Test Definition: MPNJM

Myeloproliferative Neoplasm, JAK2 V617F with Reflex to CALR and MPL, Bone Marrow

Overview

Useful For

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

Evaluating for variants in *JAK2*, *CALR*, and *MPL* genes in an algorithmic process

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CALJM	CALR, Gene Mutation, Exon 9, BM	No	No
MPLJM	MPL Exon 10 Mutation Detection, BM	No	No

Testing Algorithm

This test sequentially evaluates for the common major gene variants associated with non-*BCR-ABL1*-positive myeloproliferative neoplasms until a variant is identified. The testing sequence is based on the reported frequency of gene variants in this disease group. Initial testing evaluates for the presence of the *JAK2* V617F variant. If this result is negative or very low positive (0.06%-0.6%), testing proceeds with assessment for *CALR* gene variants. If the *CALR* result is also negative, then testing proceeds to evaluate for variants in exon 10 of the *MPL* gene. If either *JAK2* V617F (>0.6%) or *CALR* variants are detected in the process, the testing algorithm ends; therefore, the complete reflex is followed only in the event of sequential negative variant. An integrated report is issued with the summary of test results.

Method Name

MPNJM: Quantitative Polymerase Chain Reaction (PCR)
CALJM: PCR/Fragment Analysis
MPLJM: Sanger Sequencing

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.

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 (PV) and 50% to 60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET) cases. 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

An interpretive report will be provided.

Interpretation

The results will be reported as 1 of the 4 following states:
-Positive for *JAK2* V617F variant
-Positive for *CALR* variant
-Positive for *MPL* variant
-Negative for *JAK2* V617F, *CALR*, and *MPL* variants

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

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

Results below the laboratory cutoff for positivity are of unclear clinical significance at this time.

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.

In rare cases, a variant other than *JAK2* V617F may be present in an area that interferes with primer or probe binding, which may cause a false-negative result.

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

JAK2 analysis:
Genomic DNA is extracted, and 2 polymerase chain reactions (PCR) are used for each sample. In each reaction, a short fragment of genomic DNA, including the variant site, is amplified using quantitative PCR in a real-time PCR instrument. In one reaction, the reverse primer matches the altered sequence, and the PCR conditions are such that it will only bind altered DNA. In the second reaction, the reverse primer matches the wild-type sequence, and the PCR conditions are such that it will only bind the wild-type sequence. In both reactions, the PCR is monitored using TaqMan probe chemistry. The amount of altered DNA and the amount of wild-type DNA is measured for each sample. In each run, the amount of altered and wild-type DNA in a calibrator DNA sample is also measured.

The final result is reported as percent *JAK2* V617F of total *JAK2*.(Unpublished Mayo method)

CALR analysis:
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.(Unpublished Mayo method)

MPL analysis:
Polymerase chain reaction (PCR) amplification of *MPL* exon 10 is performed on DNA isolated from the patient sample. The entire exon 10 sequence is obtained using Sanger sequencing with analysis on an automated genetic analyzer.(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

81270-JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
81219-CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9 (if appropriate)
81339-MPL (myeloproliferative leukemia virus oncogene, thrombopoietin receptor, TPOR) (eg, myeloproliferative disorder), exon 10 sequence (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MPNJM	MPN (JAK2 V617F, CALR, MPL), BM	53761-3

Result ID	Test Result Name	Result LOINC® Value
614536	Final Diagnosis	22637-3
614537	Method	85069-3
614538	Additional Information	48767-8
614539	Signing Pathologist	19139-5
606805	MPNJM Result	No LOINC Needed
614543	Disclaimer	62364-5