Overview

Useful For
Rapid and sensitive detection of insertion and deletion-type mutations in exon 9 of CALR

An aid in distinction between reactive thrombocytosis and leukocytosis versus a myeloproliferative neoplasm (MPN), especially essential thrombocythemia (ET) and primary myelofibrosis (PMF), and is highly informative in cases in which JAK2 and MPL testing are negative

Especially helpful to the pathologist in those bone marrow cases with ambiguous etiology of thrombocytosis, equivocal bone marrow morphologic findings of MPN, and unexplained reticulin fibrosis

An aid in prognostication of PMF and thrombosis risk assessment in ET

Testing Algorithm
The following algorithms are available in Special Instructions:

- Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood
- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation

Special Instructions

- Myeloproliferative Neoplasm: A Diagnostic Approach to Peripheral Blood Evaluation
- Myeloproliferative Neoplasm: A Diagnostic Approach to Bone Marrow Evaluation
- Hematopathology Patient Information

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 (168 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:** Peripheral blood

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

**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:** Ambient (preferred)/Refrigerate

**Specimen Type:** Bone marrow

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

**Specimen Volume:** 2 mL

**Collection Instructions:**

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

**Specimen Stability:** 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:** Label specimen as extracted DNA from blood or bone marrow and include indication of volume and concentration of the DNA.

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

1 mL

**Reject Due To**

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<tr>
<th>Specimen Type</th>
<th>Reason</th>
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<tbody>
<tr>
<td>Hemolysis</td>
<td>Mild OK; Gross reject</td>
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<tr>
<td>Lipemia</td>
<td>NA</td>
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<tr>
<td>Icterus</td>
<td>NA</td>
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<tr>
<td>Other</td>
<td>Paraffin-embedded bone marrow aspirate clot, bone marrow biopsies, slides, or paraffin shavings, moderately to severely clotted</td>
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**Specimen Stability Information**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tbody>
<tr>
<td>Varies</td>
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**Clinical and Interpretive**

**Clinical Information**

The most frequent genetic mutation in BCR-ABL1-negative myeloproliferative neoplasm (MPN), essential thrombocytopenia (ET), and primary myelofibrosis (PMF) is the JAK2V617F mutation, which is present in approximately 50% to 60% of patients. It serves as a confirmatory molecular marker of these diseases. Mutations in the MPL gene are found in an additional 5% to 10% of ET and PMF cases. It was recently discovered that somatic mutation (insertions and deletions) in exon 9 of the CALR gene is the second most frequent somatic mutation after JAK2 in ET and PMF patients, and it is mutually exclusive of JAK2 and MPL mutations.\(^{(1,2)}\) It has a frequency of approximately 49% to 88% in JAK2 and MPL-wild type (WT) ET and PMF, and is not found in polycythemia vera (PV) patients.\(^{(1-4)}\) Therefore, CALR mutation serves as an important diagnostic molecular marker in ET and PMF.

The CALR gene encodes for calreticulin, a multifunctional protein with a C-terminus rich in acidic amino acids and a KDEL ER-retention motif. All the pathologic CALR mutations reported to date are out-of-frame insertion and/or deletions (indel) in exon 9, generating a 1 base-pair (bp) frame shift and a mutant protein with a novel C-terminus rich in basic amino acids and loss of the KDEL ER-retention signal. The most common mutation types are 52-bp deletion (c.1092_1143del, L367fs*46) and 5-bp insertion (c.1154_1155insTTGCC, K385fs*47), and they comprise approximately 85% of CALR mutations in MPN.\(^{(1,2)}\) CALR mutations have been found in hematopoietic stem and progenitor cells in MPN patients\(^{(2)}\) and may activate the STAT5 signaling pathway.\(^{(1)}\) They are associated with decreased risk of thrombosis in ET \(^{(1,3-5)}\), and better survival in PMF compared to JAK2 mutations.\(^{(5)}\)

**Reference Values**

An interpretive report will be provided

**Interpretation**

An interpretive report will be issued.
The results will be reported as 1 of the 3 states if DNA amplification is successful (see Cautions):

-Positive. A deletion/insertion-type mutation was detected in CALR, exon 9.

-Negative. No deletion or insertion was detected in CALR, exon 9.

-Equivocal. A small amplicon suspicious for a deletion/insertion type mutation was detected in CALR, exon 9.

Positive mutation status is highly suggestive of a myeloid neoplasm, but must be correlated with clinical and other laboratory and morphologic features for definitive diagnosis.

Negative mutation status does not exclude the presence of a myeloproliferative neoplasm or other neoplastic disorders.

**Cautions**

A positive result is not specific for a particular myeloproliferative neoplasm (MPN) diagnosis and clinicopathologic correlation is necessary in all cases.

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

This test is a fragment analysis assay, and only detects insertions and deletions (indels). It will not detect point mutations. However, all reported pathologic mutations in MPN described to date are insertions and/or deletions.

This test may not differentiate between out-of-frame and in-frame indels in rare cases. However, in-frame indel mutations are very rare (<0.5%), and have only been reported in few healthy individuals and myeloproliferative neoplasm patients with JAK2V617F mutation or out-of-frame CALR mutation. Most of the rare in-frame indels are considered germline mutations and represent non-pathogenic polymorphisms.

Infrequently, amplification failure can be encountered in a given sample, due to inadequate DNA, poor DNA quality, or a PCR inhibitor. In these circumstances, the assay will be reattempted and if persistently unsuccessful, the report will be issued with an “Invalid” result.

**Clinical Reference**


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 3130xl 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 mutation-containing cells in 100 total cells) in most mutation 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)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday; 8 a.m.

Analytic Time

3 days

Maximum Laboratory Time

5 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

LOINC® Information

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