Evaluation of a Quantitative Multiplex BCR-ABL1 Assay Anchored Directly to the WHO Primary Standard Reference Materials to Report Expression on the International Scale

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SUMMARY

- The BCR-ABL1 assay showed sensitive, multiplex detection of e13a2, e14a2, and ABL1 on the ABI 7500 Fast Dx with direct reporting on the International Scale (IS).
- The assay demonstrated the preliminary performance required for quantitative measurement of BCR-ABL1 expression on the IS.
- The assay showed excellent correlation against results from an IS-aligned comparison method.

INTRODUCTION

Chronic Myeloid Leukemia is a genetic disorder that results when a translocation between chromosome 9 and chromosome 22 lead to an active fusion gene BCR-ABL1.The resulting BCR-ABL1 fusion gene is now active and proliferates in the body. The BCR-ABL1 fusion gene t(9:22) is a constitutively active tyrosine kinase, leading to uncontrolled proliferation of cells. Sensitive detection of BCR-ABL1 major breakpoint transcripts (e13a2 and e14a2, M-BCR) of t(9;22) (Philadelphia Chromosome) is important in human CML patients. The Quantidex™ CMR IS kit is a multiplex RT-qPCR method which allows for sensitive detection of BCR-ABL1 and ABL1, and is directly reportable on the International Scale (IS), also expressible as a molecular reduction value (MR, a logarithmic decrease from the common baseline of 100%IS or MR0). The kit contains all necessary reagents for both reverse transcription as well as subsequent qPCR reaction. The kit also is equipped with High Positive Control (CONH), Low Positive Control (CONL), and Negative Control (CONN). All standard curve materials and controls are composed of Armored RNA Quant technology, which allows for highly stable, nuclease-resistant RNA material. Newer generation of Tyrosine Kinase Inhibitors (TKIs) create deeper responses more quickly, necessitating molecular based assays that are detect even lower levels of BCR-ABL1 fusion transcripts. Molecular Reduction (MR) at deeper levels yields more durable remission. It is important to assess the need for a simple multiplex molecular based assay for these extreme low levels of fusion transcript. The need for assays designed to detect down to MR4.5 is crucial in giving patients the best care possible. The Quantidex™ BCR-ABL IS CMR Kit is designed to detect fusion transcript below MR4.5. The assay is designed to simultaneously detect BCR-ABL1 and ABL1 with high sensitivity and specificity.

AIM

We set out to evaluate a novel multiplexed RT-qPCR assay for the simultaneous amplification and detection of two Major BCR-ABL1 transcripts (e13a2 and e14a2) as well as ABL1 (as the endogenous control gene) developed by Asuragen, Inc. Total RNA was extracted from human leukocytes from blood collected in EDTA for evaluation of the assay. The assay was challenged with numerous samples through the full linear dynamic range of the assay. The assay is designed to meet performance requirements for simple ease of use, analytical sensitivity, and traceability to a higher order standard reference material. This study is intended to challenge the linear dynamic range of the assay, as well as precision, reproducibility, sensitivity, and specificity studies designed to verify claims of the assay.

METHODS

We tested whether this prototype BCR-ABL1 fusion quantification assay could be an alternative to current clinical diagnostic tools for monitoring CML patients for minimal residual disease. We compared patient test results obtained with the Asuragen assay to our current clinical laboratory developed test (LDT) to define correlation and concordance between the two methods. Clinical and cell line RNA samples were used to study additional performance characteristics of the assay, including analytical sensitivity (7-member human RNA panel over 3 days), specificity (cell line RNAs and in vitro transcripts), linearity (7-point human RNA dilution), precision (3-member human RNA panel over 5 days), and clinical accuracy (20 human RNA specimens over 2 runs). EDTA blood-based RNA specimens were evaluated for purity ($OD_{260/280}$ >1.6) and concentration (100-500 ng/uL). RT-qPCR was performed and analyzed on the ABI 7500 Fast Dx with SDS software version 1.4. Armored RNA Quant (ARQ) technology was provided in the kit as 7 different blends of BCR-ABL1 and ABL1 RNA transcripts to calibrate and control the system. A single four-point standard curve using ARQ blends mimicked the WHO Primary BCR-ABL1 reference materials. Excel templates were used to generate results reported in MR and %IS values.

RESULTS

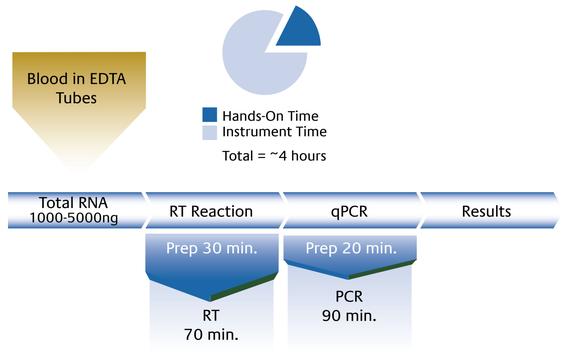

Figure 1. Assay workflow. The Quantidex™ BCR-ABL IS CMR Kit is designed to have a simple workflow. Whole blood in EDTA is obtained and a target total RNA of 1000-5000 ng is needed for accurate measurement of BCR-ABL1 and ABL1 targets. A concentration of 100-500 ng/µL is desired for the assay. Total RNA 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 is estimated at 3 hours.

