MAYO CLINIC LABORATORIES

Test Definition: MPNR

Myeloproliferative Neoplasm, JAK2 V617F with Reflex to CALR and MPL, Varies

Overview

Useful For

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

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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CALX	CALR, Gene Mutation, Exon 9, Reflex	No, (bill only)	No
MPLR	MPL Exon 10 Mutation Detection, R	No, (bill only)	No

Testing Algorithm

This reflexive 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%-2%), 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 (>2%) 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.

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

Quantitative Polymerase Chain Reaction (qPCR)

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 solution 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 solution 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 whole 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
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 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).

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). It has also been

described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome. Detection of *JAK2* V617F helps 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 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 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 currently.

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.

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.

If this test is ordered in the setting of erythrocytosis and suspicion of polycythemia vera, interpretation requires correlation with a concurrent or recent prior bone marrow evaluation.

Supportive Data

Analytical sensitivity is determined at 0.06% (by dilution of a *JAK2* V617F-positive cell line into a negative cell line DNA).

Clinical Reference

1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. Lancet. 2005;365(9464):1054-1061
2. James C, Ugo V, Le Couedic JP, et al. A unique clonal *JAK2* mutation leading to constitutive signaling causes polycythaemia vera. Nature. 2005;434(7037):1144-1148

3. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of *JAK2* in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779-1790
4. Steensma DP, Dewald GW, Lasho TL, et al. The *JAK2* V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and the myelodysplastic syndrome. Blood. 2005;106(4):1207-1209
5. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutation of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-2390
6. 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
7. 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
8. Pardanani AD, Levine RL, Lasho T, et al. *MPL*515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108:3472-3476
9. Kilpivaara O, Levine RL. *JAK2* and *MPL* mutations in myeloproliferative neoplasms: discovery and science. Leukemia. 2008;22(10):1813-1817
10. 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
11. Greenfield G, McMullin MF, Mils K. Molecular pathogenesis of the myeloproliferative neoplasms. J Hematol Oncol. 2021;14(1):103

Performance

Method Description

Genomic DNA is extracted, and 2 polymerase chain reaction (PCR) reactions 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 % *JAK2* V617F of total *JAK2*.(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

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 (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
MPNR	MPN (JAK2 V617F, CALR, MPL) Reflex	In Process

Result ID	Test Result Name	Result LOINC® Value
36687	Final Diagnosis	22637-3
39725	MPNR Result	No LOINC Needed