BLOODCENTER of WISCONSIN™

DNMT3A EXON 23 SEQUENCE ANALYSIS

BACKGROUND:
DNA methylation is mediated by a family of methyltransferases, including DNA (cytosine-5)-methyltransferase 3A, encoded by the *DNMT3A* gene. Abnormal DNA methylation is known to influence the initiation and progression of several cancers. Somatic mutations in *DNMT3A* exon 23 correlate with reduced patient survival and have been found in approximately 22%[1] of cases of acute myeloid leukemia (AML), 8-15%[2,3] of myelodysplastic syndrome (MDS) patients, and 18%[4] of T-cell acute lymphoblastic leukemia (T-ALL) patients. DNMT3A mutations detected at the R882 hotspot in exon 23 are found at a frequency of approximately 60%[1], 31%[2] and 23%[5] in AML, MDS, and T-ALL/Lymphoma patients, respectively. R882 positive AML cells exhibit severely reduced de novo methyltransferase activity[6]. Studies have shown R882 positive cells may be resistant to standard chemotherapy, enabling a subset of leukemic cells to survive induction therapy and potentiate AML relapse[7]. Recent studies have shown DNMT3A mutations to further refine the prognosis for normal karyotype AML patients[8]. As such, screening for DNMT3A mutations was added to the 2015 NCCN Guidelines© for AML[9].

REASONS FOR REFERRAL:
- Further refine the risk stratification and prognosis for *FLT3*-ITD positive patients.
- Aid risk stratification in conjunction with molecular markers *FLT3*-ITD, *CEBPA*, and *NPM1*.

METHOD:
The assay is performed by PCR amplification and bidirectional sequencing of the coding region and intron-exon junctions of exon 23 of the *DNMT3A* gene.

LIMITATIONS:
The lower limit of detection of the assay is approximately 20% allele burden in specimen, with sensitivity and specificity >99%.

REFERENCE INTERVAL:
Mutations are reported as not detected or detected and named according to the standard nomenclature from Human Genome Variation Society (HGVS).

PO Box 2178
Milwaukee, WI 53201-2178
Location/Sample Deliveries:
638 N. 18th St. Milwaukee, WI 53233-2121
p800-245-3117 | f414-937-6202 | www.bcw.edu

SPECIMEN REQUIREMENTS:
3-5 ml EDTA (lavender top) whole blood or 2-5 ml EDTA bone marrow or DNA, high quality, ≥ 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 Laboratory
BloodCenter of Wisconsin
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

TURNAROUND TIME: 5-10 days

CPT CODES: 81403

PANEL ORDERING:

AML post-FLT3 Comprehensive Mutation Panel	Turnaround Time: 7-10 days
NPM1 Mutation Analysis	CPT Codes: 81310
CEBPA Mutation Analysis	CPT Codes: 81403
DNMT3A Exon 23 Sequence Analysis	CPT Codes: 81403
IDH1 Exon 4 Mutation Detection	CPT Codes: 81403
IDH2 Exon 4 Mutation Detection	CPT Codes: 81403

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