Classic **BCR-ABL1**-negative Myeloproliferative Neoplasms

**Indications for Ordering**

Diagnose and manage classic **BCR-ABL1**-negative myeloproliferative neoplasms (MPNs)

- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Primary myelofibrosis (PMF)
- Other myeloproliferative disorders

**Test Description**

**JAK2** Gene, V617F Mutation, Qualitative
- Qualitative allele-specific PCR

**JAK2** Gene, V617F Mutation, Quantitative
- Allele-specific real-time PCR
- Mutant-to-wild type allele ratio is reported

**CALR** (Calreticulin) Exon 9 Mutation Analysis by PCR
- PCR and capillary electrophoresis

**JAK2** Exon 12 Mutation Analysis by PCR
- PCR amplification with subsequent sequencing to detect single and multiple base substitutions within exon 12 of the **JAK2** gene

**MPL** codon 515 Mutation Detection by Pyrosequencing, Quantitative
- PCR amplification with subsequent pyrosequencing of codon 515
- Wild type-to-mutant allele ratio is reported

**Tests to Consider**

**JAK2** Gene, V617F Mutation, Qualitative 0051245
- Reasonable screening test for routine evaluation of
  - Unexplained erythrocytosis
  - Thrombocytosis
  - **BCR-ABL1**-negative granulocytosis
  - Bone marrow (BM) fibrosis
- Appropriate screen in patients with symptoms characteristic for **BCR-ABL1**-negative MPN
  - Unusual thrombotic events (eg, splanchnic vein thrombosis, Budd-Chiari)
  - Aquagenic pruritus
  - Unexplained splenomegaly

**JAK2** Gene, V617F Mutation, Quantitative 0040168
- May be useful in assessment of residual disease after stem cell transplant
- Enhances diagnostic certainty in cases with low mutant allelic burden

**CALR** (Calreticulin) Exon 9 Mutation Analysis by PCR 2010673
- Use for diagnostic and prognostic information in patients with myeloproliferative neoplasms (MPNs) when **JAK2** testing is negative

**Myeloid Malignancies Mutation Panel by Next Generation Sequencing** 2011117
- Assess for single gene mutations, including substitutions and insertions and deletions that may have diagnostic, prognostic, and/or therapeutic significance

**JAK2** Exon 12 Mutation Analysis by PCR 2002357
- Use when MPN suspected and **JAK2** V617F testing is negative

**MPL** codon 515 Mutation Detection by Pyrosequencing, Quantitative 2005545
- May be useful when essential thrombocythemia or idiopathic myelofibrosis is suspected in **JAK2** V617F-negative individuals

**JAK2** Gene, V617F Mutation, Qualitative with Reflex to **JAK2** Exon 12 Mutation Analysis by PCR 2012085
- Use when diagnosis of PV is suspected

**JAK2** Gene, V617F Mutation, Qualitative with Reflex to **CALR** (Calreticulin) Exon 9 Mutation Analysis by PCR with Reflex to **MPL** codon 515 Mutation Detection by Pyrosequencing, Quantitative 2012084
- Use when diagnosis of ET or PMF is suspected

**Myeloproliferative Disorders Panel by FISH** 2002360
- Detect specific recurrent genomic aberrations in suspected MPNs
  - **BCR/ABL1**
  - **PDGFR**A
  - **PDGFR**B
  - **FGFR**1

**Disease Overview**

**Incidence** – 5/100,000 worldwide for combined PV, ET, and PMF
PV
• Must exclude secondary causes of erythrocytosis
• WHO diagnostic criteria (2008) – must meet both major and 1 minor criteria or the first major and 2 minor criteria
• Major criteria
  o Evidence of increased RBC volume, including ≥1 of the following
    ▪ Hgb>18.5 g/dL (men), >16.5 g/dL (women)
    ▪ Hgb or Hct >99th percentile of reference
    ▪ Red cell mass >25% above mean predicted
  ▪ Hgb>17 g/dL (men), Hgb>15 g/dL (women) if associated with sustained increase ≥2 g/dL not attributed to correction of iron deficiency anemia
  o Presence of JAK2 (V617F) or JAK2 exon 12 mutations
• Minor criteria
  o BM trilineage proliferation
  o Subnormal serum erythropoietin level
  o Endogenous erythroid colony (EEC) growth in vitro

