SUMMARY

- A multi-center clinical outcome study confirmed the statistically significant difference.
- RNA mass input was validated from 1000-5000 ng/RT (MR1.0-MR4.7).
- Linearity was observed from at least MR0.3 (50%IS) to MR4.7 (0.002%IS).
- Limits: LOB was “Undetected”. LOD and LOQ were both MR4.7 (0.002%IS).

INTERRODUCTION

Chronic Myeloid Leukemia is a disorder that results when between chromosome 9 and chromosome 22 lead to an active fusion gene BCR-ABL1. Detection of BCR-ABL1 at e13a2 or e14a2 fusion transscripts (major breakpoint, M-BCR) is important in studying tumor burden in CML. To facilitate this, the International Scale (IS) was established to standardize the reporting of these transcripts relative to a common panel of reference materials. This technical note describes the experiments in which the numbers of circulating leukemic cells, analytical sensitivity has become a critical topic in investigations into TKI relative to a common baseline. As newer TKI therapies create deeper responses with lower numbers. Detection of BCR-ABL1 in circulating leukemic cells, analytical sensitivity has become a critical topic in investigations into TKI relative to a common baseline. As newer TKI therapies create deeper responses with lower numbers.

METHODS

We developed reagents for the QuantDX qPCR BCR-ABL IS Kit, both steps performed on the ABI 7500 Fast Dx. Armored RNA Quant (ARQ) technology was employed to generate a blend of nucleic-acid resistant BCR-ABL and ABL RNA transcrpts to control and system. A single four-point standard curve using UQC blends the WHO Primary BCR-ABL1 reference materials and accounts 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 without inhibition. Software, developed including a floating, traceable log algorithm to ensure that sufficient ABL1 was detected to protect this LOD. This has led to various reporting formats and accounts 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 without inhibition. Software, developed including a floating, traceable log 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 QuantDX qPCR BCR-ABL IS Kit showed sensitive, multiplex detection of e13a2, e14a2, and ABL1 on the ABI 7500 Fast Dx with direct reporting on the International Scale (IS) and as Molecular Reduction (MR) values.
- Limits: LOB was “Undetected” and LOQ were both MR4.7 (0.002%IS).
- Linearity was observed from at least MR0.3 (50%IS) to MR4.7 (0.002%IS).
- Multi-site precision (reproducibility) was verified as a maximum SD of 0.9 at MR3.7 (0.002%IS).
- RNA mass input was validated from 1000-5000 ng/RT (MR1.0-MR4.7).

CONCLUSIONS

- The QuantDX qPCR BCR-ABL IS Kit improves workflow with its streamlined reagent formulation and multiplex assay format, facilitates assessment on the IS without conversion to a linear scale and TKI materials traceable to the WHO Primary (IS) reference materials, report results on a continuous scale (as both MR and %IS values), and has sensitivity sufficient to assess deep molecular responses.
- It has also been clinically validated for stratification by MR3 in a multi-center clinical outcome study.

BCR-ABL1 MONITORING ON THE IS USING A CLINICALLY AND ANALYTICALLY VALIDATED MULTIPLEX ASSAY DIRECTLY ALIGNED TO THE WHO PRIMARY REFERENCES THAT UNIFIES REPORTING FORMATS

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INTRODUCTION

Chronic Myeloid Leukemia is a disorder that results when between chromosome 9 and chromosome 22 lead to an active fusion gene BCR-ABL1. Detection of BCR-ABL1 at e13a2 or e14a2 fusion transscripts (major breakpoint, M-BCR) is important in studying tumor burden in CML. To facilitate this, the International Scale (IS) was established to standardize the reporting of these transcripts relative to a common panel of reference materials. This technical note describes the experiments in which the numbers of circulating leukemic cells, analytical sensitivity has become a critical topic in investigations into TKI relative to a common baseline. As newer TKI therapies create deeper responses with lower numbers.

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