

## Overview

### Useful For

As a prognostic indicator in patients with newly diagnosed acute myelogenous leukemia with normal karyotype and no *FLT3* mutation and as a leukemia-specific marker of minimal residual disease

### Testing Algorithm

Both DNA and RNA are extracted. The assay is composed of 2 parts, a RNA-based, sensitive quantitative reverse transcription real-time polymerase chain reaction (RT-PCR) that detects and quantifies the most common altered *NPM1* mRNA transcripts (A, B, D forms) in acute myeloid leukemia (AML), and a DNA-based qualitative *NPM1* exon 12 variant screen by fragment analysis that detects essentially all altered forms reported in AML, including the rare non-A, B, D forms (with lower sensitivity at the DNA level).

### Special Instructions

- [Hematopathology Patient Information](#)

### Method Name

RNA: Reverse-Transcription Quantitative PCR (RT-qPCR)

DNA: Polymerase Chain Reaction (PCR) with Fragment Analysis by Capillary Gel Electrophoresis

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Shipping Instructions

**Refrigerated specimen must arrive within 5 days of collection, and ambient specimens must arrive within 3 days of collection.** Collect and package specimen as close to shipping time as possible.

### Necessary Information

The following information is required:

1. Pertinent clinical history
2. Clinical or morphologic suspicion
3. Date of collection
4. Specimen source

### Specimen Required

Submit only 1 of the following specimens:

**Specimen Type:** Blood

**Container/Tube:** Lavender top (EDTA) or yellow top (ACD-B)

**Specimen Volume:** 10 mL

**Collection Instructions:**

1. Invert several times to mix blood.
2. Send specimen in original tube.
3. Label specimen as blood.

**Specimen Type:** Bone marrow

**Container/Tube:** Lavender top (EDTA) or yellow top (ACD-B)

**Specimen Volume:** 4 mL

**Collection Instructions:**

1. Invert several times to mix bone marrow.
2. Send specimen in original tube.
3. Label specimen as bone marrow.

**Forms**

[Hematopathology Patient Information](#) (T676) in Special Instructions

**Specimen Minimum Volume**

Blood: 4 mL

Bone marrow: 2 mL

**Reject Due To**

Gross hemolysis	Reject
Bone marrow biopsies Paraffin-embedded bone marrow clots Slides Paraffin shavings Moderately to severely clotted	Reject

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	5 days	
	Ambient	72 hours	

**Clinical and Interpretive**

## Clinical Information

Acute myeloid leukemia (AML) is a genetically heterogeneous group of neoplasms. While cytogenetic aberrations detected at the time of diagnosis are the most used prognostic feature, approximately 50% of AML cases show a normal karyotype, which is considered an intermediate-risk feature. Within this group, *FLT3* variants are considered indicators of poor prognosis. However, in the absence of a *FLT3* variant, the presence of a nucleophosmin (*NPM1*) variant is associated with a more favorable prognosis. A *NPM1* alteration is a common finding in de novo AML (25%-30% of cases) and consists of small insertion (typically 4 base pair) or insertion/deletion events involving exon 12. Three variants are highly recurrent, termed types A, B, and D and together account for approximately 90% of *NPM1* alterations in de novo AML. Thus, in patients with newly diagnosed AML, those with normal karyotype, no *FLT3* variant, and a *NPM1* alteration are considered to have a better prognosis than patients in the same group with neoplasms lacking a *NPM1* alteration. Furthermore, the presence of a *NPM1* alteration serves as a sensitive marker for evaluating minimal disease and therapeutic response following treatment.

## Reference Values

An interpretive report will be provided.

## Interpretation

The assay incorporates 2 parts: a qualitative screen for exon 12 nucleophosmin (*NPM1*) alterations, and a quantitative reverse transcription polymerase chain reaction (RT-PCR) to determine the copy number of *NPM1* transcripts (relative to *ABL1* reference mRNA). This strategy will allow for identification of the *NPM1* alteration at diagnosis, as well as a high sensitivity method to monitor patients who are post-therapy for minimal residual disease (MRD). Results will therefore be interpreted with integration of the quantitative and qualitative test results in the context of *NPM1* alteration type identified at the time of AML diagnosis if available. Because the quantitative RT-PCR component only reliably detects and quantifies the 3 most common variant types (A, B, D), there is a very small possibility that the qualitative assay may indicate the presence of *NPM1* alteration, but the quantitative assay will be (falsely) negative. In patients with newly diagnosed acute myeloid leukemia, a normal karyotype, and no *FLT3* variant, the presence of *NPM1* alteration is an indicator of a more favorable prognosis. Similarly, following chemotherapy, the presence, relative quantity and trend of change of *NPM1* mRNA transcript is associated with risk of disease relapse.

## Cautions

Because of the design of this assay, a very small number of *NPM1* alterations at diagnosis may not be detected by the more targeted quantitative PCR component. In that setting, the qualitative part of the test can be used for limited minimal residual disease assessment, although the sensitivity is much lower (approximately 5% at the DNA level).

## Clinical Reference

1. Heath EM, Chan SM, Minden MD, Murphy T, Shlush LI, Schimmer AD: Biological and clinical consequences of NPM1 mutations in AML. *Leukemia*. 2017 Apr;31(4):798-807
2. Kronke J, Schlenck RF, Jensen KO, et al: Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. *J Clin Oncol*. 2011 Jul 1;29(19):2709-2716
3. Ivey A, Hills RK, Simpson MA, et al: Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016 Feb 4;374(5):422-433
4. Shayegi N, Kramer M, Bornhauser M, et al: The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. *Blood*. 2013 Jul 4;122(1):83-92

## Performance

## Method Description

RNA is extracted from blood or bone marrow and reverse transcription is performed. Real time quantitative polymerase chain reaction (PCR) is performed from complementary DNA (cDNA) template using the LC480 instrument platform (Roche). This assay targets the most common recurrent nucleophosmin (*NPM1*) alterations in acute myeloid leukemia (AML) (A, B, and D insertion variants). The quantitative value of *NPM1* mRNA copy number is determined relative to *ABL1* as the reference transcript using the delta-delta CT method. The reproducible analytical sensitivity (limit of detection) of this part of the assay is approximately 0.01%.

DNA is extracted from blood or bone marrow and a PCR assay is performed using primers that amplify a fragment of *NPM1* DNA containing the region susceptible to insertion variant. One of the PCR primers contains a fluorescent label. The amplified fragments are size separated by capillary electrophoresis. Wild type *NPM1* produces a fragment length of 187-base pairs (bp). PCR fragments containing an insertional variant are observed as larger fragments, most typically 191-bp, as the majority of alterations are 4-bp insertions. The analytical sensitivity (limit of detection) of this part of the assay is approximately 5%. (Unpublished Mayo method)

## PDF Report

Supplemental

## Day(s) and Time(s) Test Performed

Monday through Friday; Varies

## Analytic Time

10 days

## Maximum Laboratory Time

14 days

## Specimen Retention Time

RNA and DNA 3 months

## Performing Laboratory Location

Rochester

## Fees and Codes

### Fees

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

### Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

### CPT Code Information

81310-*NPM1* (nucleophosmin) (eg, acute myeloid leukemia) gene analysis; exon 12 variants

### LOINC® Information



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Test ID	Test Order Name	Order LOINC Value
NPM1Q	NPM1 Mutation Analysis, V	54448-6

Result ID	Test Result Name	Result LOINC Value
MP053	Specimen Type	31208-2
605098	Interpretation	59466-3
605262	Signing Pathologist	19139-5