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 testing starts with a sensitive reverse transcription (RT)-quantitative PCR assay testing for NPM1 mutations form A, B, and D. If the testing is negative or low level (<0.01%), DNA will be used to test NPM1 exon 12 for any insertional events. If form A, B, or D is detected above 0.01% the testing algorithm will stop.

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

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<th>Time</th>
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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 commonly used prognostic feature, approximately 50% of AML cases show a normal karyotype, which is considered an intermediate-risk feature. Within this group, FLT3 mutations are considered indicators of poor prognosis. However, in the absence of a FLT3 mutation, the presence of a nucleophosmin (NPM1) mutation is associated with a more favorable prognosis. NPM1 mutation 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 mutation, and a NPM1 mutation are considered to have a better prognosis than patients in the same group with neoplasms lacking a NPM1 mutation. Furthermore, the presence of a NPM1 mutation 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) mutations, and a quantitative reverse transcription (RT)-PCR to determine the copy number of NPM1 transcripts (relative to ABL1 reference mRNA). This strategy will allow for identification of the NPM1 mutation 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 as positive with a corresponding quantitative NPM1 relative copy number, negative for NPM1, or suspicious for low level positive NPM1 (if present near the limit of detection of the quantitative assay). Because the quantitative RT-PCR component only detects the 3 most common mutation types (A, B, D), there is a very small possibility that at diagnosis, the qualitative assay may indicate the presence of NPM1 mutation, but the quantitative assay will be (falsely) negative. In patients with newly diagnosed acute myelogenous leukemia, a normal karyotype, and no FLT3 mutation, the presence of NPM1 mutation is an indicator of a more favorable prognosis. Similarly, following chemotherapy, the presence and relative quantity 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%).

Clinical Reference


Performance

Method Description

RNA is extracted from blood or bone marrow and reverse transcription is performed. Real time quantitative PCR is performed from cDNA template using the LC480 instrument platform (Roche). This assay targets the most common
Test Definition: NPM1Q
NPM1 Mutation Analysis, V

recurrent nucleophosmin (NPM1) alterations in acute myelogenous 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 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 mutation. 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 mutation are observed as larger fragments, most typically 191-bp, as the majority of alterations are 4-bp insertions. The 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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