Overview

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

Special Instructions
- Hematopathology Patient Information

Method Name
Point Mutation Detection in DNA Using 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) in Special Instructions

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

Specimen Minimum Volume
Extracted DNA from blood or bone marrow: 50 microliter at 20 ng/microliter

Reject Due To

<table>
<thead>
<tr>
<th>Other</th>
<th>Bone marrow biopsies, slides, paraffin shavings Frozen tissues and paraffin-embedded tissues Paraffin-embedded bone marrow aspirates Moderately to severely clotted</th>
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</thead>
</table>

Specimen Stability Information
Clinical and 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 thrombocytosis (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 (CML) 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 a 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 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


Performance
Method Description
Genomic DNA is extracted and 2 PCR reactions 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:

<table>
<thead>
<tr>
<th>Normalized ratio =</th>
<th>mutated/wild-type (sample)</th>
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<tbody>
<tr>
<td></td>
<td>mutated/wild-type (calibrator)</td>
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</tbody>
</table>

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)
Test Definition: JAK2V
JAK2 V617F Mutation Detection, V

No

Day(s) and Time(s) Test Performed
Monday through Friday; 12 p.m.

Analytic Time
2 days

Maximum Laboratory Time
5 days

Specimen Retention Time
DNA stored for 3 months

Performing Laboratory Location
Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
81270-JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant

LOINC® Information

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<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<td>JAK2 V617F Mutation Detection, V</td>
<td>43399-5</td>
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<td>31160</td>
<td>JAK2 V617F Mutation Detection, V</td>
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