**BCR-ABL1 Quantitative Testing**

**Indications for Ordering**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Indications</th>
</tr>
</thead>
</table>
| Chronic myelogenous leukemia (CML) | • Diagnosis and ongoing therapeutic monitoring  
  • Useful for tyrosine kinase inhibitor (TKI) treatment management strategy |
| Acute lymphoblastic leukemia (ALL) | • Risk stratification and treatment planning  
  • Minimal residual disease (MRD) assessment of Philadelphia chromosome positive (Ph+) ALL |

**Test Description**

Reverse transcription polymerase chain reaction (RT-PCR)  
- Performed on peripheral blood or bone marrow (BM)  
- RNA is isolated and reverse transcribed to cDNA  
  - BCR-ABL1 fusions are quantitated by real-time PCR amplification  
  - Primers detect fusions with major (p210) or minor (p190) BCR-ABL1 breakpoints  
  - Test reference gene is ABL1  
  - Copy numbers of BCR-ABL1 fusion transcripts are expressed as ratio of BCR-ABL1/ABL1

**Tests to Consider**

**Typical testing strategy**

**CML**

- At diagnosis:  
  - BM cytogenetic studies and quantitative measurement of BCR-ABL1 transcript levels are recommended before treatment initiation

- Monitoring response to TKI therapy in CML:  
  - Quantitative RT-PCR  
    - Every 3 months when treatment response is evident  
    - Every 3 months for 3 years, and every 3-6 months thereafter after complete cytogenetic response has been achieved  
    - More frequent monitoring may be required in individuals with rising BCR-ABL1 transcripts

**ALL**

- At diagnosis:  
  - Presence of recurrent genetic abnormalities should be evaluated using karyotyping and/or FISH assays

- MRD assessment:  
  - Flow cytometry and quantitative PCR testing (BM preferred)  
  - Upon completion of initial induction and additional time points, as required

**Primary tests**

- **BCR-ABL1, Qualitative with Reflex to BCR-ABL1 Quantitative 2005010**  
  - Recommended when submitting initial diagnostic specimen for CML or Ph+ ALL (no previous BCR-ABL1 testing)
  - If qualitative test is positive, the appropriate corresponding quantitative test is performed

- **BCR-ABL1, Major (p210), Quantitative 2005017**  
  - Aids in diagnosis and monitoring of individuals with CML or Ph+ ALL who have e13a2 or e14a2 transcripts (p210)

- **BCR-ABL1, Minor (p190), Quantitative 2005016**  
  - Aids in diagnosis and monitoring of individuals with Ph+ ALL who have e1a2 transcripts (p190)

**Related tests**

- **BCR-ABL1 Mutation Analysis for Tyrosine Kinase Inhibitor Resistance by Next Generation Sequencing 2008420**  
  - Order only for patients with an established diagnosis of a BCR-ABL1 positive leukemia  
  - Use to determine if a mutation is present that would interfere with response to TKI therapy in Ph+ ALL or CML  
  - Detects all common mutations, including T315I  
  - Higher sensitivity than traditional Sanger sequencing techniques  
  - Offers coverage of SH2, SH3, and kinase domains

- **Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117**  
  - Assesses for gene mutations, including substitutions and insertions and deletions that may have diagnostic, prognostic, and/or therapeutic significance

- **Acute Lymphocytic Leukemia (ALL) Panel by FISH, Pediatric 2002719**  
  - Recommended FISH panel for children with newly diagnosed ALL
Acute Lymphocytic Leukemia (ALL) Panel by FISH, Adult

2002647

- Recommended FISH panel for adults with newly diagnosed ALL

Chromosome FISH, Interphase 2002298

- Specific FISH probe for t(9;22); BCR-ABL1 must be requested

Disease Overview

CML

Treatment issues – goal of TKI therapy is to achieve a complete cytogenetic response within 12 months of initiation of therapy with goal of eventual major molecular response

- A subset of individuals will eventually achieve a complete molecular response (undetectable BCR-ABL1 transcripts using a test with 4.5 log sensitivity)

Prognostic issues – a 3-log decrease in the level of BCR-ABL1 fusion transcripts (major molecular response) within 18 months of beginning TKI therapy is an indicator of favorable outcome

- Monitoring for recurrence using quantitative measures is crucial for detecting early relapse

ALL

Incidence – 1.6/100,000

- 80% of childhood acute leukemias
  - 2-4% are Ph+
- 20% of adult leukemias
  - 25% are Ph+

Genetics

Gene – BCR-ABL1

Mutations

- Four regions
  - Adenosine triphosphate binding-loop (P-loop) mutations
  - Drug-binding sites
  - Catalytic domain
  - Activation loop
- >130 mutations

Test Interpretation

BCR-ABL1, Major (p210), Quantitative

Analytical sensitivity – 1:125,000 normal cells

Results

- Positive – BCR-ABL1 fusion transcripts (p210) detected
  - BCR-ABL1/ABL1 quantitative ratio is provided (normalized copy number)
  - Results also reported in terms of BCR-ABL1 international scale (IS)
- Weakly positive – BCR-ABL1 fusion transcripts detected below the limit of quantitation
  - BCR-ABL1 to ABL1 ratio cannot be calculated
  - IS result <0.0069%
- Not detected – no BCR-ABL1 fusion transcripts detected
  - Does not exclude BCR-ABL1 fusion transcripts (p210) below the test limit of detection
  - Does not exclude BCR-ABL1 fusion transcripts not detected by this test (p190 or p230)

Limitations

Does not detect p190 or p230 form

BCR-ABL1, Minor (p190), Quantitative

Analytical sensitivity – 1:125,000 normal cells

Results

- Positive – BCR-ABL1 fusion transcripts (p190) detected
  - BCR-ABL1/ABL1 quantitative ratio is provided (normalized copy number)
- Weakly positive – BCR-ABL1 fusion transcripts detected, but below the limit of quantitation
  - BCR-ABL1 to ABL1 ratio cannot be calculated
- Not detected – no BCR-ABL1 fusion transcripts detected
  - Does not exclude BCR-ABL1 fusion transcripts (p190) below the test limit of detection
  - Does not exclude BCR-ABL1 fusion transcripts that are not detected by this test (p210 or p230)

Limitations

Does not detect p210 or p230