MAYO CLINIC LABORATORIES

Test Definition: PVJAK

Polycythemia Vera, JAK2 V617F with Reflex to JAK2 Exon 12-15, Sequencing for Erythrocytosis, Varies

Overview

Useful For

Aiding in the distinction between the myeloproliferative neoplasm polycythemia vera and other secondary erythrocytosis

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
JAKXR	JAK2 Exon 12-15 Sequencing, Reflex	Yes, (order JAKXB-blood or JAKXM-bone marrow), Bill Only	No

Testing Algorithm

The test starts with a highly sensitive DNA-based *JAK2* V617F test by allele-specific polymerase chain reaction. If the *JAK2* V617F result is negative or very low positive (0.06%-2%), *JAK2* exon 12-15 Sanger sequencing will be performed on the stored RNA sample. If a *JAK2* V617F mutation (>2%) is detected, no further testing will be performed.

The Sanger sequencing covers *JAK2* exons 12 through the first 90% of exon 15, which spans the region containing essentially all mutations reported in myeloproliferative neoplasms. For more information see:
-Erythrocytosis Evaluation Testing Algorithm
-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
- Erythrocytosis Evaluation Testing Algorithm

Method Name

Allele-Specific Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Specimen must arrive within 5 days of collection.

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Blood
Container/Tube: Lavender top (EDTA) or yellow top (ACD-B)
Specimen Volume: 10 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 blood.

Specimen Type: Bone marrow aspirate
Container/Tube: Lavender top (EDTA) or yellow top (ACD-B)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send bone marrow specimen in original tube. **Do not aliquot.**
3. Label specimen as bone marrow.

Forms

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

Specimen Minimum Volume

Blood: 8 mL; Bone marrow: 2 mL

Reject Due To

Gross hemolysis	Reject
Paraffin-embedded bone marrow aspirate clot or biopsy blocks Slides Paraffin shavings Moderately to	Reject

severely clotted	

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	5 days	
	Ambient	5 days	

Clinical & Interpretive

Clinical Information

The Janus kinase 2 (*JAK2*) gene 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. The *JAK2* V617F mutation is located in exon 14 and present in 50% to 60% of primary myelofibrosis and essential thrombocythemia and in 95% to 98% of polycythemia vera (PV). In the rest of the PV cases, over 50 different mutations have been reported within exons 12 through 15 of *JAK2*, and essentially all non-V617F *JAK2* mutations have been identified in PV. These mutations include point alterations and small insertions or deletions. Several of the exon 12 mutations have been shown to have biologic effects similar to those caused by the V617F mutation such that it is currently assumed other nonpolymorphic mutations have similar clinical effects. However, some mutations may not be well characterized and require further clinical and research evaluation.

Reference Values

An interpretive report will be provided.

Interpretation

The results will be reported as 1 of the 3 following states:
-Positive for *JAK2* V617F mutation
-Positive for *JAK2* mutation (other than V617F)
-Negative for *JAK2* mutations

If the result is positive, a description of the mutation at the nucleotide level and the altered protein sequence are reported.

A positive mutation status is highly suggestive of a myeloid neoplasm and may support a diagnosis of polycythemia vera in the appropriate clinical setting. Correlation with clinicopathologic findings and other laboratory results is necessary in all cases.

A negative mutation status makes a diagnosis of polycythemia vera highly unlikely, although it does not completely exclude this possibility, other myeloproliferative neoplasms, or other neoplasms.

Cautions

A positive result is not specific for a particular diagnosis. Correlation with clinicopathologic findings and other laboratory results 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.

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. doi:10.1016/S0140-6736(05)71142-9
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. doi:10.1038/nature03546
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. doi:10.1056/NEJMoa051113
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. doi:10.1182/blood-2005-03-1183
5. Ma W, Kantarjian H, Zhang X, et al. Mutation profile of *JAK2* transcripts in patients with chronic myeloid neoplasias. J Mol Diagn. 2009;11(1):49-53
6. Kilpivaara O, Levine RL. *JAK2* and *MPL* mutations in myeloproliferative neoplasms: discovery and science. Leukemia. 2008;22(10):1813-1817. doi:10.1038/leu.2008.229
7. Kravolics R: Genetic complexity of myeloproliferative neoplasms. Leukemia. 2008;22(10):1841-1848. doi:10.1038/leu.2008.233
8. 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
9. Tefferi A. The classic myeloproliferative neoplasms: Chronic myelogenous leukemia, polycythemia vera, essential thrombocythemia, and primary myelofibrosis. In: Valle DL, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, eds. The Online Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill; 2019, Accessed January 5, 2024. Available at https://ommbid.mhmedical.com/content.aspx?sectionid=225078035&bookid=2709

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 *JAK2* V617 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 *JAK2* V617F mutated sequence and the PCR conditions are such that it will only bind the 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 and a negative cell line and used to mitigate the run-to-run variation. Following each reaction, 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 % *JAK2* V617F of total *JAK2* (ie, [mutated/mutated + wild type] x 100%).(Unpublished Mayo method)

For the Sanger sequencing, total RNA is extracted from whole blood or bone marrow and complementary DNA synthesized from *JAK2* messenger RNA. A fragment spanning exons 12 through 15 is then amplified using standard PCR and the sequence is obtained using Sanger sequencing with analysis on an automated genetic analyzer.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
7 to 10 days

Specimen Retention Time
Blood/Bone marrow: 2 weeks; Extracted DNA and RNA: 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.

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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 V617
0027U (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PVJAK	PV (JAK2 V617F, Exon 12-15) Reflex	In Process

Result ID	Test Result Name	Result LOINC® Value
MP037	Specimen Type	31208-2
42394	Final Diagnosis	50398-7
42395	PV Reflex Result	43399-5