

CALR Mutation Detection

Subhead

The *BCR/ABL1*-negative myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).¹ The *JAK2* V617F point mutation occurs in >95% of cases of PV and approximately 50-60% of cases of ET and PMF. In ET and PMF lacking the *JAK2* V617F mutation, approximately 10-20% contain a mutation in *MPL* exon 10 while 60-80% of cases have a mutation in *CALR*.^{2,3} The identification of a *JAK2*, *MPL* or *CALR* mutation is diagnostically useful to separate MPN from a reactive leukocytosis that may mimic a myeloid neoplasm. Cases of ET and PMF with mutations of *JAK2*, *MPL* or *CALR* may also show prognostic differences.⁴⁻⁷

Approximately 80% of *CALR* mutations can be classified as either type 1 (a 52-bp deletion) or type 2 (a 5-bp insertion). The remaining mutations represent other, variably sized insertions and deletions. All *CALR* mutations (type 1, type 2 or other) create a frame shift with production of an altered C-terminus of the calreticulin protein.

Cleveland Clinic Laboratories has developed, validated and implemented a sensitive PCR assay for the detection of *CALR* mutations in peripheral blood, bone marrow or formalin-fixed, paraffin-embedded tissues.

Clinical Indications

CALR mutation testing is useful in the workup of suspected MPN, especially those that are negative for *JAK2* V617F.

Interpretation

Normal results are reported as “*CALR* mutation not detected.” Positive results are reported as “*CALR* mutation detected” and an interpretation is provided that includes a description of the mutation (type 1, type 2 or other).

Methodology

Genomic DNA is extracted from the sample and *CALR* exon 9 is amplified by PCR. Fragment length analysis is performed to assess for insertion/deletion mutations.

Limitations

This assay has a sensitivity of 5% mutant alleles. This assay detects only insertion/deletion mutations in *CALR* exon 9, and a negative result does not exclude the possibility of a MPN.

References

1. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press; 2008.
2. Nangalia J, Massie CE, Baxter EJ, et al. Somatic *CALR* mutations in myeloproliferative neoplasms with nonmutated *JAK2*. *N Engl J Med* 2013;369:2391-405.
3. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med* 2013;369:2379-90.
4. Tefferi A, Lasho TL, Finke CM, et al. *CALR* vs *JAK2* vs *MPL*-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia* 2014;28:1472-7.
5. Tefferi A, Wassie EA, Lasho TL, et al. Calreticulin mutations and long-term survival in essential thrombocythemia. *Leukemia* 2014;28:2300-3.
6. Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of *JAK2*, *CALR*, or *MPL* in primary myelofibrosis. *Blood* 2014;124:1062-9.
7. Rumi E, Pietra D, Ferretti V, et al. *JAK2* or *CALR* mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood* 2013;123:1544-1551.

Test Overview

Test Name	CALR (Calreticulin) Exon 9 Mutation Detection
Ordering Mnemonic	CALR
Methodology Name and Description	Polymerase Chain Reaction with fragment length analysis by capillary electrophoresis.
Specimen Requirements	<p>Whole blood:</p> <ul style="list-style-type: none"> • Volume: 4 ml • Container: EDTA (Lavender) • Transport temperature: Refrigerated <p>Bone marrow:</p> <ul style="list-style-type: none"> • Volume: 2 ml • Container: EDTA (Lavender) • Transport temperature: Refrigerated <p>Formalin-fixed paraffin embedded tissue/bone marrow clot</p> <ul style="list-style-type: none"> • Size: one block • Transport: Ambient
Minimum Specimen Requirements	<p>Whole blood:</p> <ul style="list-style-type: none"> • Volume: 2 ml <p>Bone marrow:</p> <ul style="list-style-type: none"> • Volume: 1 ml
Stability	<p>Ambient:</p> <ul style="list-style-type: none"> • Blood/bone marrow: 24 hours • Formalin-fixed paraffin embedded tissue/bone marrow clot: indefinitely <p>Frozen:</p> <ul style="list-style-type: none"> • Blood/bone marrow: unacceptable • Formalin-fixed paraffin embedded tissue/bone marrow clot: indefinitely <p>Refrigerated:</p> <ul style="list-style-type: none"> • Blood/bone marrow: 5 days • Formalin-fixed paraffin embedded tissue/bone marrow clot: indefinitely
Billing Code	89979
CPT Codes	81479, G0452

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