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

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

Summary

- 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.

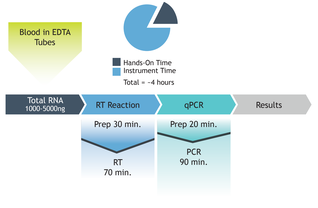
Introduction

Quantification of BCR-ABL1 Major fusion transcripts of t(9;22) assesses tumor burden in CML. Preparing RNA for testing within as little time as possible after venipuncture has been reported as a critical preanalytical step. No controlled studies for timing between collection and RNA isolation have been published (per Foroni 2011). However, multiple publications recommend an ideal of 24 hours (see Hughes 2006 and Soverini 2016). As patient management has moved from specialty care institutions to more locally managed care, the amount of time between blood collection and RNA processing 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 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-draw. Subsequent targets of 48, 60, and 72 hours were collected for the primary study. Three CML-negative samples were included in a supplemental study to allow for a baseline time point, tighter control of time points to 96 hours, and simultaneous processing in parallel. All measurements were generated using the QuantideX® qPCR BCR-ABL IS Kit (IVD). Briefly, Armored RNA Quant® (ARQ) technology was employed to generate a blend of nuclease-resistant BCR-ABL1 and ABL1 RNA transcripts to calibrate and control the system. A single four-point standard curve using ARQ blends mimics the WHO Primary BCR-ABL1 reference materials and accounts for the relative batch run-specific efficiency of the RT step. A lot-specific 2-ΔCt calculation provides ABL1 copies/qPCR for evaluation of endogenous control levels. Included software automatically analyzes raw SDS files for ratios and contains a logic algorithm that flags any specimen requiring further review. Studies herein were performed on the ABI 7500 Fast Dx for both RT and qPCR steps.

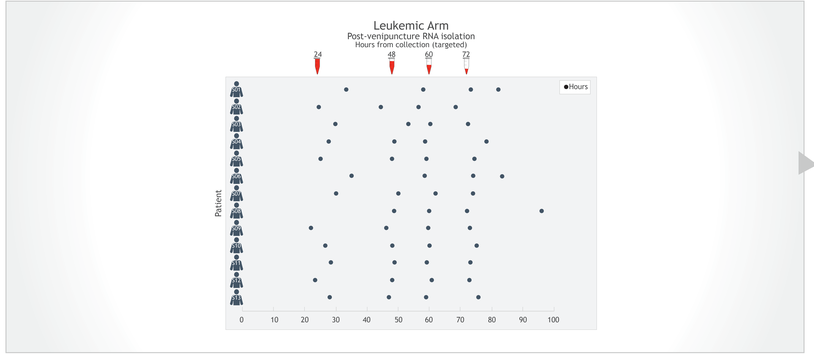
1A



1B

Response designation	MR	%IS
Baseline	0.0	100
	0.5	32
PCyR	1.0	10
	1.5	3.2
CCyR	2.0	1
	2.5	0.32
MMR	3.0	0.1
	3.5	0.032
	4.0	0.01
DMR	4.5	0.0032
	4.7	0.002
	5.0	0.001

Figure 1. Assay workflow and reportable values. A) 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 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. **B)** The numerical value of the WHO International Scale is %IS, the ratio, expressed as a percentage, of BCR-ABL1 expression to the expression of a control gene (ABL1 for the test). The MR value is the $\log_{10}$ reduction from the internationally standardized baseline (100%IS). 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 - $\log_{10}$(%IS)) and their corresponding %IS values (%IS= 10^(2-MR)) for reference.



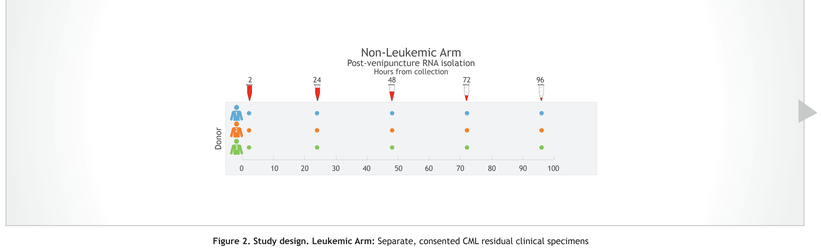


Figure 2. Study design. Leukemic Arm: Separate, consented CML residual clinical specimens were obtained under IRB-approved clinical protocols for all studies. Thirteen different patients were drawn independently from one another over a course of ~18 months. Collection occurred at the same time as the routine monitoring visits occurring for each patient. 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-draw. Subsequent targets of 48, 60, and 72 hours were collected for the primary study. Actual times were recorded. The variable nature of the collection and shipment times led to variability in the subsequent time points. **Non-Leukemic Arm:** Three CML-negative samples were included in a supplemental study to allow for a shorter baseline RNA isolation (2 hours), tighter control of time points to 96 hours, and parallel processing. These specimens were collected in one sitting from 3 non-leukemic, consented donors.

