

**Overview****Useful For**

Aiding in the distinction between a reactive cytosis and a chronic myeloproliferative disorder

Evaluating for variants in *CALR* in an algorithmic process

**Method Name**

Only orderable as a reflex. For more information see MPNJM / Myeloproliferative Neoplasm, *JAK2* V617F with Reflex to *CALR* and *MPL*, Bone Marrow.

Polymerase Chain Reaction (PCR)/Fragment Analysis

**NY State Available**

No

**Specimen****Specimen Type**

Bone Marrow

**Specimen Required**

Only orderable as a reflex. For more information see MPNJM / Myeloproliferative Neoplasm, *JAK2* V617F with Reflex to *CALR* and *MPL*, Bone Marrow.

**Container/Tube:**

**Preferred:** Lavender top (EDTA)

**Acceptable:** None

**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.

**Note:** Extracted DNA from bone marrow is **not** acceptable.

**Specimen Minimum Volume**

1 mL

**Reject Due To**

Gross hemolysis	Reject
Moderately to severely clotted	Reject
Extracted DNA from outside laboratory	Reject

### Specimen Stability Information

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

### Clinical & Interpretive

#### Clinical Information

The Janus kinase 2 gene (*JAK2*) codes for a tyrosine kinase (*JAK2*) that is associated with the cytoplasmic portion of a variety of transmembrane cytokine and growth factor receptors important for signal transduction in hematopoietic cells. Signaling via *JAK2* activation causes phosphorylation of downstream signal transducers and activators of transcription (STAT) proteins (eg, STAT5) ultimately leading to cell growth and differentiation. *BCR-ABL1*-negative myeloproliferative neoplasms (MPN) frequently harbor an acquired single nucleotide variant in *JAK2* characterized as c.G1849T; p. Val617Phe (V617F). *JAK2* V617F is present in 95% to 98% of polycythemia vera and 50% to 60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET). It has also been described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome. Detection of *JAK2* V617F is useful to help establish the diagnosis of 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 variant (20%-30% of PMF and ET) and *MPL* exon 10 variant (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 PMF patients. A triple negative (*JAK2* V617F, *CALR*, and *MPL*-negative) genotype is considered a high-risk molecular signature in PMF.

#### Reference Values

Only orderable as a reflex. For more information see MPNJM / Myeloproliferative Neoplasm (MPN), *JAK2* V617F with Reflex to *CALR* and *MPL*, Bone Marrow.

An interpretive report will be provided.

#### Interpretation

The interpretive report includes an overview of the findings.

#### Cautions

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

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

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

This test may not differentiate between out-of-frame and in-frame delins in rare cases. However, in-frame delins are very rare (<0.5%) and have only been reported in few healthy individuals and in MPN patients with *JAK2V617F* variant or out-of-frame *CALR* variant. Most of the rare in-frame delins are considered germline variants and represent nonpathogenic alterations.

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.

### **Supportive Data**

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-base pair deletion, which has a sensitivity of approximately 20%.

### **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. doi:10.1056/NEJMoa1311347
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. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol.* 2020;95(12):1599-1613
4. Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. *Blood.* 2023;141(16):1909-1921
5. Tefferi A, Vannucchi AM, Barbui T. Essential thrombocythemia: 2024 update on diagnosis, risk stratification, and management. *Am J Hematol.* 2024;99(4):697-718

### **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 Applied Biosystems Genetic Analyzer for fragment analysis to detect insertions and deletions.(Unpublished Mayo method)

#### **PDF Report**

No

**Day(s) Performed**

Monday through Friday

**Report Available**

2 to 10 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-CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9

**LOINC® Information**

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

Result ID	Test Result Name	Result LOINC® Value
614540	Final Diagnosis	22637-3