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

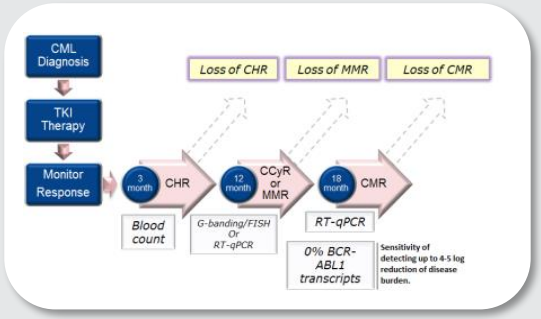

Figure 1. Current strategy for monitoring CML response to TKI therapy.

Monitoring Minimal Residual Disease after TKI therapy

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 lifelong therapy with tyrosine kinase inhibitors as suboptimal or loss of response can be an indicator of treatment failure.
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 adaption of the qPCR method that could enable simple, standardised quantification of nucleic acids, primarily due to its non-reliance on calibration curves, that could improve accuracy when measuring RNA transcripts. dPCR was used to quantify the ERM®-AD623 reference material that can be used either for the calibration of secondary 'in-house' control materials or to traceably calibrate *BCR-ABL1* copy numbers. dPCR can also be used directly for the absolute quantification of the *BCR-ABL1* copy numbers

Aim
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 102 samples; 70 CML patients undergoing TKI therapy and 32 non-CML individuals. Three commercially available RT-dPCR platforms (QS3D, QX200 and RainDrop) were compared with the routinely used RT-qPCR platform using a modified version of the Europe Against Cancer (E.A.C.) assay.

Experiential design

Target = BCR-ABL1 and ABL1

Template = cDNA

Platform = QS3D vs RainDrop vs BioRad

Assay = MGB modified E.A.C Duplex Assay

Range:	Ratio on IS	BCR-ABL1 copy/Rxn	# of samples	# of Rxns	# of Rxns
	20%	2000-8000	10	3	1
	10%	200-2000	10	3	1
	1%	20-200	10	3	1
	0.1%	5-50	10	3	1
	0.01%	5-15	10	9	1
	0.001%	1-5	10	9	1
	0%	0	10	9	1
	Neg. control	0	32	9	1

Figure 2. experimental design.

Results

ABL1 transcript copy numbers measured on all three RT-dPCR platforms showed good correlation with RT-qPCR across all sample groups (R2 = 0.91, 0.93 and 0.95 for QS3D, QX200, RainDrop, respectively; Figure 3).

RT-dPCR quantification of the *BCR-ABL1* transcript copy numbers correlated well with RT-qPCR across all three platforms only down to ≥0.1% (R2 = 0.85, 0.94 and 0.92 for QS3D, QX200, RainDrop, respectively; Figure 3). The reason was the prance 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*[5] level <0.001% (Figure 3).

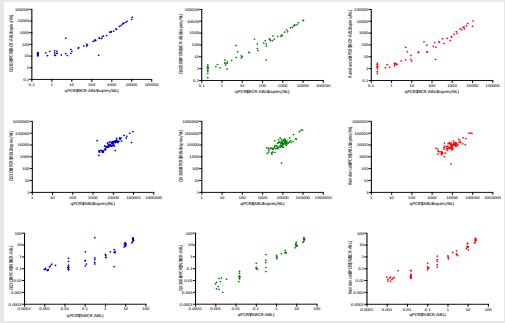

Figure 3. Comparison of RT-qPCR with RT-dPCR for the quantification of *BCR-ABL1* and *ABL1* transcript copy numbers in clinical samples. Scatter plots demonstrating the linear relationship between the quantification of (A) *BCR-ABL1*, (B) *ABL1* and (C) %*BCR-ABL1*[6]. Quantification of the cDNA derived from clinical samples by RT-qPCR (x-axis) was compared with the QS3D (blue), QX200 (green) and RainDrop (red) platforms. Each data point represents the mean value derived from either triplicate or nine replicate reactions. Correlation coefficiencies were as follows: (R2 = 0.91, 0.93, 0.95 for QS3D, QX200, RainDrop, respectively) for *ABL1* transcript copy numbers; (R2 = 0.85, 0.94, 0.92 for QS3D, QX200, RainDrop, respectively) for *BCR-ABL1* transcript copy numbers; (R2 = 0.89, 0.92, 0.97 for QS3D, QX200, RainDrop, respectively) for %*BCR-ABL1*[5].

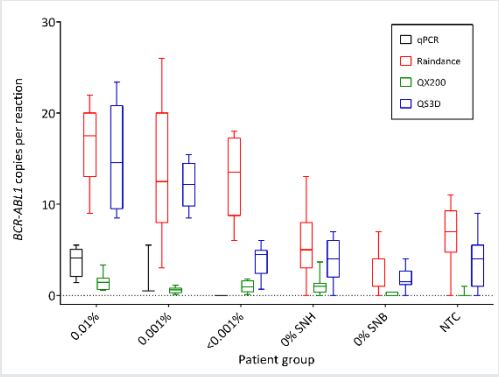

Figure 4. Comparison of the *BCR-ABL1* transcript copy numbers measured per reaction in low disease level patient (≤0.01%) and matched control samples. The three low disease level patients as measured by RT-qPCR (0.01%, 0.001% and <0.001%) are shown with the matched non-CML controls (SNH and SNB) that are nominally 0% *BCR-ABL1*[5]. The NTC reactions were generated with water in place of cDNA. The box plots show the median and interquartile range with the whiskers showing the min and max data points from nine replicates for each platform. The dashed horizontal line indicates zero *BCR-ABL1* transcript copies per reaction.

Points to consider

Several factors need to be considered when applying RT-dPCR 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 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 numbers quantified by RT-dPCR. We recommend RT-dPCR results be expressed per volume of cDNA 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.

Advantages of using the RainDrop platform

- 5-10 million partitions in 25-50ul reaction volumes allowing a large dynamic range
- Largest sample volume (10-28ul) per reaction
- High level *ABL1* 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.

Conclusions

1- RT-dPCR 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-dPCR was able to perform sensitive measurements without calibration curve.

3- The E.A.C. assay has a considerable false positive rate on all dPCR platforms which affects the limit of detection.

4- Adaptions 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.

References

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