Validation of BCR-ABL1 Test Performance from Whole Blood Stored up to 72 Hours Facilitates Operational Flexibility and Expanding Locally Managed CML Monitoring

Ion Beidorth, Keri Jefferson, Marie Fahey, Adam Ruskin, Bernard Andruss, and Justin T Brown
Asuragen, Inc., Austin, TX

Abstract

• MR values were obtained from CML patients ≥72 hours post-venipuncture that were indistinguishable across time points.
• ABL1 results were obtained from non-leukemic donors from 2 to 96 hours post-venipuncture that were consistent in the test across time points.
• QuantideX® qPCR BCR-ABL IS Kit facilitates testing decentralized specimens.

Introduction

Quantification of BCR-ABL1 fusion transcripts can be used to monitor disease activity. Preparing RNA for testing within as little time as possible after venipuncture has been reported as a critical pre-analytical phase. This prospective study involving testing and collection of whole blood samples has been published (Generson 2011). However, multiple publications recommend an ideal of 1 hour (Hughes 2011; Deininger 2011) as patient management has moved from specialty care facilities to more locally managed care, the amount of time between blood collection and RNA isolation has increased.

We describe the analytical validation of BCR-ABL1 monitoring results in a time course following collection of whole blood specimens.

Methods

Separate, consented CML residual clinical specimens were obtained under IRB-approved clinical protocols for all studies. Test time points were calculated in hours from time of blood draw. Time of receipt was used as the analytical baseline as it was not feasible to receive and extract blood in under 24 hours post-venipuncture. The test reports MR values and %IS, traceable to the WHO primary reference materials. The IS is most normally distributed after logarithmic transformation. MR values represent such a transformation. The table provides a summary of several MR values (MR = 2 – log10(%IS)) and their corresponding %IS values (%IS= 10^(2-MR)).

Results

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Post-venipuncture RNA isolation was performed on whole blood samples and samples were subjected to a reverse transcription reaction to yield cDNA for subsequent qPCR analysis. Total hands-on-time is estimated at 1 hour and total on board instrument time was established as ≤4 hours.

Whole blood in EDTA is obtained and a leukocyte-enriched RNA is prepared at 100-500 ng/μL for a total of 1000-5000 ng input. Total RNA is

Figure 1. Assay workflow and reportable values. A) BCR-ABL1 qPCR BCR-ABL IS Kit, this finding therefore facilitates testing specimens that take longer to ship, process, and arrive into RNA than was previously possible.

Table 1. Non-Leukemic Arm of Whole Blood Stability Study. Left Panel: Summary of MR values for plasma and whole blood specimens taken from 3 distinct non-leukemic donors.

<table>
<thead>
<tr>
<th>Hours from collection</th>
<th>MR</th>
<th>95%CI</th>
<th>Mean</th>
<th>Valid Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>332</td>
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<td>48</td>
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Each of the 5 test points was run in 9 replicates (n=135 possible measurements). Box plots are

Figure 4. Non-Leukemic Arm of Whole Blood Stability Study. Left Panel: Summary of %IS values for plasma and whole blood specimens taken from 3 distinct non-leukemic donors.

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Each of the 5 test points was run in 9 replicates (n=135 possible measurements). Box plots are

Figure 3. Leukemic Arm of Whole Blood Stability Study. Left Panel: Summary of MR values for plasma and whole blood specimens taken from 3 distinct leukemic donors.

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Each of the 5 test points was run in 9 replicates (n=135 possible measurements). Box plots are

Figure 2. Leukemic Arm of Whole Blood Stability Study. Left Panel: Summary of %IS values for plasma and whole blood specimens taken from 3 distinct leukemic donors.

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Each of the 5 test points was run in 9 replicates (n=135 possible measurements). Box plots are

Conclusions

• MR values from CML patients generated overlapping distributions across all time points ≥72 hours post-venipuncture, even beyond 96.
• ABL1 Ct values from 3 distinct non-leukemic donors were indistinguishable across time points from 2 to 96 hours post-venipuncture.
• In the context of the QuantideX® qPCR BCR-ABL IS Kit, this finding therefore facilitates testing specimens that take longer to ship, process, and arrive into RNA than was previously possible.

References


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