Test Definition: NPM1Q

Nucleophosmin (NPM1) Mutation Analysis, Varies

Overview

Useful For

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

Testing Algorithm

The assay is composed of 2 parts:
-RNA-based, sensitive quantitative real-time, reverse transcription polymerase chain reaction (RT-PCR) that detects and quantifies the most common altered *NPM1* messenger RNA transcripts (A, B, D forms) in acute myeloid leukemia (AML)
-DNA-based qualitative *NPM1* exon 12 variant screening 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

1. Refrigerated specimens must arrive within 5 days of collection, and ambient specimens must arrive within 3 days of collection.
2. 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. Specimen source (blood or bone marrow)

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 whole blood specimen in original tube. **Do not aliquot.**
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 bone marrow specimen in original tube. **Do not aliquot.**
3. Label specimen as bone marrow.

Forms

1. Hematopathology Patient Information (T676)
2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Specimen Minimum Volume

Blood: 8 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 & 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 *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 pairs) or deletion/insertion 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 *NPM1* alterations and a quantitative reverse transcription polymerase chain reaction (RT-PCR) assay to determine the copy number of *NPM1* transcripts (relative to *ABL1* reference messenger RNA [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. 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 an *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 an *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 polymerase chain reaction 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;31(4):798-807. doi:10.1038/leu.2017.30

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;29(19):2709-2716. doi:10.1200/JCO.2011.35.0371
3. Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016;374(5):422-433. doi:10.1056/NEJMoa1507471
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;122(1):83-92. doi:10.1182/blood-2012-10-461749

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 template using the LC480 instrument platform (Roche). This assay targets the most common recurrent *NPM1* alterations in acute myeloid leukemia (A, B, and D insertion variants). The quantitative value of *NPM1* messenger RNA 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) Performed

Monday through Saturday

Report Available

10 to 14 days

Specimen Retention Time

Blood/bone marrow: 2 weeks; Extracted DNA and RNA: 3 months

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

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

LOINC® Information

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