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 Descriptions**

- **JAK2 Gene, V617F Mutation, Qualitative**
  - Polymerase chain reaction (PCR)
- **JAK2 Gene, V617F Mutation, Quantitative**
  - PCR
  - Mutant-to-wild type allele ratio is reported
- **CALR (Calreticulin) Exon 9 Mutation Analysis by PCR**
  - PCR/capillary electrophoresis
- **Myeloid Malignancies Mutation Panel by Next Generation Sequencing**
  - Massively parallel sequencing
- **JAK2 Exon 12 Mutation Analysis by PCR**
  - Allele-specific PCR amplification with subsequent sequencing of positive products to detect single and multiple base substitutions within exon 12 of the JAK2 gene
- **MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative**
  - PCR/pyrosequencing
  - Wild type-to-mutant allele ratio is reported
- **Myeloproliferative Disorders Panel by FISH**
  - Fluorescence in situ hybridization

**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*
  - *PDGFRα*
  - *PDGFRβ*
  - *FGFR1*

**Disease Overview**

**Incidence** – 5/100,000 worldwide for combined PV, ET, and PMF

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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**
    - 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
    - Presence of JAK2 (V617F) or JAK2 exon 12 mutations
  - **Minor criteria**
    - BM trilineage proliferation
    - Subnormal serum erythropoietin level
    - Endogenous erythroid colony (EEC) growth in vitro

ET
- Must exclude secondary causes of thrombocytosis
- **WHO diagnostic criteria (2008)** – must meet all 4 criteria
  - Sustained platelet count ≥450 x 10^9/L before treatment
  - BM proliferation of enlarged, mature megakaryocytes
  - Does not meet WHO criteria for BCR-ABL1-positive CML, PV, PMF, MDS, or myeloid malignancy
  - 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**
    - 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
    - Does not meet the WHO criteria for BCR-ABL1-positive CML, PV, ET, MDS, or myeloid malignancy
    - Presence of JAK2 V617F or other clonal marker or no evidence of reactive marrow fibrosis
  - **Minor criteria**
    - Leukoerythroblastosis
    - Increased serum LDH
    - Anemia
    - Palpable splenomegaly

Mutations
- **JAK2 V617F mutation**
  - Present in classic MPNs
    - PV – 90-95%
    - ET – ~50%
    - 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 thrombocythemia (ET) – 67% of JAK2 and MPL negative cases (Nangalia, 2013)
- 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 (Nangalia, 2013)
- **CALR** mutations appear restricted to patients with ET and PMF and are not found in patients with PV (Klampfl, 2013; Nangalia, 2013)

Prognostic issues
- **CALR** mutated ET and PMF are associated with increased overall survival when compared with JAK2 and MPL mutated ET/PMF (Rotunno, 2013)
- **CALR** mutated ET is associated with a decreased incidence of thrombosis when compared to JAK2 mutated ET (Rotunno, 2013)

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
  ○ ET – 3-4%
  ○ PMF – 8-10%
  ○ PV – rare
• W515A and W515R – rare

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

JAK2 Exon 12 Mutation Analysis by PCR
Clinical sensitivity
• PV – 2%

Results
• Positive – meets a diagnostic criterion for JAK2 MPN
• Not detected – no evidence of the JAK2 exon mutation

Limitations
• Only exon 12 mutations are detected
• Limit of detection is 1/1,000 cells

MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative
Clinical sensitivity
• ET – 3-4%
• PMF – 8-10%
• PV – very rare

Results
• Detected
  ○ Meets a diagnostic criterion for JAK2 MPN
  ○ MPL codon 515 mutation present (W515K or W515L)
  ○ Mutant allele burden is reported
• Not detected
  ○ MPL codon 515 mutation not detected

Limitations
• Does not detect mutations in other locations within the MPL gene
• Limit of detection for this test is 5% mutant allele

References