Overview

Useful For
Evaluating patients with chronic myelogenous leukemia and Philadelphia chromosome positive B-cell acute lymphoblastic leukemia receiving tyrosine kinase inhibitor (TKI) therapy, who are apparently failing treatment

Preferred initial test to identify the presence of acquired BCR-ABL1 mutations associated with TKI-resistance

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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</thead>
<tbody>
<tr>
<td>BADX</td>
<td>BCR/ABL1, RNA-Qual, Diagnostic</td>
<td>Yes</td>
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</table>

Testing Algorithm

If BCR/ABL1 fusion type (p210, p190, p205 or p230) is not provided, BADX / BCR/ABL1, Qualitative, Diagnostic Assay will be performed at an additional charge.

In the event that no fusion form (p190, p205, p210, p230) is identified by BADX testing, BAKDM testing will be cancelled.

This is the preferred initial test to identify the presence of acquired BCR/ABL1 mutations associated with TKI-resistance.

See BCR/ABL1 Ordering Guide for Blood and Bone Marrow in Special Instructions.

Special Instructions

- Hematopathology Patient Information
- BCR/ABL1 Ordering Guide for Blood and Bone Marrow

Method Name
Reverse Transcription-Polymerase Chain Reaction (RT-PCR) with Analysis of PCR Products by Sanger Sequencing

NY State Available
Yes

Specimen

Specimen Type
Varies

Advisory Information
This is the preferred initial test to identify the presence of acquired BCR/ABL1 mutations associated with tyrosine kinase inhibitor (TKI)-resistance.

Shipping Instructions
1. Refrigerated specimens must arrive within 5 days (120 hours) of collection, and ambient specimens must
arrive within 3 days (72 hours) of collection.

2. Draw and package specimen as close to shipping time as possible.

**Necessary Information**

The following information is required:

1. Patient's fusion type (p210, p190, p205 or p230)
2. Pertinent clinical history
3. Clinical or morphologic suspicion
4. Date of collection
5. Specimen source (blood or bone marrow)

**Specimen Required**

Submit only 1 of the following specimens:

**Preferred:**

**Specimen Type:** Whole blood

**Container/Tube:** EDTA (lavender top)

**Specimen Volume:** 10 mL

**Collection Instructions:**

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

**Acceptable:**

**Specimen Type:** Bone marrow

**Container/Tube:** EDTA (lavender top)

**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.
Test Definition: BAKDM
BCR/ABL1 Mutation, Sequencing

Forms
1. Hematopathology Patient Information (T676) in Special Instructions

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

Specimen Minimum Volume
Blood: 4 mL
Bone Marrow: 2 mL

Reject Due To

<table>
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<tr>
<th>Gross hemolysis</th>
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<td>Other</td>
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Specimen Stability Information

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<tr>
<td></td>
<td>Ambient</td>
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Clinical and Interpretive

Clinical Information

Chronic myelogenous leukemia (CML) is characterized by the presence of the t(9:22) BCR/ABL1 abnormality, resulting in formation of a fusion BCR/ABL1 mRNA and protein. The ABL1 component of this oncoprotein contains tyrosine kinase activity and is thought to play a central role in the proliferative phenotype of this leukemia.

Recent advances have resulted in a number of therapeutic drugs that inhibit the ABL1 tyrosine kinase, as well as other protein tyrosine kinases. Imatinib mesylate (Gleevec, Novartis) is the prototype of these tyrosine kinase inhibitors (TKIs), which are capable of inducing durable hematologic and (in most patients) cytogenetic remissions. Unfortunately, a significant subset of patients can develop functional resistance to TKIs, due in a large number of cases (approximately 50%) to the acquisition of point mutations in the kinase domain (KD) of the chimeric ABL1 gene. To date, over 50 distinct mutations have been described, although a smaller subset of these (<20) account for the majority of patients with clinical resistance to TKIs, or have well documented in vitro data in the published literature.

