing cells in 100 total cells) in most variant types, except for the rare type of 1-bp deletion, which has a sensitivity of approximately 20%.(Unpublished Mayo method)

Genomic DNA is extracted, and Sanger sequencing is used to evaluate for variants in *MPL*, exon 10. The sensitivity of this assay is approximately 20%, such that samples containing lower percentages of altered DNA will appear negative.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
7 to 10 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
81339 -MPL (MPL proto-oncogene, thrombopoietin receptor) (eg, myeloproliferative disorder) gene analysis; sequence analysis, exon 10 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MPNCM	MPN (CALR, MPL) Reflex	In Process

Result ID	Test Result Name	Result LOINC® Value

42393	MPNCM Reflex Result	82939-0
MP036	Specimen Type	31208-2
42392	Final Diagnosis	50398-7