Molecular Genetic Testing For **BCR-ABL1** Fusion Gene

**Background:**
The Philadelphia chromosome (Ph), a derivative chromosome 22 resulting from a translocation between chromosomes 9 and 22 causing the **BCR-ABL1** fusion, is present in approximately 95% of chronic myeloid leukemia (CML) and 25-30% of adult acute lymphoblastic leukemia (ALL) cases. This fusion is also seen in 2-4% of children with ALL. In general, the ratio of the fusion to a reference control can be used for diagnosis, prognosis, and ongoing therapeutic monitoring.

The **BCR-ABL1** rearrangement results in the generation of fusion protein with constitutive tyrosine kinase activity. Based on the specific breakpoints of the rearrangement, several different isoforms of the **BCR-ABL1** fusion protein can be generated, which correlate with different leukemic phenotypes. According to recommendations from the National Comprehensive Cancer Network, “identification of specific recurrent genetic abnormalities is critical for disease evaluation, optimal risk stratification and treatment planning” (NCCN Guidelines Version 1, 2020, “Acute Lymphoblastic Leukemia”). In approximately 95% of CML cases, the major rearrangement is present and the p210 fusion protein is produced. In most children with Ph+ ALL, the minor rearrangement generates the p190 fusion protein. Adults with Ph+ ALL may have either the p190 or the p210 transcript. The p230 isoform is rare and commonly associated with the neutrophilic variant of CML.

For CML patients, associations have been established between **BCR-ABL1/ABL1** international scale (IS) values and assessment of treatment milestones and therapeutic efficacy. For the p210 fusion transcript, **BCR-ABL1/ABL1**: IS values indicating a major molecular response (MMR) correlate with excellent progression-free survival.

For Ph+ ALL patients, associations for p190 IS values have not been established. For the p190 fusion transcript, an increasing **BCR-ABL1/ABL1** ratio may indicate a poor initial response in Ph+ ALL patients.

**Available Tests from Cincinnati Children’s Diagnostic Laboratories:**

**BCR-ABL1 FISH cytogenetic testing** detects the presence of the 9;22 translocation and the **BCR-ABL1** fusion gene within somatic cells. This test may also identify an additional copy of Ph in cells or an atypical 9;22 translocation. FISH cytogenetic testing is indicated as an initial evaluation for patients suspected to have CML or ALL and then for monitoring disease status, however, it does not differentiate between the different isoforms (p190, p210, and p230).

**Qualitative **BCR-ABL1** testing** is indicated as an initial evaluation for patients known to have a positive FISH cytogenetic test for **BCR-ABL1**. The qualitative **BCR-ABL1** test can determine the specific type (isoform) of the Philadelphia chromosome present which is important for appropriate diagnosis and treatment. Our qualitative **BCR-ABL1** test detects the presence of the p190, p210 and p230 isoforms; however, the qualitative test does not measure the levels of the transcripts.

**Quantitative **BCR-ABL1** testing** is indicated for monitoring of disease for any patient positive for the p210 or p190 **BCR-ABL1** fusion gene by qualitative assay. Our laboratory uses a methodology that measures the level of the **BCR-ABL1** p210 or p190 fusion gene present relative to the level of ABL1 transcripts in the same sample. Comparing the ratio of disease fusion gene to the reference gene transcripts allows physicians to have a numerical measure of response to therapy and allows for a more sensitive monitoring for possible disease relapse.
### Diagnostic Algorithm:

**Testing Strategy:**

**Initial evaluation:**

**BCR-ABL1 FISH cytogenetic testing** is performed to detect the BCR-ABL1 fusion gene as well as to provide an estimate of the percentage of cells carrying the fusion gene. **Qualitative BCR-ABL1 testing** is then performed to determine which isoform is present. A baseline sample should then be sent for **quantitative BCR-ABL1 testing** for the isoform identified.

**Monitoring of disease status:**

If the patient has the p190 or p210 isoform of the BCR-ABL1 fusion gene, **quantitative BCR-ABL1 testing** should be used for continued monitoring of disease. An atypical 9;22 translocation can be monitored by **BCR-ABL1 FISH cytogenetic testing.**

**Testing Methodology:**

**BCR-ABL1 FISH cytogenetic testing:** Fluorescence in situ hybridization with probes for the 9;22 translocation.

**Qualitative BCR-ABL1 testing:** RNA isolation with reverse transcription nested PCR and gel detection of PCR products.

**Quantitative BCR-ABL1 testing:** RNA isolation with RQ-PCR reaction to precisely quantitate the level of control and fusion gene transcripts.

**Sensitivity and Accuracy:**

**BCR-ABL1 FISH cytogenetic testing:** For initial analysis, 250 nuclei are counted. For disease monitoring, with a previously abnormal result, up to 500 nuclei may be counted.

**Qualitative BCR-ABL1 testing:** One cell with fusion transcripts per 10,000 normal cells.
Quantitative **BCR-ABL1 (p210)** testing: Sensitivity studies within the laboratory have shown that 0.002%IS (MR4.7) can be detected. Because of variability of the RNA quantity within a specimen and assay variability, only changes of 0.5 log or greater should be considered significant.

Quantitative **BCR-ABL1 (p190)** testing: Sensitivity studies within the laboratory have shown that 5-10 fusion copies can be detected. Because of variability of the RNA quantity within a specimen and assay variability, only changes of 0.5 log or greater should be considered significant.

**Specimens:**

**BCR-ABL1 FISH cytogenetic testing:** 1-3 ml bone marrow OR peripheral blood in green (Na Hep) tube.

**Qualitative **BCR-ABL1 testing:**** 3 mls peripheral blood OR bone marrow in purple (EDTA) tube (Please note that samples must be received within 48 hours after collection).

**Quantitative **BCR-ABL1 testing:**** 5-10 mls peripheral blood or 3-5 mls bone marrow in purple (EDTA) tube (please note that samples must be received within 24 hours after collection).

**Turn-Around Time:**

- **BCR-ABL1 FISH cytogenetic testing:** 1 day.
- **Qualitative **BCR-ABL1 testing:**** 7 days.
- **Quantitative **BCR-ABL1 testing:**** 10 days.

**CPT Codes:**

- **BCR-ABL1 FISH cytogenetic testing:** 88271(x2), 88275 (add 88237 if cells need to be cultured)
- **Qualitative **BCR-ABL1 (p190, p210, & p230) testing:** 81206, 81207, 81208
- **Quantitative **BCR-ABL1 (p210) testing:** 81206
- **Quantitative **BCR-ABL1 (p190) testing:** 81207

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

**Results:**

Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for the clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

**Shipping Instructions:**

Please enclose test requisition with sample. **All information must be completed before sample can be processed.**

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

**Ship to:**

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

**References:**