ET
• Must exclude secondary causes of thrombocytosis
• WHO diagnostic criteria (2008) – must meet all 4 criteria
  o Sustained platelet count >450 x 10^9/L before treatment
  o BM proliferation of enlarged, mature megakaryocytes
  o Does not meet WHO criteria for BCR-ABL1-positive CML, PV, PMF, MDS, or myeloid malignancy
  o Demonstration of JAK2 (V617F), MPL gene mutations (eg, W515L and W515K), or no evidence of reactive thrombocytosis

PMF
• WHO diagnostic criteria (2008) – must meet all 3 major and 2 minor criteria
• Major criteria
  o Megakaryocyte proliferation with atypia in association with reticulin and/or collagen fibrosis or, in the absence of reticulin fibrosis, an increase in BM cellularity associated with granulocyte proliferation and often decreased erythrocyte proliferation
  o Does not meet the WHO criteria for BCR-ABL1-positive CML, PV, ET, MDS, or myeloid malignancy
  o Presence of JAK2 V617F or other clonal marker or no evidence of reactive marrow fibrosis
• Minor criteria
  o Leukoerythroblastosis
  o Increased serum LDH
  o Anemia
  o Palpable splenomegaly

Genetics
Gene – JAK2

Structure/function
• Located on 9p24
• Nonreceptor tyrosine kinase involved in cytokine receptor signaling

Mutations
JAK2 V617F mutation
• Present in classic MPNs
  o PV – 90-95%
  o ET – ~50%
  o PMF – 50%
• Mutations rarely found in non-MPNs such as AML, MDS
• Mutation is common in the rare MDS/MPN subtype RARS-T (refractory anemia with ring sideroblasts and thrombocytosis)

JAK2 exon 12 mutation
• Present in the majority of patients with PV who are JAK2 V617F negative
• Rarely occurs in PMF, ET

Gene – CALR

Structure/function
Chromosome 19p13.3-13.2
• CALR encodes a calcium-binding chaperone protein involved in glycoprotein folding and calcium homeostasis

Mutations
Exon 9 mutations
• A variety of insertions/deletions (indels) have been identified
• Net result of the exon 9 indels is a frame shift

Prevalence
• Essential thrombocytethmia (ET) – 67% of JAK2 and MPL negative cases
• Primary myelofibrosis (PMF) – 88% of JAK2 and MPL negative cases

Diagnostic issues
• CALR somatic mutations are seen in a subset of patients with MPNs who lack JAK2 and MPL mutations
• CALR mutations appear restricted to patients with ET and PMF and are not found in patients with PV

Prognostic issues
• CALR mutated ET and PMF are associated with increased overall survival when compared with JAK2 and MPL mutated ET/PMF
• CALR mutated ET is associated with a decreased incidence of thrombosis when compared to JAK2 mutated ET

Gene – MPL

Structure/function
• Located on 1p34
• Thrombopoietin receptor; mutation causes downstream signaling and contributes to megakaryocytic myeloproliferation

Mutations
Several acquired mutations in exon 10 have been reported
• W515L and W515K – most common
Test Interpretation

**JAK2 Gene, V617F Mutation, Qualitative**

**Clinical sensitivity**
- PV – 95%
- ET – 50%
- PMF – 50%

**Results**
- Positive – meets a diagnostic criterion for JAK2 MPN
- Not detected – no evidence of the JAK2 V617F point mutation

**Limitations**
- Only one point mutation is detected
- Limit of detection is 0.5% mutant allele

**JAK2 Gene, V617F Mutation, Quantitative**

**Clinical sensitivity**
- PV – 95%
- ET – 50%
- PMF – 50%

**Results**
- The percentage of JAK2 alleles positive for the V617F mutation (allele burden) is reported
  - 0% – no evidence of the JAK2 V617F mutation
  - Lower JAK2 V617F allele burden in PMF is associated with worse outcome

**Limitations**
- Only the one point mutation is detected
- Limit of detection is 0.2% mutant allele

**CALR (Calreticulin) Exon 9 Mutation Analysis by PCR**

**Results**
- Positive – a mutation in CALR exon 9 is detected
  - Highly suggestive of MPN in conjunction with appropriate clinical and histological findings
- Negative – a mutation in CALR exon 9 is not detected

**Limitations**
- Detects only exon 9 indel mutations and does not detect mutations in other regions of the CALR gene
- Results must be interpreted in the context of morphological and other relevant data
- Test should not be used alone to diagnose malignancy

**References**