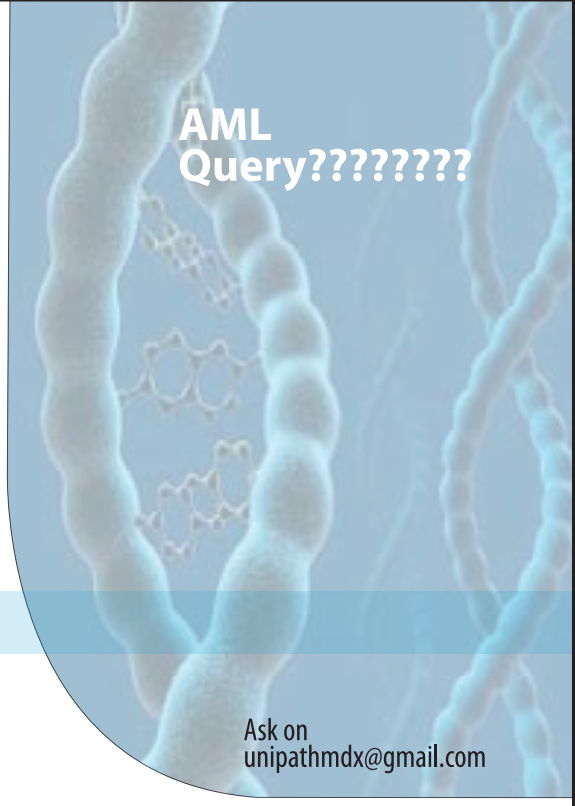
Unipath
SPECIALTY LABORATORY Ltd.



ACUTE MYELOGENOUS LEUKEMIA (AML)

Department of Molecular Biology

Ask on
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NPM1 MUTATION

Insertion mutations in the NPM1 gene occur in acute myeloid leukemia (AML) and predominantly associate with those cases demonstrating a normal karyotype. Testing for these mutations can aid in prediction of clinical outcome. Small insertion mutations in

NPM1 exon 12 were first identified in AML due to the cytoplasmic mislocalization of the mutated NPM1 protein. The affected cases were referred to as NPM1c-positive, and were associated with CD34 negativity and the absence of recurrent cytogenetic abnormalities. NPM1 mutations are now considered the most common known genetic lesion in AML, occurring in about 30% of adult de novo cases, and 50-60% of AMLs with normal karyotype. NPM1 mutations in the absence of FLT3 mutations confer a more favorable prognosis in AML. This test detects virtually all reported NPM1 mutations.

FLT3 MUTATION

The FLT3 gene encodes a cell surface receptor tyrosine kinase that is expressed on early hematopoietic stem cells. Activating mutations in FLT3 occur in approximately 25-30% of patients with acute myelogenous leukemia (AML). The mutation generally occurs as either an internal tandem duplication (ITD) within the juxtamembrane domain, or as a missense point mutation within the tyrosine kinase domain (TKD) at codon 835. This test detects both the FLT3-ITD and FLT3-TKD (codon 835) mutations. The presence of a FLT3-ITD mutation has been associated with a significantly poorer outcome in normal karyotype AML.

CEBPA MUTATION

Mutations within the CEBPA gene occur in approximately 5-10% of patients with newly diagnosed acute myeloid leukemia (AML), and are most frequent in cases with a normal karyotype. The presence of CEBPA mutations has been associated with a more favorable clinical outcome. Two types of CEBPA mutations are most frequently observed: N-terminal frame shift mutations that lead to truncation of the full-length protein, and C-terminal bZIP domain region mutations that are in-frame insertions or deletions. Both mutation types usually occur simultaneously (double mutations), although up to a third of cases may exhibit only one type (single mutation). This DNA sequencing test will detect single and double mutations within the entire CEBPA coding region from blood or bone marrow specimens. Testing for CEBPA mutations may aid in prediction of clinical outcome.

IDH1 AND IDH2 MUTATIONS

IDH1 or IDH2 gene mutations occur in approximately 15% of acute myeloid leukemias (AML). The presence of an IDH mutation has been associated with a poorer prognosis in the better-risk NPM1 mutant, FLT3-ITD negative group. This test detects all mutations at codon 132 of IDH1 and codons 140 and 172 of IDH2. Specimens should contain at least 30% neoplastic cells to enable mutation detection.

