

AML Panel: *FLT3* & *NPM1* Mutation Detection by PCR

Indications for Testing

- Stratifying high and low risk AML
- At initial diagnosis of AML
- Reoccurrence of leukemia after induction therapy on patients not initially screened for *FLT3* or *NPM1* mutations.

Test Code

AMLPL

Specimen Requirements

5 ml of Peripheral Blood in EDTA or ACD
3 ml of bone marrow in EDTA or ACD
1 µg of previously isolated DNA

Specimen Transport

Ambient or Cool
Do not Freeze

CPT Codes

83891, 83898×3, 83892,
83909×3, 83912

Turn-Around-Time

3-5 Days

Description of Testing

Primers targeting the area surrounding the ITD and D835 regions of the *FLT3* gene and exon 12 of the *NPM1* gene are used to amplify the patient's DNA. The size of the ITD and *NPM1* PCR product is determined by capillary electrophoresis. The D835 PCR product is digested with EcoRV and the presence of the mutation is assessed using capillary electrophoresis.

References

1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, (V.1.2010) National Comprehensive Cancer Network.
2. Wertheim G, et al. (2008) Nucleophosmin (*NPM1*) mutations in acute myeloid leukemia: an ongoing (cytoplasmic) tale of dueling mutations and duality of molecular genetic testing methodologies. *J Mol Diagn* 10(3):198-202.
3. Gale R, et al. (2008) The impact of *FLT3* internal tandem duplication mutant level, number, size and interaction with *NPM1* mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood* 111:2776-2784.
4. Falini B, et al. (2007) Translocations and mutations involving the nucleophosmin (*NPM1*) gene in lymphomas and leukemias. *Haematologica* 92(4):519-532.
5. Mrozek, K, et al. (2007) Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification. *Blood* 109(2):431-448.
6. Gilliland, DG, and Griffin, JD. (2002) The roles of *FLT3* in hematopoiesis and leukemia. *Blood* 100(5):1532-1542.
7. Nakao, M, et al. (1996) Internal tandem duplication of the *FLT3* gene found in acute myeloid leukemia. *Leukemia* 10(12):1911-1918.

* *NPM1* testing is covered by a United States patent licensed from Xenomics, Inc.

Clinical Utility of the AML Panel

The *FLT3* tyrosine kinase is one of the most commonly mutated genes in AML, occurring in about 30% of AML patients at diagnosis. Clinical studies have determined *FLT3*-ITD mutations are associated with higher numbers of leukemic cells in both blood and bone marrow, increased incidence of relapse, and decreased overall survival. A mutation in the codon for the aspartic acid residue at position 835 (D835) in the *FLT3* kinase domain occurs in about 8% of AML patients. This mutation has been linked to poor survival in some studies, but less strongly than the ITD mutation.

The Nucleophosmin (*NPM1*) gene is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in about 35% of AML patients at diagnosis, and in approximately 60% of adult cytogenetically normal AML (CN-AML). Although *NPM1* mutations are associated with a favorable response to induction therapy, presence of the *NPM1* mutation is not an independent factor when predicting response to therapy. *NPM1* mutations, in the absence of the *FLT3* mutation, are associated with a good response to induction therapy and have a 60% improved 5 year survival.

Clinical studies have confirmed the importance of determining the mutation status of both *FLT3* and *NPM1* genes in order to effectively stratify cytogenetically normal (CN-AML) AML patients. Current guidelines stratify patients into at least three different prognostic groups based upon patient's combined *FLT3* + *NPM1* mutation status. A more favorable prognosis is associated with presence of a *NPM1* mutation in the absence of a *FLT3* mutation (*NPM1*-mut; *FLT3*-wt). An intermediate prognosis is assigned to patients with wild type *NPM1* and *FLT3* (*NPM1*-wt; *FLT3*-wt); and in patients with mutations in both *NPM1* and *FLT3* (*NPM1*-mut; *FLT3*-mut). A poor prognosis is indicated in cases of CN-AML that lack an *NPM1* mutation if *FLT3* is mutated (*NPM1*-wt; *FLT3*-mut).