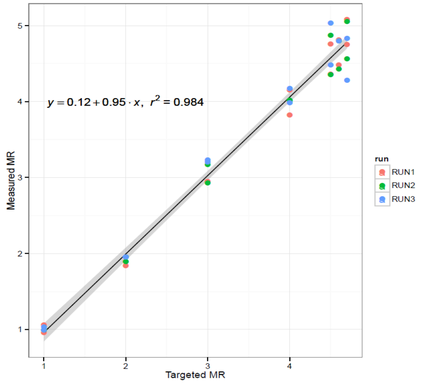

Figure 2. Linearity and Sensitivity study. Sample dilutions were prepared using known human RNA targeted between 100-500 ng/µL at measured MR values for linearity and sensitivity studies. MR values of MR1, MR2, MR3, MR4, MR4.5, MR4.6, and MR4.7 are shown. 7 samples were run over 3 days on the ABI 7500 Fast Dx and compared to targeted values. The assay shows good linearity throughout the dynamic range. Assay sensitivity shows good linearity even at levels above MR 4.5 (<0.032% IS).

Sample Name	ABL Ct	BA Ct
NOMO-1 (MLL-MLLt3)	24.5	Undetermined
NOMO-1 (MLL-MLLt3)	25.2	Undetermined
RCH-ACV (cALL)	22.9	Undetermined
RCH-ACV (cALL)	23.3	Undetermined
MV4-11 (AML FAB M5)	23.2	Undetermined
MV4-11 (AML FAB M5)	23.2	Undetermined
NB4 (APL)	23.1	Undetermined
NB4 (APL)	27.1	Undetermined
RS4;11 (ALL L2)	23.1	Undetermined
RS4;11 (ALL L2)	23.7	Undetermined
KASUMI-1 (AML FABM2/AMLI-ETO)	23.6	Undetermined
KASUMI-1 (AML FABM2/AMLI-ETO)	23.8	Undetermined
e1a2 (IVS-0032)	21.1	Undetermined
e1a2 (IVS-0032)	21.0	Undetermined

Figure 3. Specificity. Known non-M-BCR cell lines and *in vitro* transcript RNAs were prepared at targeted concentration of 100-500 ng/µL. Six (6) Philadelphia-negative samples as well as 1 minor BCR-ABL1 breakpoint (e1a2) were run using the Quantidex BCR-ABL IS CMR Kit™. All samples were undetected, indicating good analytical specificity. Targets showed sufficient amounts of the control gene ABL1.

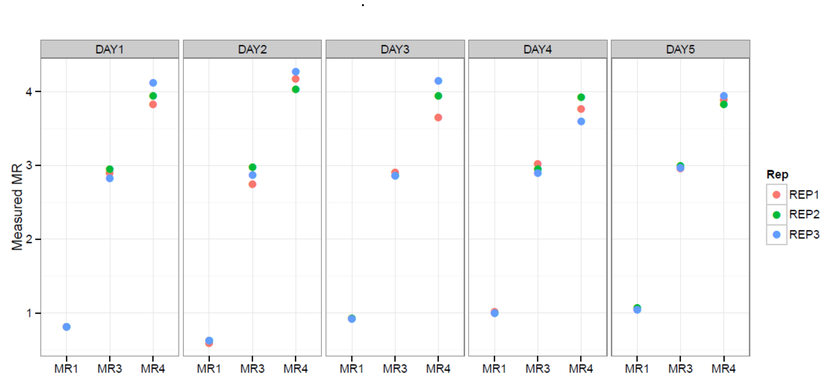

Figure 4. Precision study. 3-member targeted Human RNA panel was used to assess precision of the assay. 3 known human RNA samples were run in triplicate over 5 days to test for precision at targeted MR values. Measured MR values show good precision at targeted MR1, MR3, and MR4 with SD values of 0.16, 0.07, and 0.19 respectively.

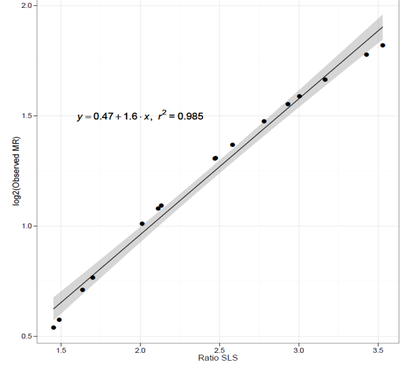

Figure 5. Accuracy using method comparison. Asuragen's assay showed good intra- and inter-laboratory correlation, with a Pearson R correlation coefficient of 0.992. Moreover, using assay comparison criteria proposed by Müller et al. (Leukemia 2009), the Asuragen assay was considered comparable to our current laboratory developed test, suggesting good clinical accuracy (when considering the prior result in the LDT as truth).

CONCLUSIONS

The Quantidex™ BCR-ABL IS CMR Kit quantifies BCR-ABL1 major fusion transcripts and ABL1 in a single reaction using a streamlined RT-qPCR workflow (**Figure 1**) and shows excellent correlation with the comparator method. All samples can be directly reported on the IS as the standard curves are directly traceable to the WHO Primary Reference materials. In this study, 100% of samples tested were positive at MR4.7. The high sensitivity of the assay and its ability to report directly on the IS enables labs to have an assay that detects deep molecular response in keeping pace with advances in TKI therapy.