MAYO CLINIC LABORATORIES

Test Definition: JAK2M

JAK2 V617F Mutation Detection, Bone Marrow

Overview

Useful For

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

Testing Algorithm

For information see Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation.

Special Instructions

- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation
- Hematopathology Patient Information

Method Name

Quantitative Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Bone Marrow

Shipping Instructions

Specimen must arrive within 7 days of collection.

Specimen Required

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Yellow top (ACD)

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.**

Forms

1. Hematopathology Patient Information (T676)
2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Gross hemolysis	Reject
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Bone Marrow	Ambient (preferred)	7 days	PURPLE OR PINK TOP/EDTA
	Refrigerated	7 days	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

The Janus kinase 2 (*JAK2*) gene codes for a tyrosine kinase (JAK2) 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). This variant is identified overall in approximately two-thirds of all MPN,(1-3) but the prevalence varies by MPN subtype. The *JAK2* V617F variant is present in 95% to 98% of polycythemia vera patients, 50% to 60% of primary myelofibrosis (PMF) patients, and 50% to 60% of essential thrombocythemia (ET) patients. It has infrequently been described in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome.(4) This variant is not seen in chronic myelogenous leukemia or reactive conditions with elevated blood counts. Detection of the *JAK2* V617F variant is useful to help establish the diagnosis of MPN. However, a negative *JAK2* V617F result does not indicate absence of an 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.

Reference Values

An interpretive report will be provided.

Interpretation

The results will be reported as 1 of the 2 states:
-Negative for *JAK2* V617F variant
-Positive for *JAK2* V617F variant

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 neoplasm.

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 and clinicopathologic correlation is necessary in all cases. 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.

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

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

Supportive Data

Analytical sensitivity is determined at 0.06% (by dilution of a *JAK2* V617F-positive cell line DNA 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 Mar;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 Apr 28;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 Apr 28;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 syndromes. Blood. 2005 Aug 15;106(4):1207-1209
5. Gong JZ, Cook JR, Greiner TC, et al: Laboratory practice guidelines for detecting and reporting JAK2 and MPL mutations in myeloproliferative neoplasms: a report of the Association for Molecular Pathology. J Mol Diagn. 2013 Nov;15(6):733-744

Performance

Method Description

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 the first reaction, the 5' terminal base of the reverse primer matches the mutated sequence, and the PCR conditions are such that it will only bind mutated DNA. In the second reaction, the 5' terminal base of the reverse primer matches the wildtype 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 mutated DNA and the amount of wildtype DNA is measured for each sample. In each run, the amount of mutated and wild-type DNA in a calibrator DNA sample is also measured. The calibrator is a mixture of DNA from a positive cell line (HEL) and a negative cell line (HL60) that is frozen

in aliquots and expected to give an identical result in each run. Deviations in the calibrator result are assumed to be due to deviations in the run conditions and the sample results are corrected accordingly. Following each reaction, Relative Quantification Software is used to calculate the normalized mutated:wildtype ratio, which is expressed as a unitless ratio following correction with the calibrator data.

The formula for the normalized ratio is as follows:

$$\text{Normalized ratio} = \frac{\text{mutated/wildtype (sample)}}{\text{mutated/wildtype (calibrator)}}$$

The final result is reported as percent *JAK2* V617F of total *JAK2* (ie, [mutated/mutated + wildtype] x 100%), calculated from the normalized mutated:wildtype ratio.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
2 to 5 days

Specimen Retention Time
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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value

JAK2M	JAK2 V617F Mutation Detection, BM	72333-8

Result ID	Test Result Name	Result LOINC® Value
31157	JAK2 V617F Mutation Detection, BM	72333-8
39723	JAK2 Result	53761-3