

## *KIT* Exons 8 and 17 Mutation Analysis for Acute Myeloid Leukemia

### Background Information

Using 2008 WHO criteria, acute myeloid leukemia is subclassified based upon a combination of clinical, morphologic, phenotypic, cytogenetic and molecular findings. Several cytogenetic and/or molecular abnormalities define distinct clinical entities that are associated with differences in prognosis. For example, acute myeloid leukemias containing the core-binding factor (CBF) translocations, t(8;21)(q22;q22) and inv(16)(p13q22) are recognized as distinct neoplasms with characteristic clinical and morphologic features and an overall favorable prognosis.<sup>1</sup>

Mutations in the *KIT* gene are identified in approximately 20-40% of CBF acute myeloid leukemias. Mutations consist predominantly of point mutations in exon 17 or, less frequently, insertions and deletions occurring in exon 8. The presence of a *KIT* mutation is reported to abrogate the favorable prognosis associated with CBF acute leukemias, especially t(8;21).<sup>2-4</sup>

The identification of *KIT* mutations in CBF leukemias assists in prognostic assessment and selection of appropriate therapy, and *KIT* mutation analysis is recommended for risk stratification of CBF leukemias in current National Comprehensive Cancer Network (NCCN) guidelines.<sup>5</sup> The Cleveland Clinic Department of Molecular Pathology has developed, validated and implemented a sequencing assay for the detection of mutations in *KIT* exons 8 and 17.

### Clinical Indications

*KIT* Exons 8 and 17 Mutation Analysis is useful in the workup of suspected acute myeloid leukemia, especially in cases carrying the CBF translocations, t(8;21)(q22;q22) or inv(16)(p13q22).

### Interpretation

Normal results are reported as “*KIT* mutations are not detected.” Positive results are reported using Human

Genome Variation Society (HGVS) nomenclature, and an interpretation provided.

### Methodology

DNA is extracted and *KIT* exons 8 and 17 are amplified by PCR. The PCR product is subjected to bidirectional cycle sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) on the ABI 3730 Genetic Analyzer. Sequences are aligned to wild type reference sequence and assessed for the presence of mutations.

### Limitations of the Assay

The lower limit of reliable mutation detection is 15-20% mutant alleles. Formalin-fixed, paraffin-embedded tissue is not accepted.

### References

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3. Cairoli R, Beghini A, Grillo G, *et al.* Prognostic impact of c-*KIT* mutations in core binding factor leukemias: an Italian retrospective study. *Blood.* 2006;107:3463-8.
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5. O'Donnell MR, Abboud CN, Altman J, *et al.* Acute myeloid leukemia. *J Natl Compr Canc Netw.* 2012;10:984-1021.
6. Levine RL, Wadleigh M, Cools J, *et al.* Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. *Cancer Cell.* 2005;7:387-397.
7. Ma W, Kantarjian H, Zhang X, *et al.* Mutation profile of JAK2 transcripts in patients with chronic myeloproliferative neoplasias. *J Mol Diagn.* 2009;11:49-53.
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9. Scott LM, Tong W, Levine RL, *et al.* JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med.* 2007;356: 459-468.

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#### Test Overview

<b>Test Name</b>	KIT-AML Exons 8 and 17 Mutation Analysis for Acute Myeloid Leukemia
<b>Reference Range</b>	Normal results are reported as "KIT mutations are not detected."
<b>Specimen Requirements</b>	Peripheral Blood: 5mL EDTA (Lavender); Transport Temperature: Refrigerated
<b>Alternative Specimen Requirements</b>	Bone Marrow: 1-2mL. EDTA (Lavender); Transport Temperature: Refrigerated
<b>Ordering Mnemonic</b>	KITAML
<b>Billing Code</b>	84158
<b>CPT Code</b>	81404

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