

BACKGROUND:

Acute myeloid leukemia with mutated *NPM1*, in the absence of a coexisting internal tandem duplication in the FMS-like tyrosine kinase 3 gene (FLT3 ITD), is associated with a favorable prognosis in patients with normal cytogenetics and other intermediate risk karyotypes.¹⁻⁴ The *NPM1* gene is located at chromosome 5q35 and contains 12 exons. *NPM1* encodes for nucleophosmin, a 37 kDa nucleolar chaperone protein that shuttles between the nucleus and cytoplasm. Somatic heterozygous length mutations in *NPM1* exon 12 are present in approximately 35% of adult AML cases, including up to 60% of those with normal cytogenetics, but are found in only 8% of childhood AML.^{5,6} *NPM1* exon 12 mutations produce C-terminus changes that result in abnormal cytoplasmic localization of nucleophosmin. These mutations are associated with FLT3 ITD in approximately 40% of cases.⁵

In adults, *NPM1*-mutated AML is strongly associated with acute myelomonocytic and acute monocytic leukemias. Approximately 80 – 90% of acute monocytic leukemias have *NPM1* length mutations.¹

REASONS FOR REFERRAL:

Risk stratification in patients with cytogenetically normal AML.

METHOD:

NPM1 exon 12 mutations are detected by PCR amplification with fluorescently labeled primers and capillary electrophoresis-based fragment analysis.

LIMITATIONS:

This assay will only detect known length mutations in *NPM1* exon 12. Rare mutations in other exons will not be detected. The lower limit of detection of the assay is approximately 5%. This assay is expected to detect >99% of *NPM1* exon 12 length mutations that are present at a level of 5% or greater at >99% specificity.

REFERENCE INTERVAL:

No mutation detected.

Sequence variations are reported using standard nomenclature.

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: 3-6 days

CPT CODES: 81310

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

AML Mutation Panel Turnaround Time: 7-10 days

FLT3 Mutation Analysis	CPT Codes: 81245, 81246
NPM1 Mutation Analysis	CPT Codes: 81310
CEBPA Mutation Analysis	CPT Codes: 81403

REFLEX ORDERING:

AML Mutation Panel - Reflex

FLT3 Mutation Analysis	CPT Codes: 81245, 81246	Turnaround Time: 7-10 days
NPM1 Mutation Analysis (if indicated)	CPT Codes: 81310	Turnaround Time: add 3-6 days
CEBPA Mutation Analysis (if indicated)	CPT Codes: 81403	Turnaround Time: add 5-10 days

NPM1 Mutation Analysis with Reflex to CEBPA

NPM1 Mutation Analysis	CPT Codes: 81310	Turnaround Time: 3-6 days
CEBPA Mutation Analysis (if indicated)	CPT Codes: 81403	Turnaround Time: add 5-10 days

REFERENCES:

1. Arber D, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: WHO Press, 2008:110-23.
2. Schlenk, et al. N Engl J Med 2008;358:1909-18.
3. Haferlach C, et al. Blood 2009; May 8, Epub ahead of print.
4. Thiede C, et al. Blood 2006;107:4011-20.
5. Falini B, et al. Blood 2007;109:874-85.
6. Brown P, et al. Blood 2007;110:979-85.

ADDITIONAL REFERENCES:

Renneville A, Roumier C, Biggio V, et al. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. Leukemia 2008;22:915-31.
Schlenk RF, Dohner K, Krauter J, et al. Mutations and Treatment Outcome in Cytogenetically Normal Acute Myeloid Leukemia. N Engl J Med 2008;358:1909-18.