Background:
The **Philadelphia chromosome (Ph)**, a derivative chromosome 22 resulting from a translocation between chromosomes 9 and 22 causing the **BCR-ABL** 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. Chronic neutrophilic leukemia (CNL) is also associated with the Philadelphia chromosome and a specific **BCR-ABL** transcript. In general, the ratio of the fusion to a reference control can be used for diagnosis, prognosis, and ongoing therapeutic monitoring.

The **BCR-ABL** 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-ABL** 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 2.2012, “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. CNL is characterized by the p230 isoform and has a significantly different clinical course than CML or ALL, which further demonstrates the importance of distinguishing the different isoforms.

For CML patients, associations have been established between **BCR-ABL/ABL** 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-ABL/ABL** ratio may indicate a poor initial response in Ph+ ALL patients.

Available Tests from Cincinnati Children’s Diagnostic Laboratories:

- **BCR-ABL FISH cytogenetic testing** detects the presence of the 9;22 translocation and the **BCR-ABL** 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-ABL testing** is indicated as an initial evaluation for patients known to have a positive FISH cytogenetic test for **BCR-ABL**. The qualitative **BCR-ABL** test can determine the specific type (isoform) of the Philadelphia chromosome present which is important for appropriate diagnosis and treatment. Our qualitative **BCR-ABL** 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-ABL testing** is indicated for monitoring of disease for any patient positive for the p210 or p190 **BCR-ABL** fusion gene by qualitative assay. Our laboratory uses a methodology that measures the number of copies of the p210 or p190 **BCR-ABL** fusion gene present relative to the number of **ABL** gene transcripts in the same sample. Comparing the ratio of disease fusion gene copies to normal gene copies 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-ABL FISH cytogenetic testing* is performed to detect the *BCR-ABL* fusion gene as well as to provide an estimate of the percentage of cells carrying the fusion gene. *Qualitative BCR-ABL testing* is then performed to determine which isoform is present. A baseline sample should then be sent for *quantitative BCR-ABL testing* for the isoform identified.

**Monitoring of disease status:**

Disease can be monitored by *BCR-ABL FISH cytogenetic testing* until the assay can no longer detect the fusion gene, and then a sample for *quantitative BCR-ABL testing* should be sent for continued monitoring of disease, if the patient has the p190 or p210 isoform of the *BCR-ABL* fusion gene.

**Testing Methodology:**

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

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

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

**Sensitivity and Accuracy:**

*BCR-ABL 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-ABL testing*: One cell with fusion transcripts per 10,000 normal cells.
Quantitative BCR-ABL (p210) testing: Sensitivity studies within the laboratory have shown that a ratio as low as 0.02% 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. Therefore, results of 1% BCR-ABL1/ABL1 would be considered equivalent to any results between 3% and 0.3%.

Quantitative BCR-ABL (p190) testing: Sensitivity studies within the laboratory have shown that a ratio as low as 0.02% 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. Therefore, results of 1% BCR-ABL1/ABL1 would be considered equivalent to any results between 3% and 0.3%.

Specimens:

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

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

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


