

## 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 the following algorithms are available:

- [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 blood.

**Specimen Stability Information:** Ambient (preferred)/Refrigerate 7 days

**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 specimen.
2. Send bone marrow specimen in original tube. **Do not aliquot.**
3. Label specimen as bone marrow.

**Specimen Stability Information:** Ambient (preferred)/Refrigerate 7 days

**Specimen Type:** Extracted DNA from blood or bone marrow

**Container/Tube:** 1.5 to 2 mL tube

**Specimen Volume:** Entire specimen

**Collection Instructions:**

1. Indicate volume and concentration of DNA
2. Label specimen as extracted DNA from blood or bone marrow.

**Specimen Stability Information:** Frozen (preferred)/Refrigerate/Ambient

**Forms**

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

**Specimen Minimum Volume**

[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 Slides Paraffin shavings 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 mutation 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 mutation 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. *MPL515* 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

### **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**

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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