**BCR-ABL1** Mutation Analysis for Tyrosine Kinase Inhibitor Resistance

**Indications for Ordering**

Detect mutations that may impart tyrosine kinase inhibitor (TKI) resistance in either chronic myelogenous leukemia (CML) or Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL)

**Test Description**

Next generation sequencing (NGS)
- RNA extracted from whole blood or bone marrow aspirate
- PCR amplification of BCR-ABL1 SH2, SH3, and kinase domains
- Mutations identified by massively parallel sequencing

**Test to Consider**

*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 domain

**Disease Overview**

**Treatment issues**

CML
- CML is characterized by BCR-ABL1 translocations
- Initial treatment protocol is TKI therapy
  - Imatinib (Gleevec) – inhibits tyrosine kinase activity caused by the BCR-ABL1 gene fusion
  - Dasatinib (Sprycel) – dual specific SRC/ABL inhibitor
  - Nilotinib (Tasigna) – imatinib derivative with 30-fold potency compared to imatinib
- Resistance to TKI therapy may result from
  - Acquired point mutations within the ABL kinase domain
  - BCR-ABL1 amplification
  - Low bioavailability
  - Quiescent CML stem cells
- Resistance may be overcome with
  - Dose adjustments
  - Change in therapy

- Newer drugs may be useful when resistance develops
  - Bosutinib (Bosulif) – dual SRC/ABL inhibitor active in low nanomolar range
  - Ponatinib (Iclusig) – pan BCR-ABL1 inhibitor (includes T315I mutant inhibition)
- Use of NGS improves ability to detect low-level clones across larger sections of the gene

**ALL**

- BCR-ABL1 mutation is present in a subset of ALL patients
  - More common in adults than children
- Detection of mutations in BCR-ABL1 is important in helping to determine potential response to TKI therapy

**Genetics**

- **Gene fusion – BCR-ABL1**
  - **Mutations**
    - Four regions tested
      - Adenosine triphosphate binding-loop (P-loop) mutations
      - Drug-binding sites
      - Catalytic domain
      - Activation loop
    - >130 mutations currently identified

**Test Interpretation**

**Results**

- Detected – mutation detected in the SH2, SH3, or kinase domain (ABL1 amino acid residues 46-542)
- Not amplified – multiple attempts to amplify the BCR-ABL1 translocation were unsuccessful by PCR
- Not detected – no mutation detected

**Limitations**

- Negative result does not exclude mutations below the level of detection or outside the sequenced region
  - Sensitivity of this assay may be limited
  - Sequencing may not be possible in patient samples containing low tumor burden (ie, low levels of BCR-ABL1 fusion transcript by International Scale [IS]% or normalized copy number [NCN])
- This assay is not intended to be used for detection or quantification of BCR-ABL1 fusion transcripts
## Analytical Sensitivity

<table>
<thead>
<tr>
<th>Variant class</th>
<th>No. Variants Tested</th>
<th>Positive Percent Agreement (PPA)</th>
<th>PPA, 95% Tolerance at 95% Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nucleotide variant (SNV)</td>
<td>396</td>
<td>96.5%</td>
<td>94.3-98.0%</td>
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</tbody>
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## Related 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)
  - When 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 and a subset of individuals with 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)

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

**Chromosome FISH, Interphase 2002298**
- Use to order individual or multiple FISH probes when standard FISH panels are not desired
  - Specific FISH probe for t(9;22); BCR-ABL1 must be requested

**Acute Lymphocytic Leukemia (ALL) Panel by FISH, Adult 2002647**
- Recommended panel for adults with newly diagnosed ALL