BACKGROUND:
Genetic profiling of hematologic malignancies is increasingly useful for patient care, generating information that can support diagnosis, prognosis, risk stratification, and the use of targeted therapy. The HemeOnc panel utilizes next-generation sequencing (NGS) to detect variants within selected coding and junctional regions of 30 genes as listed in the table which are either prognostic or diagnostic for myeloid hematologic malignancies including: acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN) including mixed myeloproliferative/myelodysplastic syndromes such as juvenile myelomonocytic leukemia and refractory anemia with ringed sideroblasts associated with marked thrombocytosis.

For every specimen, BloodCenter of Wisconsin will provide a comprehensive report including up to date information on the clinical significance of sequence variants, potential therapeutic options and applicable clinical trials.

Table. The NGS HemeOnc panel contains 30 genes associated with several myeloid hematological malignancies.

REASONS FOR REFERRAL:
- Identification of pathogenic genetic variants in myeloid hematologic malignancies.
- Risk stratification, possible treatment decisions, and applicable clinical trials in 10 different myeloid hematologic malignancies.
METHOD:
Specific coding and junctional regions of the following 30 genes are sequenced in this test: (ABL1, ASXL1, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, GATA2, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTPN11, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, TET2, TP53, U2AF1, WT1). Multiplex PCR-based amplification is used to enrich genomic DNA, followed by paired-end sequencing on the Illumina MiSeq. Reference sequences are available from the laboratory upon request. Data analysis is performed using NextGene/Geneticist Assistant (Soft Genetics) and variants are reported in accordance with ACMG NGS standards.  

Sanger sequencing is used to analyze either GC rich or poorly covered regions (CEBPA exon 1, GATA2 exon 3 and 5, RUNX1 exon 4 & 9, SETBP1 exon 4, and SH2B3 exon 2). All pathogenic and likely pathogenic sequence variants identified by NGS are confirmed by Sanger sequencing or alternative methods. If a sequence variant cannot be confirmed due to the limitations in sensitivity of Sanger sequencing technology the report will indicate that the ‘result is based on NGS only’.

LIMITATIONS:
Using NGS technology, the lower limit of detection is ≥10% at ≥ 200X coverage. When coverage of <200X is observed, Sanger sequencing is performed. Excluding the GC-rich or poorly covered regions described above, a coverage of >200X is expected for ≥98% of the remaining 142 target areas assessed with the panel. Any target areas where Sanger sequencing is performed due to coverage <200X will be reported. Target regions sequenced by Sanger sequencing due to high GC content or poor coverage have a lower limit of detection of approximately 20%.

REFERENCE INTERVAL:
No variants detected. Sequence variants are reported using standard nomenclature from HGVS recommendation (www.hgvs.org). Sequence variants are classified using current recommendations from ACMG.

REPORTING OF RESULTS:
The comprehensive diagnostic report includes the latest information on clinical significance of sequence variants, potential therapeutic options and applicable clinical trials.

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 packages to:

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

TURNAROUND TIME: 21 days
CPT CODES: 81450

REFERENCES: