Test Definition: JAKFM

JAK2 V617F Mutation Detection, Bone Marrow

Overview

Useful For

Diagnosis or differential diagnosis of myeloproliferative disorders by *JAK2* V617F variant detection in bone marrow specimens

Method Name

Quantitative Polymerase Chain Reaction (qPCR)

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.

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	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Mutations in the *JAK2*, *CALR*, and *MPL* genes are considered driver events in the BCR::ABL1 negative myeloproliferative neoplasms (MPN), including polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET). The *JAK2* V617F mutation occurs in 95% to 98% of patients with PV, 50% to 60% of patients with PMF and 50% to 60% of patients with ET respectively at diagnosis. Other *JAK2* mutations in exons 12 through 15 occur in the remaining patients with PV. Mutations in the *CALR* gene occur in 20% to 30% of patients with PMF and 20% to 30% of patients with ET at diagnosis. A 52 base pair (bp) deletion (53%) and a 5 bp deletion (32%) are the most common mutations in the *CALR* gene while other types of mutations may occur in the remaining cases. MPL exon 10 mutations occur in 5% to 10% of patients with PMF and 5% to 10% of patients with ET. Mutations in *JAK2*, *CALR*, and *MPL* are mutually exclusive. The *JAK2* V617F mutation is detected by quantitative polymerase chain reaction (qPCR). The *CALR* mutations are detected by PCR targeting exon 9. The *MPL* mutations in exon 10 are detected by Sanger sequencing. All mutations in *JAK2*, *CALR*, and *MPL* can also be detected by next-generation sequencing (NGS). In addition to the mutations in *JAK2*, *CALR*, and *MPL*, mutations in many other genes including *ASXL1*, *TET2*, *DNMT3A*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *EZH2*, *IDH1*, *IDH2*, *CBL*, *KRAS*, *NRAS*, *STAG2*, and *TP53* can occur in MPN. These additional mutations are more frequent in PMF and advanced disease, as compared to PV and ET. It is known that mutations in the *ASXL1*, *SRSF2*, *U2AF1*, *EZH2, IDH1*, and *IDH2* are correlated with a poor prognostic risk. While a single gene test on *JAK2*, *CALR*, and *MPL* can be clinically useful, all above mentioned gene mutations can be detected by NGS.

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 have 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 V617F may be present in an area that interferes with primer or probe binding, causing 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. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutation of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-2390
2. Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544-1551
3. Greenfield G, McMullin MF, Mils K. Molecular pathogenesis of the myeloproliferative neoplasms. J Hematol Oncol. 2021;14(1):103
4. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-1719

Performance

Method Description

Genomic DNA is extracted and 2 polymerase chain reactions (PCR) are performed. 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 3' 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 3' 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) which 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 mutated:wild-type ratio using the delta-delta Ct method, which is expressed as a unitless ratio following correction with the calibrator data.

The final result is reported as percent *JAK2* V617F of total *JAK2* (ie, [mutated/mutated + wild-type] x 100%), calculated from the relative quantification value.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
1 to 8 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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
JAKFM	JAK2 V617F Mutation, BM	72333-8

Result ID	Test Result Name	Result LOINC® Value
618647	JAK2 Result	72333-8
618648	Final Diagnosis	69047-9
618649	Method	85069-3
618650	Signing Pathologist	18771-6
618652	Additional Information	48767-8
618653	Disclaimer	62364-5