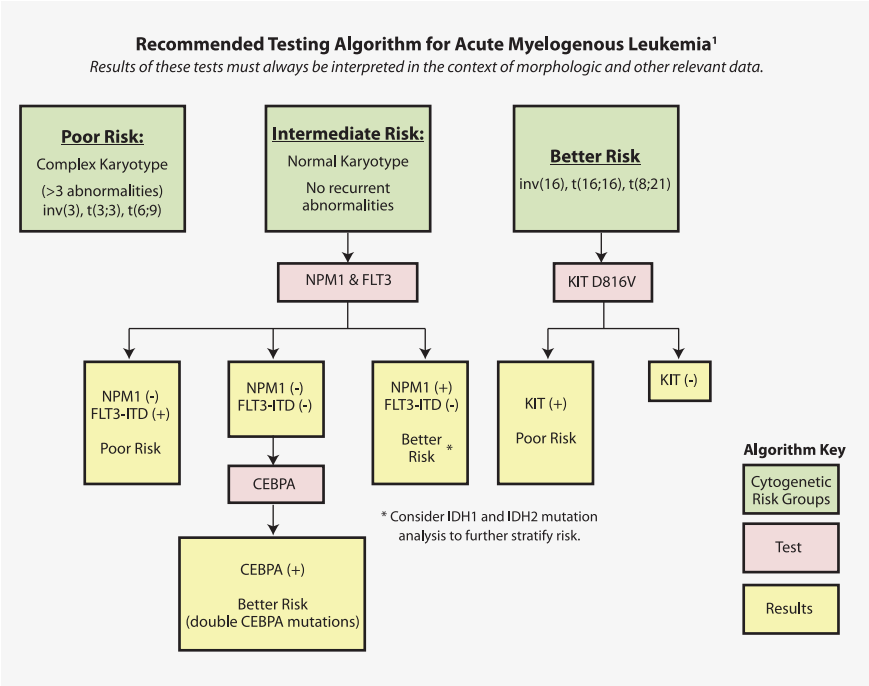
KIT D816V MUTATION

This test detects the KIT c.2447A>T(D816V) mutation found in most adults (>80%) with systemic mastocytosis and in some classes of acute myeloid leukemia. Detection of the KIT D816V mutation can aid in diagnosis of systemic mastocytosis. In AML with the t(8:21) karyotype, and to a lesser extent inv(16), the presence of a KIT D816V mutation has been associated with a higher risk of relapse.

DNMT3A MUTATION

DNMT3A protein contains an N-terminal and a smaller C-terminal part encoded by the DNMT3A gene located at chromosome 2p23. Somatic mutations in DNMT3A are described in AML cases and are shown to have an overall poor survival outcome.

ACUTE MYELOGENOUS LEUKEMIA (AML) ALGORITHM



The Department of Molecular Biology at Unipath utilizes a testing algorithm for Acute Myelogenous Leukemia to stratify patients with either favorable or unfavorable genetic markers associated with AML prognosis. The Unipath AML algorithm will

- Consolidate AML testing in a single source laboratory.
- Streamline efficiency of testing, decreasing turnaround time.
- Deliver highly accurate diagnostically relevant results.
- Improve cost efficiency.

As a leader in the rapidly evolving molecular diagnostic field, we hope that the following AML Testing Algorithm will aid in your selection of the most informative and diagnostically-relevant tests.

ACUTE PROMYELOCYTIC LEUKEMIA
PML/RARA t (15;17) TRANSLOCATION, QUALITATIVE

Greater than 95% of acute promyelocytic leukemia (APML) cases harbor a t(15;17) translocation. This rearrangement results in the fusion of the PML and retinoic acid receptor alpha (RARA) genes located on chromosomes 15 and 17 respectively. Three breakpoint regions within the PML gene can be involved, resulting in three possible PML/ RARA fusion types: bcr1 (long), bcr2 (variable), and bcr3 (short). This test qualitatively detects all three PML/RARA transcript variants in peripheral blood and bone marrow. Testing for PML/RARA can assist in the diagnosis, clinical management, and monitoring of APL.

1 Ref. O'Donnell MR, Abbound CN, Altman J, Appelbaum FR, et al. Acute Myeloid Leukemia. Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2011 Mar;9(3):280-317

Frequency and effect of AML mutations

Gene	Mutation Frequency	Risk
NPM1	~50	Good
CEBPA	10-18	
DNMT3A	~33	Adverse
FLT – ITD	28-42	
FLT – TKD	7-14	
IDH 1 & 2	~20	

TAT AND ORDERING INFORMATION:

TEST NAME	METHOD	SPECIMEN	REPORTING TIME
PCR NPM 1 Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
PCR FLT 3 (ITD & D835Y) Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
PCR CEBPA Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
PCR IDH 1&2 Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
PCR DNMT3A Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
KIT D816V Mutation Detection	PCR SEQUENCING	EDTA WB/BM	7 DAYS
PCR PML RARA (15;17)Translocation	NESTED RT PCR	EDTA WB/BM	3RD DAY

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