



CPAL

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BCR/ABL t(9;22) Translocation by FISH

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Test Name (LabNexus order code): BCR/ABL FISH (BCRFISH)

Specimen:

Peripheral blood or bone marrow with EDTA (preferred) or sodium heparin as anticoagulant. Minimum volume for both specimen types is 1 mL.
Specimens should be transported and stored at 2-8 degrees C.
The order for this test should be placed in the LabNexus ordering portal.

Intended Use:

To detect the t(9;22) translocation, also known as the Philadelphia chromosome, in interphase nuclei of whole blood and bone marrow. Greater than 90% of patients with chronic myelogenous leukemia (CML) demonstrate these translocations. In CML cases lacking a karyotypically detectable translocation, the BCR/ABL1 fusion can still almost always be detected by FISH methodology. BCR/ABL1 fusions also occur in a portion of acute lymphocytic leukemia cases and more rarely in acute myeloid leukemia and are often associated with less favorable outcomes in these cases.

Testing Schedule:

Typically set up Mondays and Wednesdays, with cases posted in SoloWeb on Tuesdays and Thursdays. The testing schedule may be adjusted week to week to

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maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 2-4 days from receipt of testing order at CPAL.

Method:

Fluorescent In Situ Hybridization (FISH).

This test utilizes the Vysis BCR/ABL1/ASS1 Tri-color DF Translocation FISH Probes (Abbott Molecular). In about 15 to 20 percent of CML cases, the t(9;22) results in the loss of genetic material flanking the BCR and/or ABL1 breakpoints on the derivative 9 chromosome. This loss can prevent the production of the highly specific two-fusion signal patterns expected of dual fusion probes and balanced translocations. If both BCR and ABL1 targets are deleted on the der(9) chromosome, low-level random overlap of orange and green signals within normal cells (producing a 1 orange, 1 green, 1 fusion pattern) cannot be discriminated from low-level true BCR/ABL1 fusions producing the same pattern. The Tri-Color design of this test uses a probe in a third color (aqua) on the centromeric side of the ABL1 breakpoint, which co-localizes with the orange signal in a random orange/green signal fusion, but is absent from a true BCR/ABL1 molecular fusion on the der(22) chromosome. The probes have been used in published papers to detect low levels of positive cells in CML patients who were undergoing therapy and had deletions of FISH signals on the derivative chromosome 9. Automated signal counting on the BioView Duet Image Analysis System is combined with manual confirmation and selection of target nuclei for ultimate scoring/interpretation by the ordering pathologist.

Reference Ranges/Interpretation:

The sample is considered positive for the BCR/ABL translocation if the percentages of defined abnormal signal patterns exceed the laboratory-established cutoff for that pattern (see validation data for listing of specific patterns/cutoffs).

The sample is considered negative for the BCR/ABL translocation if percentages of defined abnormal signal patterns fall below the cutoff value.

Clinical Background:

A proliferation of cells with a t(9;22)(q34;q11.2) occurs in the bone marrow and peripheral blood of more than 90% of patients with chronic myeloid leukemia (CML); in approximately 6% of children and 17% of adults with acute lymphoblastic leukemia (ALL); and approximately 1% of patients with acute myeloid leukemia (AML) with immature granulocytes. The abnormal chromosome 22 derived from this translocation is called the Philadelphia (Ph) chromosome.

The remaining 10% of patients with CML have a variant Ph chromosome. The classic Ph and all variants result in fusion of part of the Abelson (*ABL1*) oncogene from 9q34 with the breakpoint cluster region (*BCR*) at 22q11.2.

The molecular consequence of this translocation is a chimeric gene that produces a protein with enhanced tyrosine kinase activity. This constitutively activated enzyme activity is a major factor in the pathophysiology of CML. The drug imatinib (Gleevec) works by blocking this TK activity.

Limitations of Procedure:

This FISH assay does not rule out other chromosomal abnormalities.

FISH assay results may not be informative if the specimen quality is inadequate.

References:

Vysis LSI DNA Probes Package Insert, February 2007, 30-608314/RJ, Abbott Molecular Inc., Des Plaines, IL <https://www.abbottmolecular.com/products/oncology/fish/vysis-lsi-bcr-abl-9q34-tricolor-dual-fusion-translocation-probe.html>

Dewald GW: Interphase FISH studies for chronic myeloid leukemia. In *Methods in Molecular Biology*. Vol 204. *Molecular Cytogenetics: Protocols and Applications*. Edited by YS Fan. Totowa, NJ, Humana Press, USA, 2002 pp 311-342.

Frater JL, Tallman MS, Variakojis D, Druker BJ, Resta D, Riley MB, Hrisinko MA and Peterson LC.: Chronic Myeloid Leukemia Following therapy with Imatinib Mesylate (Gleevec); (2003). *Am. J. Clin. Pathol.*, **119**, 833–841.