**Overview**

**Useful For**
Aiding in the distinction between a reactive cytosis and a myeloproliferative neoplasm when JAK2V617F testing result is negative

**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>MPNML</td>
<td>MPL Exon 10 Sequencing, Reflex</td>
<td>No, (bill only)</td>
<td>No</td>
</tr>
</tbody>
</table>

**Testing Algorithm**

This test reflexively evaluates for variants in the CALR and MPL genes commonly associated with BCR/ABL1-negative myeloproliferative neoplasms. The testing sequence is based on the reported frequency of gene variants in this disease group. It is usually ordered when a JAK2 V617F result is known to be negative. Initial testing evaluates for the presence of the CALR insertions and deletions. If out-of-frame CALR insertions or deletions are detected, the testing algorithm ends. If the CALR result is negative or an in-frame CALR insertion or deletion is identified, then testing proceeds, at an additional charge, to evaluate for variants in exon 10 of the MPL gene by Sanger sequencing. An integrated report is issued with the summary of test results.

The following algorithms are available in Special Instructions:

- 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

**Method Name**
Polymerase Chain Reaction (PCR) and Fragment Analysis

**NY State Available**
Yes

**Specimen**

**Specimen Type**
Varies

**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**
Submit only 1 of the following specimens:

**Specimen Type:** Blood

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

**Specimen Volume:** 3 mL

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

**Specimen Stability Information:** Ambient (preferred)/Refrigerate

**Specimen Type:** Bone marrow aspirate

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

**Specimen Volume:** 2 mL

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

**Specimen Stability Information:** Ambient (preferred)/Refrigerate

**Specimen Type:** Extracted DNA from blood or bone marrow

**Container/Tube:** 1.5 to 2 mL tube

**Specimen Volume:** Entire specimen

**Collection Instructions:**
1. Indicate volume and concentration of DNA
2. Label specimen as extracted DNA from blood or bone marrow.

**Specimen Stability Information:** Frozen (preferred)/Refrigerate/Ambient

**Forms**

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

**Specimen Minimum Volume**

Blood or Bone marrow: 0.5 mL

**Reject Due To**

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin-embedded bone marrow aspirate clot or biopsy blocks Slides Paraffin shavings Moderately to severely clotted</td>
<td>Reject</td>
</tr>
</tbody>
</table>

**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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</thead>
<tbody>
<tr>
<td>Varies</td>
<td>Varies</td>
<td>7 days</td>
<td></td>
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</table>

**Clinical and Interpretive**

**Clinical Information**

*JAK2 V617F* variant is present in 95% to 98% of polycythemia vera (PV) patients, 50% to 60% of primary myelofibrosis (PMF) patients, and 50% to 60% of essential thrombocythemia (ET) patients. Detection of the *JAK2 V617F* variant helps establish the diagnosis of a myeloproliferative neoplasm (MPN). However, a negative *JAK2 V617F* result does not indicate the absence of MPN. Other important molecular markers in *BCR-ABL1*-negative MPN include *CALR* exon 9 variants (20%-30% of PMF and ET) and *MPL* exon 10 variants (5%-10% of PMF and 3%-5% of ET). Variants in *JAK2*, *CALR*, and *MPL* are essentially mutually exclusive. A *CALR* variant is associated with decreased risk of thrombosis in both ET and PMF, and confers a favorable clinical outcome in PMF patients. A triple negative (*JAK2 V617F, CALR, and MPL*-negative) genotype is considered a high-risk molecular signature in PMF.

**Reference Values**

An interpretive report will be provided.

**Interpretation**

The results will be reported as 1 of the 3 following states:

- Positive for *CALR* variant
- Positive for *MPL* variant
- Negative for *CALR* and *MPL* variants

Positive variants status is highly suggestive of a myeloid neoplasm and clinicopathologic correlation is necessary in
Test Definition: MPNCM
MPN (CALR, MPL) Reflex

all cases.

Negative variant status does not exclude the presence of a myeloproliferative neoplasm or other neoplasms.

Cautions

A positive result is not specific for a particular subtype of myeloproliferative neoplasm and clinicopathologic correlation is necessary in all cases.

A negative result does not exclude the presence of a myeloproliferative neoplasm or other neoplastic process.

Clinical Reference


Performance

Method Description

PCR amplification of CALR exon 9 is performed on DNA isolated from the patient sample. The PCR product is then run on an ABI Genetic Analyzer for fragment analysis to detect insertions and deletions. An unmutated CALR will show an amplicon at 266 bp, a mutated CALR with insertion will show an amplicon greater than 266 bp, and a mutated CALR with deletion will show an amplicon smaller than 266 bp. This assay has an analytical sensitivity of approximately 6% (ie, 6 variant-containing cells in 100 total cells) in most variant types, except for the rare type of 1-bp deletion, which has a sensitivity of approximately 20%. This is a laboratory developed test using analyte-specific reagents and research use only (RUO) reagents.(Unpublished Mayo method)

Genomic DNA is extracted and Sanger sequencing used to evaluate for variants in MPL, exon 10. The sensitivity of this assay is approximately 20%, such that samples containing lower percentages of mutated DNA will appear negative.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available
Test Definition: MPNCM
MPN (CALR, MPL) Reflex

7 to 10 days

**Specimen Retention Time**
DNA: 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**
81219-CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9
81403-MPL (myeloproliferative leukemia virus oncogene, thrombopoietin receptor, TPOR) (eg, myeloproliferative disorder), exon 10 sequence (if appropriate)

**LOINC® Information**

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tr>
<td>MPNCM</td>
<td>MPN (CALR, MPL) Reflex</td>
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<td>MPNCM Reflex Result</td>
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<td>MP036</td>
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<td>42392</td>
<td>Final Diagnosis</td>
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