Overview

Useful For
Aiding in the distinction between the myeloproliferative neoplasm polycythemia vera (PV) and other secondary erythrocytosis

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAKXR</td>
<td>JAK2 Exon 12-15 Sequencing, Reflex</td>
<td>No, (Bill Only)</td>
<td>No</td>
</tr>
</tbody>
</table>

Testing Algorithm

Both DNA and RNA are extracted. The algorithm starts with a highly sensitive DNA-based JAK2 V617F test by allele specific PCR. If the JAK2 V617F result is negative or very low positive (0.06%-0.6%), JAK2 exon 12-15 Sanger sequencing test will be performed on the stored RNA sample. If a JAK2 V617F mutation (>0.6%) is detected, the algorithm stops and 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. The following algorithms are available in Special Instructions.

- 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 (AS-PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Shipping Instructions

Specimen must arrive within 5 days (120 hours) 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
Submit only 1 of the following specimens:

Specimen Type: Blood

Container/Tube: EDTA (lavender top) or ACD-B (yellow top)

Specimen Volume: 10 mL

Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.
3. Label specimen as blood.

Specimen Type: Bone marrow aspirate

Container/Tube: EDTA (lavender top) or ACD-B (yellow top)

Specimen Volume: 4 mL

Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube.
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: 4 mL
Bone Marrow: 2 mL

Reject Due To
Test Definition: PVJAK
PV (JAK2 V617F, Exon 12-15) Reflex

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

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td></td>
<td>Ambient</td>
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Clinical and 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 is located in exon 14 and present in 50% to 60% of primary myelofibrosis and essential thrombocytopenia, and 95% to 98% of polycythemia vera (PV). In the rest of the polycythemia vera cases, over 50 different mutations have been reported within exons 12 through 15 of JAK2 and essentially all of the non-V617F JAK2 mutations have been identified in polycythemia vera. These mutations include point mutations 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 requires 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

Performance

Method Description

Genomic DNA and RNA are extracted. 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. 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, 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:

\[
\text{Normalized ratio} = \frac{\text{mutated}}{\text{wild-type (sample)}}
\]
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 cDNA synthesized from JAK2 mRNA. 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) and Time(s) Test Performed
Monday through Friday

Analytic Time
7 days

Maximum Laboratory Time
10 days

Specimen Retention Time
DNA and RNA: 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 V617
0027U (if appropriate)

LOINC® Information

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<th>Order LOINC Value</th>
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