MAYO CLINIC LABORATORIES

Test Definition: JAK2V

JAK2 V617F Mutation Detection, Varies

Overview

Useful For

Aiding in the distinction between a reactive blood cytosis and a chronic myeloproliferative disorder using extracted DNA specimens

Special Instructions

- Hematopathology Patient Information

Method Name

Quantitative Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Specimen Required

Specimen Type: Extracted DNA from blood or bone marrow
Container/Tube: 1.5- to 2-mL tube with indication of volume and concentration of the DNA
Specimen Volume: Entire specimen
Collection Instructions: Label specimen as extracted DNA from blood or bone marrow and indicate volume and concentration of the DNA.
Specimen Stability Information: Refrigerated/Ambient

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

50 microliter at a concentration of 20 ng/microliter

Reject Due To

Bone marrow biopsies Slides	Reject

Paraffin shavings Frozen tissues and paraffin-embedded tissues Paraffin-embedded bone marrow aspirates Moderately to severely clotted	

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

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 mutation in *JAK2* characterized as c.G1849T; p. Val617Phe (V617F). This mutation is identified overall in approximately two-thirds of all MPN,(1-3) but the prevalence varies by MPN subtype. The *JAK2* V617F is present in 95% to 98% of polycythemia vera, 50% to 60% of primary myelofibrosis (PMF), and 50% to 60% of essential thrombocythemia (ET). It has also been described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome.(4) This mutation is not seen in chronic myelogenous leukemia or in reactive conditions with elevated blood counts. Detection of the JAK2 V617F 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 mutation (20%-30% of PMF and ET) and *MPL* exon 10 mutation (5%-10% of PMF and 3%-5% of ET). Mutations 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 mutation
-Positive for *JAK2* V617F mutation

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

Negative mutation 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 mutation other than *JAK2* 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;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;352:1779-1790
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;106:1207-1209
5. Stuckey R, Gomez-Casares MT. Recent advances in the use of molecular analyses to inform the diagnosis and prognosis of patients with polycythaemia vera. Int J Mol Sci. 2021;22(9):5042. doi:10.3390/ijms22095042

Performance

Method Description

Genomic DNA is extracted and 2 polymerase chain reaction (PCR) amplifications are used for each sample. In each reaction, a short fragment of genomic DNA, including the mutation site, is amplified using quantitative PCR in a real-time PCR instrument (LightCycler 480, Roche). 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 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 mutated DNA and the amount of wild-type 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, LightCycler 480 Relative Quantification Software is used to calculate the normalized mutated:wild-type ratio, which is expressed as a unitless ratio following correction with the calibrator data.

The formula for the normalized ratio is as follows:

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

The final result is reported as percent *JAK2* V617F of total *JAK2*, ie [mutated/mutated + wild type] x 100%, calculated from the normalized mutated:wild-type ratio.(Instruction manual: Roche Applied Science Technical Note No. LC 13/2001. Relative Quantification; LightCycler 480, 2006)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
2 to 5 days

Specimen Retention Time
3 months

Performing Laboratory Location
Rochester

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
JAK2V	JAK2 V617F Mutation Detection, V	43399-5

Result ID	Test Result Name	Result LOINC® Value
31160	JAK2 V617F Mutation Detection, V	43399-5
39724	JAK2 Result	53761-3