INTRODUCTION

Chronic Myeloid Leukemia is a genetic disorder that results when a translocation between chromosomes 9 and 22 leads to an active fusion gene BCR-ABL. The resulting BCR-ABL fusion gene is now active and proliferates in the body. The BCR-ABL 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) and ABL1 on the ABI 7500 Fast Dx with direct reporting on the International Scale (IS).

RESULTS

Figure 1. Assay workflow. The Quantidex™ BCR-ABL IS CMR Kit is designed to have a simple workflow.

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).

Figure 3. Specificity. Known non-ABL cell line 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 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.