MAYO CLINIC LABORATORIES

Test Definition: BAKDM

BCR/ABL1, Tyrosine Kinase Inhibitor Resistance, Kinase Domain Mutation Screen, Sanger Sequencing, Varies

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

Test Id	Reporting Name	Available Separately	Always Performed
BADX	BCR/ABL1, RNA-Qual, Diagnostic	Yes	No

Testing Algorithm

If *BCR::ABL1* fusion type (p210, p190, p205 or p230) is not provided, the qualitative, diagnostic assay for *BCR::ABL1* will be performed at an additional charge.

If no fusion form (p190, p205, p210, p230) is identified by qualitative testing, this test will be canceled.

Special Instructions

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

Method Name

Reverse Transcription Polymerase Chain Reaction (RT-PCR) with Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

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

associated with tyrosine kinase inhibitor (TKI)-resistance.

Additional testing options are available. For ordering guidance see BCR/ABL1 Ordering Guide for Blood and Bone Marrow.

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

Pertinent clinical history including if the patient has a diagnosis of chronic myelogenous leukemia or other *BCR::ABL1*-positive neoplasm is required.

Specimen Required

Submit only 1 of the following specimens:

Preferred:
Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA)
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.

Acceptable:
Specimen Type: Bone marrow
Container/Tube: Lavender top (EDTA)
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
Moderately to severely clotted	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	5 days	PURPLE OR PINK TOP/EDTA
	Ambient	72 hours	PURPLE OR PINK TOP/EDTA

Clinical & Interpretive

Clinical Information

Chronic myeloid leukemia (CML) is characterized by the presence of the t(9:22) *BCR::ABL1* abnormality, resulting in formation of a fusion *BCR::ABL1* messenger RNA (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 (TKI), which can induce durable hematologic and (in most patients) cytogenetic remissions. Unfortunately, a significant subset of patients can develop functional resistance to TKI, 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 TKI 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 TKI 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 polymerase chain reaction (PCR) products, following reverse transcription PCR (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) 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 messenger RNA 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* kinase domain (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 messenger RNA (mRNA) template is decreased with a low abundance of target. If the *BCR::ABL1* quantitative polymerase chain reaction (PCR) level is too low, reverse transcription-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 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 due to 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

1. Hughes T, Deininger M, Hochhaus A, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28-37. doi:10.1182/blood-2006-01-0092
2. Press RD, Kamel-Reid S, Ang D. BCR-ABL1 RT-qPCR for Monitoring the Molecular Response to Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia. J Mol Diagn. 2013;15(5):565-576. doi:10.1016/j.jmoldx.2013.04.007
3. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic

myeloid leukemia: 2013. Blood. 2013;122(6):872-884. doi:10.1182/blood-2013-05-501569
4. Jones D, Kamel-Reid S, Bahler D, et al. Laboratory practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. J Mol Diagn. 2009;11(1):4-11. doi: 10.2353/jmoldx.2009.080095
5. Iezza M, Cortesi S, Ottaviani E, et al. Prognosis in chronic myeloid leukemia: Baseline factors, dynamic risk assessment and novel insights. Cells. 2023;12(13):1703. doi:10.3390/cells12131703

Performance

Method Description

Total RNA is extracted from the sample using an extraction kit. Complementary DNA is transcribed, and polymerase chain reaction (PCR) 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) Performed

Monday through Saturday

Report Available

5 to 7 days

Specimen Retention Time

Blood, bone marrow: 2 weeks; Extracted 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

Test Definition: BAKDM

BCR/ABL1, Tyrosine Kinase Inhibitor Resistance, Kinase Domain Mutation Screen, Sanger Sequencing, Varies

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

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

Test ID	Test Order Name	Order LOINC® Value
BAKDM	BCR/ABL1 Mutation, Sequencing	55135-8

Result ID	Test Result Name	Result LOINC® Value
MP004	Specimen Type	31208-2
MOFF	BCRABL Fusion (210, 190, 205, 230)	55135-8
19824	Final Diagnosis:	34574-4