Background:

The Philadelphia chromosome (Ph), a derivative chromosome 22 resulting from a translocation between chromosomes 9 and 22 causing the \textit{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 \textit{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 \textit{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 \textit{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 \textit{BCR-ABL/ABL} international scale (IS) values and assessment of treatment milestones and therapeutic efficacy. For the p210 fusion transcript, \textit{BCR-ABL1/ABL1: IS} values indicating a major molecular response (MMR) correlate with excellent progression-free survival. For patients who are suspected to have emerging drug resistance to tyrosine kinase inhibitors (TKIs) or who fail to achieve a MMR initially, evaluation of \textit{ABL1} kinase domain mutations may identify the cause of resistance and potentially guide treatment changes.

For Ph+ ALL patients, associations for p190 IS values have not been established. For the p190 fusion transcript, an increasing \textit{BCR-ABL/ABL} ratio may indicate a poor initial response in Ph+ ALL patients. Evaluation of \textit{ABL} kinase domain mutations in recurrent Ph+ ALL can help guide changes in therapy.

Available Tests from Cincinnati Children’s Diagnostic Laboratories:

\textbf{BCR-ABL FISH cytogenetic testing} detects the presence of the 9;22 translocation and the \textit{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).

\textbf{Qualitative BCR-ABL testing} is indicated as an initial evaluation for patients known to have a positive FISH cytogenetic test for \textit{BCR-ABL}. The qualitative \textit{BCR-ABL} test can determine the specific type (isoform) of the Philadelphia chromosome present which is important for appropriate diagnosis and treatment. Our qualitative \textit{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.

**p210 isoform International Scale** A major molecular response is considered to be a 3-log reduction in the ratio from baseline of diagnosis (for example, 100% BCR-ABL/ABL ratio must reduce to 0.1% BCR-ABL/ABL ratio or 10 copies of fusion gene compared to 10,000 copies of the ABL gene). To facilitate comparison of quantitative RT-PCR results for the p210 BCR-ABL fusion gene between laboratories and platforms, International Scale (IS) reference materials were established by the World Health Organization and results of this assay are reported on the IS. IS reference materials have not been established for the p190 BCR-ABL fusion gene.

**ABL1 kinase domain mutation analysis** is indicated for Ph+ patients who develop resistance to a first-line TKI inhibitor, such as imatinib (Gleevec®). A proportion of patients treated with imatinib relapse due to the development of imatinib-resistant CML clones from acquired mutations in the ABL1 kinase domain (KD) of the BCR-ABL1 gene. A patient with imatinib resistant CML may respond to a second-generation TKI such as dasatinib or nilotinib. This assay uses PCR followed by Sanger DNA sequencing to identify TKI-resistant mutations in the kinase domain of the BCR-ABL1 fusion protein.

**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.

Indications for **ABL1 kinase domain mutation analysis**:
- A CML patient who progresses to the accelerated phase or blast phase of CML while treated with imatinib (or other TKI).
- A CML patient with a hematologic relapse, a loss of complete cytogenetic response, or a 1 log or greater increase in the **BCR-ABL1** transcript levels while treated with imatinib (or other TKI).
- A patient in the chronic phase of CML who has a suboptimal initial response to imatinib (or other TKI) as defined as a failure to achieve a complete hematologic response at 3 months, a failure to achieve a minimal cytogenetic response at 6 months, or a failure to achieve a major cytogenetic response at 12 months.
- A CML patient with a previously detected **ABL1** kinase domain mutation. In this setting, testing can be performed after three months to determine the response to a change in therapy.

**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 quantify the amount of control and fusion gene transcripts.

**ABL1 kinase domain mutation analysis:**
Extraction of total cellular RNA and cDNA from peripheral blood or bone marrow samples using reverse transcriptase, followed by PCR amplification of **BCR-ABL1** fusion gene and targeted Sanger sequencing for TKI-resistant mutations.

**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%.

**ABL1 kinase domain mutation analysis:**
ABL1 kinase domain mutations are detectable if present at greater than 20% of **BCR-ABL1** transcript level. Sequencing detects about 80-90% of reported mutations in this region.

**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).

**ABL1 kinase domain sequence analysis:**
3-5 mls blood or bone marrow in purple (EDTA) tube (please send unprocessed samples to the laboratory within 24 hours of collection).
Turn-Around Times:

**BCR-ABL FISH cytogenetic testing:** 1 day.

**Qualitative BCR-ABL testing:** 7 days.

**Quantitative BCR-ABL testing:** 10 days.

**ABL1 kinase domain mutation analysis:** 10 days.

Costs:

Please call 1-866-450-4198 for pricing or with any billing questions.

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

**ABL1 kinase domain mutation analysis:** 81403

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:


