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Assessment of droplet digital polymerase chain reaction for measuring *BCR-ABL1* in chronic myeloid leukaemia in an international interlaboratory study

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Summary

Measurement of BCR activator of RhoGEF and GTPase -ABL proto-oncogene 1, non-receptor tyrosine kinase (*BCR-ABL1*) mRNA levels by reverse transcription quantitative polymerase chain reaction (RTqPCR) has been critical to treatment protocols and clinical trials in chronic myeloid leukaemia; however, interlaboratory variation remains a significant issue. Reverse transcriptase droplet digital PCR (RTddPCR) has shown potential to improve testing but a large-scale interlaboratory study is required to definitively establish this. In the present study, 10 *BCR-ABL1*-positive samples with levels ranging from molecular response (MR)$^{1·0}$–MR$^{5·0}$ were tested by 23 laboratories using RTddPCR with the QXDX BCR-ABL %IS kit. A subset of participants tested the samples using RTqPCR. All 23 participants using RTddPCR detected *BCR-ABL1* in all samples to MR$^{4·0}$. Detection rates for deep-response samples were 95·7% at MR$^{4·5}$, 78·3% at MR$^{4·7}$ and 87·0% at MR$^{5·0}$. Interlaboratory coefficient of variation was indirectly proportional to *BCR-ABL1* level ranging from 29·3% to 69·0%. Linearity ranged from 0·9330 to 1·000 (average 0·9936). When results were compared for the 11 participants who performed both RTddPCR and RTqPCR, RTddPCR showed a similar limit of detection to RTqPCR with reduced interlaboratory variation and better assay linearity. The ability to detect deep responses with RTddPCR, matched with an improved linearity and reduced interlaboratory variation will allow improved patient management, and is of particular importance for future clinical trials focussed on achieving and maintaining treatment-free remission.

Keywords: *BCR-ABL1*, external quality assessment (EQA), Quality, CML, RTddPCR.

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Introduction

Sequential measurement of BCR activator of RhoGEF and GTPase-ABL proto-oncogene 1, non-receptor tyrosine kinase (*BCR-ABL1*) mRNA levels by reverse transcription quantitative polymerase chain reaction (RTqPCR) is now embedded in standard patient management protocols for chronic myeloid leukaemia (CML)[1] and is a validated surrogate outcome

for traditional clinical outcomes in trials of new therapies for patients with CML.[2] It has played a seminal role in the remarkable improvement in patient survival seen in this disease, where the majority of patients with chronic phase disease now have a normal life expectancy.[3]

This improvement in life expectancy has led to a change in emphasis for the clinicians and researchers treating CML, from extending patient survival to curing the disease. Current aims for treatment in CML are to achieve a faster molecular response (MR), preventing progression to blastic phase disease allowing a stable deep MR and tyrosine kinase inhibitor (TKI) treatment discontinuation for treatment-free remission (TFR).[4,5] With RTqPCR being used to monitor the speed of response, and define eligibility for TFR ever more accurate and precise measurement of *BCR-ABL1* is desirable, to ensure that these important treatment decisions are made on the best data available with optimal comparability between laboratories. Efforts to standardise the current 'gold standard' approach for *BCR-ABL1* measurement, RTqPCR,[6,7] including the development of the International Scale (IS),[8–10] has reduced inter- and intralaboratory variation in *BCR-ABL1* measurement;[11] however, the inherent complexity of RTqPCR means that inter- and intralaboratory variation remains a significant issue for laboratories,[8,11] that still needs to be addressed.

It has been speculated that reverse transcriptase droplet digital PCR (RTddPCR) may overcome some of the variability inherent to RTqPCR, particularly when measuring very low *BCR-ABL1* transcript levels. RTddPCR separates a bulk PCR reaction into thousands of droplet-based single molecule reactions, allowing a more accurate quantification of the number of target molecules. In contrast to RTqPCR, RTddPCR does not rely on the indirect relationship between the amplification of the *BCR-ABL1* transcript in patient samples and a calibration curve to quantify *BCR-ABL1* levels, a complex approach that is vulnerable to potential error. Instead, it is a direct measurement of the number of copies of the target molecule. Encouragingly, initial studies have displayed a lower limit of detection (LOD) and limit of quantification compared to RTqPCR.[12–15] The Life After Stopping TKIs (LAST) study showed that the superior LOD of RTddPCR over RTqPCR may allow for less frequent monitoring, resulting in less hospital visits for patients.[16] Large-scale, multicentre studies are required to assess if the benefits of RTddPCR shown in single-centre studies can be replicated in a multicentre study.

Methods

A total of 10 cell-line based *BCR-ABL1* positive samples (labelled A–J in a random order) with *BCR-ABL1* levels ranging from $MR^{1·0}$ (10% $BCR\text{-}ABL^{IS}$)–$MR^{5·0}$ (0·001% $BCR\text{-}ABL1^{IS}$) were manufactured from a mixture of *BCR-ABL1* (e14a2)-positive K562 cells in a background of *BCR-ABL1*-negative HL60 cells. K562 and HL60 cell lines were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). The HL60 and K562 cultures tested negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human T-lymphotrophic virus type I and II (HTLV-I/II), human herpes virus 8 (HHV-8) and mycoplasma by PCR. Cell lines were grown in RPMI 1640 (Thermo Fisher, Waltham, USA) medium supplemented with 10% fetal bovine serum (Thermo Fisher). Pre-defined dilutions of K562 cells in HL60 cells were then prepared and freeze dried in 3-ml glass ampoules to contain 9×10^6 cells. Samples were dried for 24 h. Prior to distribution, to establish that the manufactured samples accurately represented the target values, and to ensure sample quality and homogeneity, a minimum of three selected samples were subjected to (phenol chloroform) RNA extraction, complementary DNA (cDNA) synthesis and *BCR-ABL1* quantification. This was performed using the Qiagen Ipsogen BCR-ABL1 Mbcr kit (Qiagen, Hilden, Germany) and the Applied Biosystems 7500 RQ-PCR machine (Thermo Fisher) and the QXDX BCR-ABL %IS kit (Bio-Rad, Hercules, CA, USA) and QX200 Auto DG system (Bio-Rad). Data analysis was performed in line with UK recommendations.[17] Sample quality was defined as RNA OD260/280 ratio of between 1·8 and 2·2 and *ABL1* levels >100 000/replicate. The percentage $BCR\text{-}ABL1^{IS}$ results from pre issue testing were required to be within 1·2-fold of the target value, as this is approaching the degree of reproducibility seen within laboratories[8,18] (Table SI). Stability of trial samples was ensured by measuring *ABL1* levels on a further three vials at trial closure. Samples A–G were formulated to contain *BCR-ABL1* levels between $MR^{1·0}$ (10% $BCR\text{-}ABL^{IS}$)- and $MR^{4·0}$ (0·01% $BCR\text{-}ABL^{IS}$)- at half log intervals, important to detect within standard treatment protocols. Samples H–J represented 'deep-response' levels [$MR^{4·5}$ (0·0033% $BCR\text{-}ABL^{IS}$)-, $MR^{4·7}$ (0·002% $BCR\text{-}ABL^{IS}$)- and $MR^{5·0}$ (0·001% $BCR\text{-}ABL^{IS}$)-], important to detect in treatment discontinuation protocols.

The samples were shipped at ambient temperature to 26 study participants in 15 countries in Europe, Asia and North America. All participants in the study were asked to extract RNA and perform reverse transcription using their standard in-house protocol and test the samples with the QXDX BCR-ABL %IS kit, using either the QX200 auto or manual DG system. Results from the QXDX BCR-ABL %IS kit are reported on the IS by using an assay specific conversion factor determined by comparing the assay to an IS reference value. Samples A–G used two reaction wells. Samples H–J used four reaction wells. Participants were asked to independently analyse RTddPCR data using the QuantaSoft (version 1.7.4) and QXDx Reporter Tool software. Where participants were currently reporting *BCR-ABL1* quantification results using RTqPCR, all study samples were run using local methodology for processing and analysis for comparison to the RTddPCR results (n = 11).

Table I. Summary of all participants reverse transcriptase droplet digital polymerase chain reaction (RTddPCR) results for samples A–J.

	Sample F ($MR^{1·0}$)	Sample E ($MR^{1·5}$)	Sample C ($MR^{2·0}$)	Sample D ($MR^{2·5}$)	Sample G ($MR^{3·0}$)	Sample B ($MR^{3·5}$)	Sample A ($MR^{4·0}$)	Sample J ($MR^{4·5}$)	Sample I ($MR^{4·7}$)	Sample H ($MR^{5·0}$)
n	23	23	23	23	22†	23	23	23	23	23
Average*	11·348	4·011	1·265	0·401	0·134	0·039	0·015	0·005	0·003	0·003
SD	3·792	1·211	0·377	0·117	0·041	0·016	0·008	0·003	0·002	0·002
CV, %	33·4	30·2	29·8	29·3	30·5	40·4	52·9	59·7	61·6	69·0

CV, coefficient of variation; MR, molecular response; *n*, number of participant results submitted; SD, standard deviation.
*Average of participant *BCR-ABL1*^{IS} results.
†One participant did not return results for sample G as it did not pass local quality control measures.

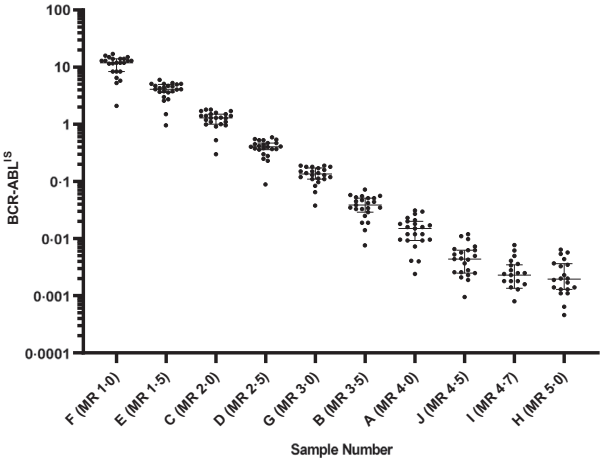

Fig 1. BCR-ABL1^{IS} results reported for samples A–J for all participants who performed reverse transcriptase droplet digital polymerase chain reaction (RTddPCR). Black dots represent individual participant results. Long horizontal line represents average. Short horizontal line represents standard deviation.

Final *BCR-ABL*^{IS} levels for each sample were then calculated and reported independently by each participant (along with methodological information) using a dedicated online data entry page. Method averages were calculated using an arithmetic mean and significance calculated using a two-tailed *t*-test. A $P < 0·05$ was defined as statistically significant.

Methodological variance was calculated using coefficient of variation (CV) with an *F*-test for two-sample variance used to calculate significance in Microsoft Excel. A $P < 0·05$ was defined as statistically significant. Assay linearity was calculated using a simple linear regression (R^2) in GraphPad Prism (version 8·3.1). $R^2 > 0·99$ was deemed satisfactory. A Fisher *r*-to-*z* transformation was used to create a *z* value that was used to assess the significance of difference between the two correlation coefficients. A $P < 0·05$ was defined as statistically significant.

Results

Results were returned by 23/26 (88·0%) participants. All returned RTddPCR data (Table I; Fig 1) with 11 participants returning both RTqPCR and RTddPCR data (Table II; Fig 2).

All 23 participants using RTddPCR detected *BCR-ABL1* in all samples down to $MR^{4·0}$. Detection rates for the deep-response samples were 95·7% at $MR^{4·5}$ (0·0033% *BCR-ABL*^{IS})-, 78·3% at $MR^{4·7}$ (0·002% *BCR-ABL*^{IS})- and 87·0% at $MR^{5·0}$ (0·001% *BCR-ABL*^{IS})- (Table SII). Interlaboratory CV ranged from 29·3% at $MR^{2·5}$ (0·33% *BCR-ABL*^{IS})- to 52·9% at $MR^{4·0}$ (0·01% *BCR-ABL*^{IS})- (Table I). For deep-response samples, the CV was 59·7% at $MR^{4·5}$ (0·0033% *BCR-ABL*^{IS})-, 61·6% at $MR^{4·7}$ (0·002% *BCR-ABL*^{IS})- and 69·0% at $MR^{5·0}$ (0·001% *BCR-ABL*^{IS})- (Table I). Linearity (R^2) was assessed for all participants using RTddPCR results from $MR^{1·0}$ (10% *BCR-ABL*^{IS})- to $MR^{4·0}$ (0·01% *BCR-ABL*^{IS})- and ranged between 0·9330 and 1·000 (average 0·9936) (Fig 3; Table SIII). Overall, 20/23 laboratories had a satisfactory assay linearity with $R^2 > 0·99$.

In total, 11 participants performed both RTddPCR and RTqPCR. When the average percentage *BCR-ABL*^{IS} results reported by participants using RTqPCR was compared to the average percentage *BCR-ABL*^{IS} result for participants using RTddPCR, the results were seen to be comparable, with no statistically significant difference demonstrated (Table II).

There was no statistically significant difference seen in the median *ABL1* control gene levels calculated from participant's RTddPCR data compared to RTqPCR (Table SIV; Table SV).

When detections rates were compared for participants who performed both RTddPCR and RTqPCR, all participants detected *BCR-ABL1* in all samples down to $MR^{4·0}$ (0·01% *BCR-ABL*^{IS})-, using both techniques. For deep-response samples, RTddPCR showed a detection rate of 90·9% at $MR^{4·5}$ (0·01% *BCR-ABL*^{IS})-, 81·8% at $MR^{4·7}$ (0·033% *BCR-ABL*^{IS})- and 81·8% at $MR^{5·0}$ (0·001% *BCR-ABL*^{IS})- compared to 90·9% at $MR^{4·5}$ (0·033% *BCR-ABL*^{IS})-, 90·9% at $MR^{4·7}$ (0·02% *BCR-ABL*^{IS})- and 72·7% at $MR^{5·0}$ (0·001% *BCR-ABL*^{IS})- for RTqPCR (Table SVI).

Interlaboratory CV was lower across all 10 samples for RTddPCR when compared to RTqPCR (Table II, Fig 2) with RTddPCR CV ranging from 18·5% for sample D [$MR^{2·5}$ (0·33% *BCR-ABL*^{IS})-] to 44·1% for sample A [$MR^{4·0}$ (0·01%

Table II. Summary of data from participant's who submitted RTqPCR and RTddPCR results for samples A–J.

		Sample F ($MR^{1.0}$)	Sample E ($MR^{1.5}$)	Sample C ($MR^{2.0}$)	Sample D ($MR^{2.5}$)	Sample G ($MR^{3.0}$)	Sample B ($MR^{3.5}$)	Sample A ($MR^{4.0}$)	Sample J ($MR^{4.5}$)	Sample I ($MR^{4.7}$)	Sample H ($MR^{5.0}$)
RTddPCR	*n*	11	11	11	11	11	11	11	11	11	11
	Average*	12·356	4·209	1·363	0·436	0·143	0·042	0·019	0·004	0·003	0·003
	SD	2·433	0·827	0·254	0·081	0·028	0·010	0·008	0·003	0·001	0·001
	CV, %	19·7	19·6	18·7	18·5	19·4	25·1	44·1	70·6	46·8	49·3
RTqPCR	*n*	11	11	11	11	11	11	11	11	11	10†
	Average*	11·733	3·955	1·331	0·393	0·123	0·035	0·013	0·006	0·003	0·002
	SD	4·659	1·390	0·501	0·155	0·051	0·021	0·008	0·007	0·002	0·001
	CV, %	39·7	35·2	37·7	39·5	41·3	58·4	59·4	116·6	75·4	71·4

CV, coefficient of variation; MR, molecular response; n, number of participant results submitted; RTddPCR, reverse transcriptase droplet digital polymerase chain reaction; RTqPCR, reverse transcription quantitative polymerase chain reaction; SD, standard deviation.
*Average of participant *BCR-ABL1*^{IS} results.
†One participant did not return results for sample H as it did not pass local quality control measures.

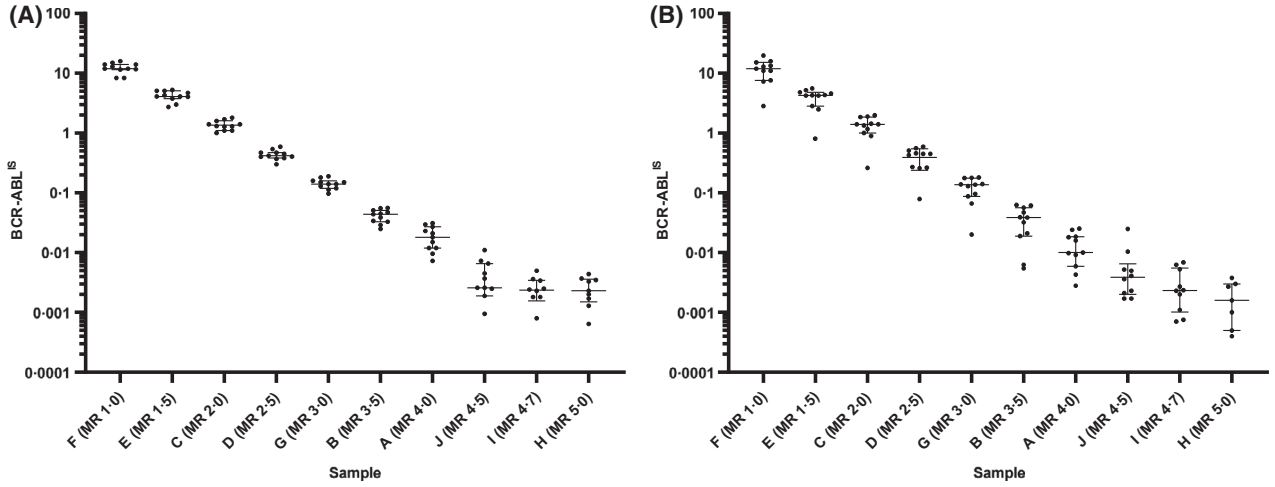

Fig 2. BCR-ABL1^{IS} results reported for samples A–J for participants who performed reverse transcriptase droplet digital polymerase chain reaction (RTddPCR) (A) and reverse transcription quantitative polymerase chain reaction (RTqPCR) (B). Black dots represent individual participant results. Long horizontal line represents average. Short horizontal line represents standard deviation.

BCR-ABL^{IS})-] compared to a range of 35·2% for sample E [$MR^{1·5}$ (3·3% *BCR-ABL*^{IS})-] to 59·4% for sample A [$MR^{4·0}$ (0·01% *BCR-ABL*^{IS})-] for RTqPCR for samples between $MR^{1·0}$ (10% *BCR-ABL*^{IS})- and $MR^{4·0}$ (0·01% *BCR-ABL*^{IS})-. For deep-response samples, the CV ranged from 46·8% for sample I [$MR^{4·7}$ (0·0033% *BCR-ABL*^{IS})-] to 70·6% for sample J [$MR^{4·5}$ (0·033% *BCR-ABL*^{IS})-] for participants using RTddPCR compared to a RTqPCR CV range of 71·4% for sample H [$MR^{5·0}$ (0·001% *BCR-ABL*^{IS})-] to 116·6% for sample J [$MR^{4·5}$ (0·033% *BCR-ABL*^{IS})-]. A statistically significant lower CV for RTddPCR ($P < 0·05$) could be demonstrated in seven of the 10 samples (Table SVII). A statistically significant lower CV for RTddPCR could not be shown in sample E [$MR^{1·5}$ (3·3% *BCR-ABL*^{IS})-], sample A [$MR^{4·0}$ (0·01% *BCR-ABL*^{IS})-] and sample H [$MR^{5·0}$ (0·01% *BCR-ABL*^{IS})-].

Nine out of 11 participants RTddPCR results showed better linearity from $MR^{1·0}$ (10% *BCR-ABL*^{IS})- to $MR^{4·0}$ (0·01% *BCR-ABL*^{IS})- (average $R^2 = 0·9996$) compared to RTqPCR (average $R^2 = 0·9940$) (Fig 4; Table SVIII) with six of these nine being shown to be a statistically significant difference (Table SVIII). One participant showed the same linearity with both RTddPCR and RTqPCR, and one participant showed better linearity with RTqPCR, although this was not a statistically significant difference. All 11 participants using RTddPCR had a satisfactory $R^2 > 0·99$; eight of 11 participants using RTqPCR had a satisfactory $R^2 > 0·99$.

Discussion

Measurement of *BCR-ABL1* has long since been central to the management of patients with CML. The European LeukemiaNET (ELN) 2020 recommendations for CML mandate patients *BCR-ABL1* levels are monitored molecularly every 3 months by qPCR, regardless of whether they have achieved

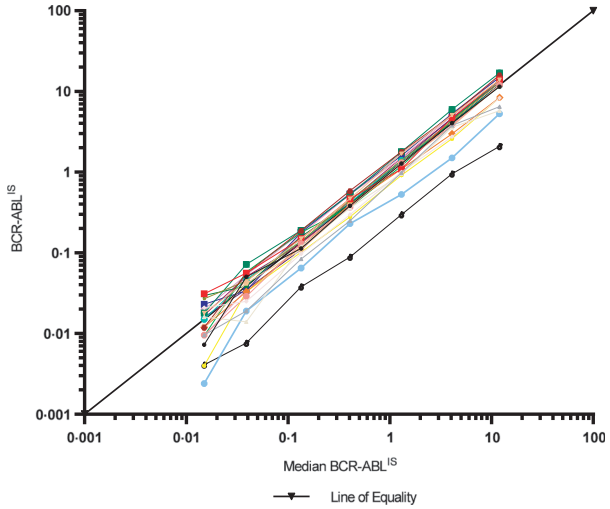

Fig 3. Linear regression for all participants who performed reverse transcriptase droplet digital polymerase chain reaction (RTddPCR) for samples A–G. Coloured lines represent individual participant's results. [Colour figure can be viewed at wileyonlinelibrary.com]

a MR$^{3.0}$ while on TKI therapy. The *BCR-ABL*^{IS} levels at 3, 6, 12 and 18 months are important assessment points and determine the response to TKI therapy, with key decisions being made on continuation or switching of treatment. Measurement *BCR-ABL1* at deep-response levels is also used in the management of pregnancy in younger patients,[1,19] as well as for bone marrow transplantation.[20] TFR is now an achievable goal for a proportion of patients with CML. Achievement of a deep MR has been a major criterion for eligibility for treatment discontinuation studies.[5]

Despite initial studies showing the potential to deliver more accurate and precise data on which these important treatment decisions could be made, RTddPCR has yet to gain widespread adoption for the measurement of *BCR-ABL1*; however, it is showing an increased uptake for the detection of other haematological variants. There has been a steady increase in participants using digital PCR in the UK National External Quality Assessment Services (NEQAS) for Leucocyte Immunophenotyping (LI) JAK2 p.Val617Phe Mutation Status external quality assessment (EQA) programme (Figure S1) (from 0·7% of participants in 2015 to 8·2% in 2020) and the UK NEQAS LI KIT p.Asp816Val in Mast Cell Disease EQA programme (from 8·3% of participants in 2015 to 24·0% in 2020); however, growth has been minimal in the *BCR-ABL1* (Major) Quantification programme (from 0·2% of participants in 2015 to 1·0% in 2020). A lack of interlaboratory validation studies, the extensive validation required for a quantitative minimal residual disease test, concerns about the comparability of results to legacy data, the lack of well-established best practise guidelines and the need to establish an IS conversion factor for laboratory developed RTddPCR tests are likely some of the limiting factors for laboratories considering adopting RTddPCR for *BCR-ABL1* measurement that have stymied its growth.

The average *BCR-ABL*^{IS} results submitted by participants using RTqPCR in the present study was compared to that from participants using RTddPCR and were seen to be comparable, with no statistically significant difference

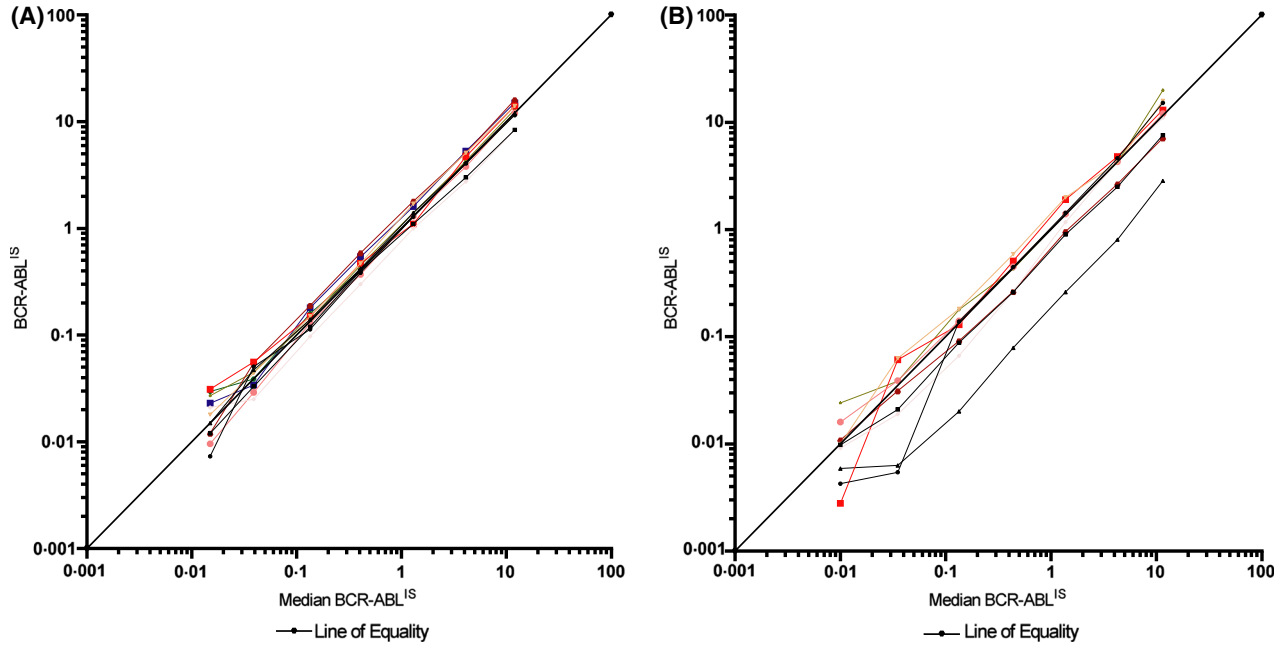

Fig 4. Linear regression for participants who performed both reverse transcriptase droplet digital polymerase chain reaction (RTddPCR) (A) and reverse transcription quantitative polymerase chain reaction (RTqPCR) (B) for samples A–G. Coloured lines represent individual participant's results. [Colour figure can be viewed at wileyonlinelibrary.com]

demonstrated. This provides confidence to laboratories looking to adopt RTddPCR that the RTddPCR percentage *BCR-ABL*^{IS} results can meaningfully compared to legacy percentage *BCR-ABL*^{IS} RTqPCR data in longitudinal analysis. Nonetheless, comparability of results must still be validated on a centre-by-centre basis. Both methods achieved sufficient *ABL1* control gene levels (median for participants using RTqPCR was >80 000 for all samples; median for participants using RTddPCR was >100 000 for all samples) (Table SIV; Table SV) to allow laboratories to adhere to current best practise requirements for measuring deep MR using both methods.[21]

