BCR-ABL1 MONITORING ON THE IS USING AN ANALYTICALLY AND CLINICALLY VALIDATED MULTIPLEX ASSAY DIRECTLY ALIGNED TO THE WHO PRIMARY STANDARDS

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SUMMARY

- The QuantideX® qPCR BCR-ABL IS Kit showed sensitive, multiplex detection of e1a2, e1a2z, and ABL1, and on the ABI 7500 Fast Dx with direct reporting on the International Scale (IS) and as Molecular Reduction (MR) Values.
- Limits LOD was "Unidentified" LOO and LOQ were both MR4 (0.00125%IS).
- Linearity was observed at least MR3 (0.035%IS) to MR4.7 (0.0015%IS).
- Multi-site precision (reproducibility) was verified as SD ≤0.1 for MR values ≤3.7.
- Whole blood specimens were stable at 2-8C for 72 hours without degradation of Test performance.
- A multi-center clinical outcome study confirmed the statistically significant difference in EFS at 32-40 months stratified by achievement of MR4 by using the Test run 12-18 months after start of TKI.

INTRODUCTION

Chronic Myeloid Leukemia is a disorder that results when a translocation between chromosomes 9 and chromosome 22 lead to an active fusion protein BCR-ABL. Detection of BCR-ABL in e1a2 or e1a2z fusion transcripts (major breakpoint, M-BCR) of 0.22 is important in studying tumor burden in CML. To facilitate this, the International Scale (IS) was established to standardize the reporting of these transscripts relative to a common baseline. As newer TKI therapies create deeper responses with lower numbers of circulating leukemic cells, analytical sensitivity has become increasingly important. The QuantideX® qPCR BCR-ABL IS Kit improves workflow with its streamlined reagent formulation and multiplex assay format, facilitates assessment on the IS without conversion to target values of BCR-ABL1 and ABL1 RNA transcripts to calibrate and control the WHO Primary BCR-ABL reference materials without requiring establishment and revalidation of a conversion factor.

METHODS

We developed reagents for the QuantideX® qPCR BCR-ABL IS Kit, both steps performed on the ABI 7500 Fast Dx. Armored QuantiGARD® (AQR) 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 ARQs mimics the WHO Primary BCR-ABL reference materials and allows for the relative batch run-specific efficiency of the RT step. cDNA generation and qPCR were optimized, including allowance of high mass of nucleic acid input. Software was developed, including a floating, traceable logic algorithm to ensure that sufficient ABL1 was detected to protect this LOD. A multi-center clinical outcome study was conducted at 3 clinical laboratories to validate clinical monitoring.

RESULTS

- The QaurtideX® qPCR BCR-ABL IS Kit was tested against 3 separate human RNA preparations that were positive for BCR-ABL IS with high mass of nucleic acid input to demonstrate the robustness of the assay.
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CONCLUSIONS

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