Recognition of TKI resistance is important in CML, as the effect of some mutations can be overcome by increasing imatinib dosage, whereas others require switching to either a different (second-generation) TKI, or alternative therapy. The common T315I KD mutation is particularly important, given that this alteration confers pan-resistance to all currently employed TKIs except ponatinib. Typically, TKI resistance is suspected in a CML patient who shows loss of initial therapeutic response (eg, cytogenetic relapse), or a significant and sustained increase in molecular BCR/ABL1 quantitative levels. Similar considerations are also present in patients with Philadelphia chromosome positive B-cell acute lymphoblastic leukemia, who can also be treated using TKI therapy.

Point mutations in the oncogenic BCR/ABL1 are typically detected by direct sequencing of PCR products, following RT-PCR amplification of the BCR/ABL mRNA transcript from a peripheral blood specimen. This approach ensures
comprehensive screening of the clinically relevant KD region. Because this technique requires inclusion of a longer region of ABL1 in the BCR/ABL1 RT-PCR product, low levels of the BCR/ABL1 mRNA transcript (below 0.01% normalized BCR/ABL1 on the International Scale: IS) may not be efficiently amplified (in contrast to similar amplicons generated by quantitative RT-PCR for diagnosis or monitoring).

**Reference Values**
An interpretive report will be provided.

**Interpretation**
The presence of one or more point mutations in the translocated portion of the ABL1 region of the BCR/ABL1 fusion mRNA is considered a positive result, indicating tyrosine kinase inhibitor (TKI) resistance. The specific type of mutation may influence the sensitivity to a specific TKI, and could be useful in guiding therapeutic options for an individual patient.

**Cautions**
This assay is comprehensive for detecting BCR/ABL1 KD mutations, but does not detect all possible mutations in ABL1; therefore, a negative result by this assay does not exclude the presence of a rare, less-well characterized, or unknown mutation that could be associated with some degree of tyrosine kinase inhibitor resistance. The clinical significance of such rarely occurring mutations is, however, uncertain.

The quantitative level of BCR/ABL1 transcript is critical for a successful assay mutation analysis, because the amplification efficiency for a longer mRNA template is decreased with a low abundance of target. If the BCR/ABL1 quantitative PCR level is too low, RT-PCR amplification of BCR/ABL1 may be unsuccessful to yield product for sequencing. Although laboratory standards are yet to be developed, a BCR-ABL1/ABL1 quantitative level above 0.1% is generally considered to be required in order to detect KD mutations by this assay.

Subclonal mutations may be difficult to identify by Sanger sequencing method, even if the BCR/ABL1 mRNA amplification was successful. This is due to the inherit sensitivity level limit of sequencing, which is typically around 15% to 20% mutant allele in a wild-type background.

EDTA blood specimens are preferred for testing. Bone marrow specimens are acceptable; there occasionally are specimen failures from bone marrow RNA, for reasons that are not completely understood. Heparin anticoagulant cannot be used because of PCR inhibition.

Assay precision does not appear to be significantly affected by specimen transport or moderate delays in processing. However, in specimens with lower levels of BCR/ABL, these conditions may cause sufficient RNA degradation to produce false-negative results. Thus, specimens should be shipped as quickly as possible. Ambient specimens over 3 days old and refrigerate specimens over 5 days old at the time of receipt are unacceptable.

**Clinical Reference**
Method Description
Total RNA is extracted from the sample using an extraction kit. cDNA is transcribed and PCR is performed using primers directed against BCR and ABL1 regions to generate a long PCR product representing the translocated allele only (p210, p190, p205 or p230 transcript types) and encompassing the ABL1 region through exon 7. Second (nested) PCR amplifications are next performed to amplify the ABL1 kinase domain region using template from the first-round PCR product. Aliquots of the nested ABL1 PCR products are analyzed by Sanger sequencing and post-sequence software interpretation.(Unpublished Mayo method)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Friday

Analytic Time
5 days

Specimen Retention Time
RNA 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
81170-ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase)(eg, acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain

LOINC® Information

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