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**

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<thead>
<tr>
<th>Gross hemolysis</th>
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<tr>
<td>Bone marrow biopsies Paraffin-embedded bone marrow clots Slides Paraffin shavings Moderately to severely clotted</td>
<td>Reject</td>
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**Specimen Stability Information**

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<th>Specimen Type</th>
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<tr>
<td>Varies</td>
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<td></td>
<td>Ambient</td>
<td>72 hours</td>
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**Clinical and Interpretive**
Test Definition: NPM1Q
NPM1 Mutation Analysis, V

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

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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