Results

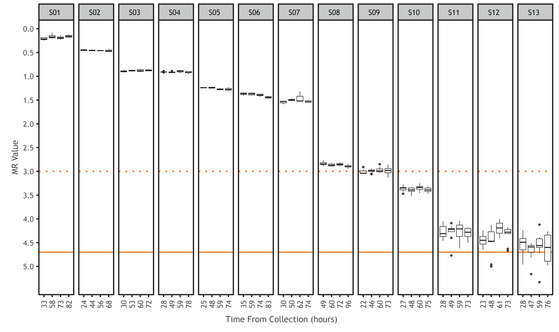
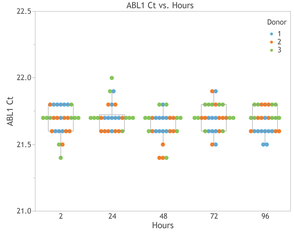


Figure 3. Leukemic Arm of Whole Blood Stability Study. Thirteen (13) primary CML specimens were collected for stability, each with 4 post-venipuncture RNA isolation time points. Actual time point of each specimen processing rounded to the nearest hour post-venipuncture is shown. Each RNA was tested a minimum of 3 times, and in some cases up to 9 times (depending on RNA yield) (n=332 valid measurements). MR values for each replicate are plotted according to the time they were processed. The median, upper, and lower quartiles are shown by the box and whisker plot per Tukey (whiskers extend to 1.5 times the upper and lower quantiles, 1st and 3rd, and open circles denote values measured outside this range). The dotted orange line is drawn at MR3 (equivalent to 0.1% and MMR). The y-axis represents measured MR (with highest BCR-ABL1 analyte level at the top), with a solid orange reference line drawn at MR4.7, the LOD of the test. The X-axis denotes the recorded time from collection for each patient sample. From a qualitative standpoint, 100% of replicates were detected as positive.



Time point (hours)	Mean ABL1 Ct	SD of Ct	Lower bound 95%CI	Upper bound 95%CI	Minimum Ct value	Maximum Ct value	Valid Observations
2	21.7	0.105	21.6	21.7	21.4	21.8	27
24	21.7	0.101	21.7	21.8	21.6	22.0	27
48	21.6	0.111	21.6	21.7	21.4	21.8	27
72	21.7	0.102	21.7	21.7	21.5	21.9	26
96	21.7	0.093	21.6	21.7	21.5	21.8	27
Pooled	21.7	0.104	21.7	21.7	21.4	22.0	134

Figure 4. Non-Leukemic Arm of Whole Blood Stability Study. Left Panel: The stability of whole blood RNA was determined for cellular ABL1 RNA by parallel processing aliquots of unique, non-leukemic human blood specimens at 2, 24, 48, 72, and 96 hours post-venipuncture. Each of the 5 test points was run in 9 replicates (n=135 possible measurements). Box plots are shown with an overlaid plot of individual data points shown with centered jitter. **Right Panel:** Estimates of imprecision of the ABL1 Ct values across post-venipuncture time points for the 3 CML-negative donors are shown. No trends were observed in the ABL1 Ct value or variability from 2 to 96 hours post-collection.

Conclusions

- MR values from CML patients generated overlapping distributions across all time points ≥72 hours post-venipuncture, even beyond MR4.
- 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, accession, and process into RNA than was previously possible.

*Presenting
QuantideX® qPCR BCR-ABL IS Kit (item 86003) is CE-marked IVD. QuantideX® qPCR BCR-ABL IS Kit (item 49574) is FDA-cleared IVD.
Conflict of Interest Disclosure. All authors have the financial relationship to disclose: Employment by Asuragen. This project has been funded in part with Federal funds from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN261201500009C.
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References
Foroni L, Wilson G, Gerrard G, et al. "Guidelines for the measurement of *BCR-ABL1* transcripts in chronic myeloid leukaemia." *British Journal of Haematology* 2011; 153:179.
Hughes T, Deininger M, Hochhaus A, et al. "Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: Review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results." *Blood* 2006;108:28.
Soverini S, De Benedittis C, Mancini M, Martinelli G. "Best Practices in Chronic Myeloid Leukemia Monitoring And Management." *The Oncologist* 2016; 21:626.

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