RT-qPCR and RT-Digital PCR: a comparison of different platforms for the evaluation of residual disease in chronic myeloid leukaemia

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Introduction

Molecular monitoring of minimal residual disease (MRD) using Reverse Transcription quantitative real-time PCR (RT-qPCR) is an essential tool in the management of CML patients in order to measure BCR-ABL1 transcript levels during tyrosine kinase inhibitor (TKI) therapy with tyrosine kinase inhibitors as adjuvant or let response to TKI treatment.

While expressing the BCR-ABL1 transcript levels as a percentage of the total number of ABL1 transcripts on the International Scale has improved inter-laboratory comparisons, it requires regular and cumbersome standardization. Digital PCR (dPCR) is an alternative method that could enable simple, standardized quantification of nucleic acids, primarily due to its non-relevance on calibration curves, that could improve accuracy when measuring RNA transcripts. dPCR was used to quantify the BCR-ABL1 reference material that can be used either for the calibration of secondary ‘hybrid’ control materials or to precisely calibrate BCR-ABL1 copy numbers. dPCR can also be used directly for the absolute quantification of the BCR-ABL1 copy numbers.

Monitoring Minimal Residual Disease after TKI therapy

In this study, we compared three different dPCR platforms and investigated whether they could be applied to a clinical setting to quantify BCR-ABL1 transcripts in CML patients.

Methods

BCR-ABL1 and ABL1 transcript copy numbers were quantified in a total of 320 samples; 70 CML patients undergoing TKI therapy and 22 normal individuals. Three commercially available RT-qPCR platforms (Q3D, Q200 and RainDrop) were compared with the routinely used RT-qPCR platform using a modified version of the Europe Against Cancer [EAC] assay.

Experimental design

Target = BCR-ABL1 and ABL1
Template = cDNA
Platform = Q3D vs RainDrop vs BioRad
Assay = MGB modified E.A.C Duplex Assay

Results

ABL1 transcript copy numbers measured on all three RT-qPCR platforms showed good correlation with RT-qPCR across all sample groups (R² > 0.95, 0.93 and 0.85 for Q3D, Q200, Raindrop, respectively; Figure 3).

RT-qPCR quantification of the ABL1-2 ABL1-3 and ABL1-4 transcript copy numbers correlated well with RT-qPCR across all three platforms only down to an ID 100 of ≥ 2.85, 3.9 and 3.5 for Q3D, Q200, Raindrop, respectively, (Figure 3). The reason was the presence of false positive background noise.

none of the platforms were able to substantially improve the sensitivity of quantification by RT-qPCR in patient samples with ≥BCR-ABL1 level ≤0.0005 (Figure 3).

Figure 3. Comparison of RT-qPCR with RT-qPCR for the quantification of ABL1-2, ABL1-3 and ABL1-4 transcript copy numbers in clinical samples. Scatter plots showing the linear relationship between the quantification of (a) ABL1-2, (b) ABL1-3 and (c) ABL1-4. Furthermore, the red line and the green line represent the detection limits of the RT-qPCR and the WHO qPCR platform, respectively. Each data point represents the mean value derived from either triplicate or nine replication reactions. Correlation coefficients are as follows: (a) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR); (b) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR); (c) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR); (d) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR); (e) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR); (f) 0.9962; 0.9981; 0.9981; 0.9981 (Q3D, Q200, Raindrop, WHO-qPCR).

Conclusions

1. RT-qPCR was able to quantify low level BCR-ABL1 transcript copies but was unable to improve sensitivity below the level of detection achieved by RT-qPCR.
2. RT-qPCR was able to perform sensitive measurements without calibration curve.
3. The E.A.C. assay has a considerable false positive rate on all qPCR platforms which affects the limit of detection.
4. Adaptations to the protocol to increase the amount of RNA measured, a redesign of the assay used and an alternative reference gene to ABL1 are likely to be necessary to improve the analytical sensitivity of BCR-ABL1 testing.

Advantages of using the Raindrop platform

- S-10 million partitions in 25-50s reaction volumes allowing a large dynamic range
- Largest sample volume (30-20ll) per reaction
- High level BCR1 quantification without compromising the low-level BCR-ABL1, i.e. one in a million sensitivity is reached in one reaction
- Minimal hands-on time.
- Sealed tube reaction.

Points to consider

Several factors need to be considered when applying RT-qPCR to MRD in CML. These are:

1. Analytical sensitivity (expressed as the LoD of an analyte) vs. clinical sensitivity (defined as the ability of the test to detect a log reduction of the ratio between BCR-ABL1 and ABL1 expressed on the international scale).
2. Assay standardisation where positive and negative controls are essential to assess false-positive and false-negative rates and to define quantification amplitude thresholds.
3. Multiplexing the high reference background and low target copy numbers in one reaction without compromising the sensitivity of the assay.
4. Quantification accuracy and precision.
5. Reporting of target gene copy number quantities by RT-qPCR. We recommend RT-qPCR results be expressed per volume of qDNA included per reaction.
6. Sampling error in cases with rare transcript concentration. When the target concentration is very rare, increasing assay sensitivity doesn’t improve the detection rate, but increased sampling (sample volume) does.

References