TRICORE REFERENCE LABORATORIES

MYELOID GENE PANEL BY NEXT GENERATION SEQUENCING

TriCore Reference Laboratories' Myeloid Gene Panel by next generation sequencing (NGS) is a cost-effective and comprehensive way to detect key mutations associated with myeloid neoplasms.

The panel permits advanced assessment of myeloid neoplasms as described in National Comprehensive Cancer Network (NCCN) and World Health Organization (WHO) guidelines. Utilizing custom Ion AmpliSeq™ reagents on the Ion Personal Genome Machine® (PGM™), the panel identifies the most common genetic alterations in critical target genes to support presumed diagnoses, direct medical management, and predict disease prognosis.

GENES ANALYZED				
ASXL1	CSF3R	IDH2	NPM1	SRSF2
BRAF	DNMT3A	JAK2	NRAS	TET2
CALR	EZH2	KIT	RUNX1	TP53
CBL	FLT3*	KRAS	SETBP1	U2AF1
CEBPA*	IDH1	MPL*	SF3B1	WT1

* *In cases of acute myeloid leukemia, the NGS testing is supplemented by CEBPA analysis by Sanger sequencing and FLT3-ITD testing by capillary electrophoresis. In cases of myeloproliferative neoplasms, the NGS analysis is supplemented by pyrosequencing of MPL.*

Myeloid neoplasms are a heterogeneous group of entities with distinct molecular profiles, and the utility of the panel differs depending on the specific diagnosis or clinical setting. Highlights include:

- **For acute myeloid leukemia (AML)**, the panel includes FLT3, NPM1, CEBPA, KIT, RUNX1, ASXL1, and TP53, permitting complete risk stratification as recommended by NCCN and European LeukemiaNet Guidelines. In addition, the panel allows complete and final subclassification of AML according to the WHO 2016 Classification. FDA-approved targeted therapies are available for some cases with mutations detected in FLT3 and IDH2.
- **For myelodysplastic syndrome (MDS)**, the panel detects mutations associated with better (SF3B1) and worse (SRSF2, DNMT3A, U2AF1, CBL, NRAS, KRAS) prognosis per NCCN guidelines. More specifically, it also includes a set of genes (TP53, EZH2, ETV6, RUNX1, ASXL1) that identifies cases with overall survival more similar to that of patients in the next higher R-IPSS risk group [PMID 21714648].
- **For myeloproliferative neoplasms (MPN)**, the panel includes JAK2 V617F and exon 12, CALR, and MPL for effective diagnosis. In addition, the included genes (ASXL1, SRSF2, IDH1, IDH2, TP53, EZH2, U2AF1, SF3B1, TET2) follow NCCN guidelines for identifying higher risk cases of polycythemia vera, essential thrombocythemia, and primary myelofibrosis.
- **For chronic myelomonocytic leukemia (CMML)**, the panel includes the mutations cited in the WHO 2016 Classification (TET2, SRSF2, ASXL1, SETBP1) as supportive evidence for establishing the proper diagnosis. It also identifies NPM1 mutations, a high risk finding in this disease setting.
- **For unexplained cytopenias**, when a diagnosis of MDS or other myeloid neoplasia is not established, the panel identifies patients with "clonal cytopenia of undetermined significance" (CCUS), including a specific subset at significantly higher risk of progression to an overt myeloid neoplasm. If a negative result is obtained using this panel, its relatively high negative predictive value (>68%) for myeloid neoplasia may prompt renewed consideration of a reactive etiology.

To assist pathologists and clinicians in using this sophisticated genetic information, each case will be accompanied by a global interpretive comment, crafted by our expert team of hematopathologists and molecular genetic pathologists, that synthesizes the clinical, morphologic, cytogenetic, and molecular genetic findings for optimal patient care. When combined with our interpretive guidance and our accessibility for consultation and discussion, TriCore's myeloid NGS panel will become an indispensable part of your workup.

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MYELOID GENE PANEL BY NEXT GENERATION SEQUENCING, continued

SPECIFIC TARGET REGIONS		
Gene	**Codons**	**RefSeq Accession**
ASXL1	574-1541	NM_015338.5
BRAF	588-617	NM_004333.4
CALR	343-417	NM_004343.3
CBL	366-477	NM_005188.2
CSF3R	576-621, 681-836	NM_000760.3
DNMT3A	428-912	NM_022552.3
EZH2	163-298, 512-732, 746-751	NM_004456.4
FLT3	666-684, 811-884	NM_004119.2
IDH1	42-148	NM_005896.2
IDH2	126-179	NM_002168.2
JAK2	520-547, 593-621	NM_004972.3
KIT	412-488, 515-549, 788-828	NM_000222.2
KRAS	1-96	NM_033360.3
NPM1	283-294	NM_002520.6
NRAS	1-96	NM_002524.4
RUNX1	21-322	NM_001754.4
SETBP1	852-894	NM_015559.2
SF3B1	603-640, 656-678, 694-734	NM_012433.2
SRSF2	73-118	NM_003016.4
TET2	1-2002	NM_001127208.2
TP53	1-355	NM_000546.4
U2AF1	16-42, 139-160	NM_001025203.1
WT1	367-416, 448-477	NM_024426.4

MYELOID DISORDERS MUTATIONS PANEL

Test Code.....MDMYE
CPT Code.....81450; G0452-26
Testing Performed.....Wednesday
Methodology.....Genomic Sequencing
Reported.....Reported in 14-21 days

COLLECTION	
Specimen	Preferred: Whole Blood or Bone Marrow
Collection Containers	Preferred: Lavender (EDTA) SQ Container Code: XL, L7 Acceptable: Pink(EDTA) SQ Container Code: PN Acceptable: Yellow (ACD) SQ Container Code: YA Acceptable: Green (NaHeparin) SQ Container Code: GN
Collection Amount	Preferred volume: 5.0mL Minimum volume: 1.0mL
PROCESSING	
Specimen Processing Instructions	Do not centrifuge. Do not open or share tube.
SHIPPING/TRANSPORT AND STORAGE	
Stabilities/Storage: (*Collection to initiation of testing*)	**Temperature** **Stability** Ambient: 5 days Refrigerated: 5 days Frozen: Unacceptable
Shipping Instructions	Ship ambient and overnight for Monday-Saturday receipt.
Rejection Criteria	Frozen Samples