BACKGROUND:
Calreticulin (CALR) somatic mutations provide a diagnostic marker in JAK2/MPL wild type essential thrombocythemia (ET) and primary myelofibrosis (PMF) with a mutation frequency of 67-71% and 56-88% respectively.6 CALR mutations were also reported in other myeloid disorders such as Myelodysplastic syndromes (MDS), Refractory anemia, Refractory anemia with ringed sideroblasts (RARS), Refractory anemia with excess blasts with mutation frequency of 8%, 9%, 11% and 12% respectively.2 In general, CALR mutations are not seen in Polycythemia Vera (PV), post-PV myelofibrosis or JAK2 V617F or MPL mutated ET or PMF.1-6

Studies showed that CALR mutations may also provide prognosis information. In ET, CALR mutations are associated with lower hemoglobin level, higher platelet count, and less thrombosis events.3,4 In PMF, CALR mutations are associated with longer overall survival compared to JAK2 mutation, while CALR-AXSL1+ and JAK2-MPL-CALR- were determined to be high-risk signatures in PMF.5 CALR therefore is a promising molecular marker for diagnosis and prognosis for these patients.6 Mutation status of CALR will aid the diagnosis of PMF and ET and risk stratification.

Calreticulin is a highly conserved Ca2+ binding chaperone protein.1,2 The calreticulin gene (CALR) contains 9 exons and is located on chromosome 19p13.2.5 The calreticulin protein consists of three domains: a globular amino terminal N-domain (residues 1 to 180), central proline-rich domain (residues 181 to 290) and an acidic carboxyl terminal C-domain (residues 291 to 400).3,5 The exact mechanism by which CALR mutations produce the myeloproliferative disease phenotype is unknown, but multiple studies have shown that CALR mutations disrupt the C-terminal endoplasmic reticulum-retention sequence (KDEL), generate a novel C-terminus, and activate the STAT5 pathway.1-5

CALR MUTATION ANALYSIS
CALR Mutation Analysis can be ordered separately or as part of our myeloproliferative neoplasm suite of tests. When ordered as part of a reflex panel, if ET or PMF are suspected, patients in whom JAK2 V617F mutations are not detected will automatically undergo CALR. If CALR mutations are not detected, they will automatically undergo MPL Exon 10.

REASONS FOR REFERRAL:
• Diagnosis in patients suspected of ET and PMF with JAK2 and MPL mutation not detected.
• Risk stratification and possible treatment decisions in diagnosed ET and PMF patients.

METHOD:
The assay is performed by PCR amplification and bidirectional sequencing of the coding region and intron-exon junctions of exon 9 of the CALR gene. If needed, fragment analysis is performed to confirm length mutations.
LIMITATIONS:
The lower limit of detection of the assay is approximately 20% (allele proportion).

REFERENCE INTERVAL:
Mutations are reported as mutation detected or mutation not detected using standard nomenclature from Human Genome Variation Society (HGVS). Polymorphisms are not reported but are available upon request.

SPECIMEN REQUIREMENTS:
3-5 ml EDTA (lavender top) whole blood or 2-5 ml EDTA bone marrow or high quality DNA (≥ 500ng at 25ng/ul).

SHIPPING REQUIREMENTS:
Place the room temperature specimen and requisition in plastic bags, seal and insert in a Styrofoam container. Seal the Styrofoam container, place in a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. Address package to:

Client Services/Molecular Oncology
BloodCenter of Wisconsin
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

TURNAROUND TIME: 5-7 days

CPT CODES: 81219

REFLEX ORDERING:
MPN (Myeloproliferative Neoplasms) Reflex - ET/PMF
JAK2 V617F Mutation Analysis CPT Codes: 81270 Turnaround Time: 5-7 days
CALR Mutation Analysis (if indicated) CPT Codes: 81219 Turnaround Time: additional 5-7 days
MPL Exon 10 Mutation Analysis (if indicated) CPT Codes: 81403 Turnaround Time: additional 14 days

REFERENCES:
5. Tefferi A et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia 2014 Jan 9. doi: 10.1038/leu.2014.3