Both RTqPCR and RTddPCR showed good linearity and sensitivity; however, less interlaboratory variation was demonstrated in RTddPCR results in all samples down to MR$^{3·0}$, with four of the five samples tested by laboratories showing a statistically significant difference. Furthermore, nine out of 11 participants using RTddPCR showed improved assay linearity compared to RTqPCR down to and beyond MR$^{3·0}$ (0·1% *BCR-ABL*^{IS}). This is indicative of the precision and accuracy inherent to digital PCR due to it being an end-point, binary enumeration method[22] that reduces the potential for under- or overestimating *BCR-ABL1* levels. RTddPCR had a reduced interlaboratory variation in all deep-response samples when compared to RTqPCR, with a statistically significant difference being demonstrated in two-thirds of the deep-response samples.

No difference in performance was seen in the ability to detect *BCR-ABL1* in 'deep-response' samples required by treatment discontinuation studies; however, stochastic sampling error (Poisson noise) limited the possibility of reliable detection of *BCR-ABL1* when potentially detecting a single molecule (Cross *et al*., 2015[21]), given the standard sample input into the assay. To demonstrate a statistically significant difference would require an impractical number of replicates in a study of this scale. The effect of increasing blood draw volumes and RNA sample input, although potentially challenging clinically and costly, should be the focus of future studies.

The present study has demonstrated that RTddPCR using the QXDX BCR-ABL %IS kit is a viable alternative to the current 'gold standard' RTqPCR. Quantifying samples directly with no reliance on standard curves, it offers decreased interlaboratory variation and better assay linearity when directly compared to RTqPCR. It suggests that RTddPCR using the QXDX BCR-ABL %IS kit will allow more comparable management of patients with CML in a diverse range of clinical scenarios. It also establishes a baseline performance for RTddPCR using the QXDX BCR-ABL %IS kit for the measurement of *BCR-ABL1* for a diverse group of laboratories in a range of different countries. This reproduces the improved accuracy of RTddPCR shown in single-centre studies,[12–15] while additionally demonstrating the robustness of the approach when applied to a diverse range of laboratories (research, clinical, translational) in a wide variety of countries. However, with only 11 laboratories testing both RTqPCR and RTddPCR, this data will need to be replicated in a larger dataset using a more diverse range of digital PCR approaches. The impact RNA extraction and cDNA synthesis diversity on RTddPCR variability was not assessed in the present study and should be the focus of future standardisation projects.

The cost of Bio-Rad's QXDX BCR-ABL %IS kit is similar to the automated Cepheid Xpert® cartridge-based systems, but is more expensive than conventional laboratory developed RTqPCR tests, especially when the extra wells for deep-response detection are considered.[23] This is off set by the lack of requirement for a standard curve and the reduced validation required when using a Conformité Européene (CE) marked kit given the imminent changes to Europe's *in vitro* diagnostic (IVD) regulations and their impact on laboratory developed tests.

The ability to detect deep responses with RTddPCR using the Bio-Rad QXDX BCR-ABL %IS kit, matched with an improved linearity and reduced interlaboratory CVs, compared to RTqPCR, offers the potential for better classification of patients according to the ELN 2020 criteria at both major MR and deep-response level.

Acknowledgments

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Percentage of participants using droplet digital PCR in UK NEQAS LI programmes by participation year.

Table SI. Study sample constitution information.

Table SII. *BCR-ABL1* detection rate in samples A–J for all participants using RTddPCR.

Table SIII. Linearity (R^2) for all participants who performed RTddPCR for samples A–G.

Table SIV. *ABL1* copy number levels reported by participants using RTqPCR.

Table SV. *ABL1* copy number levels reported by participants using RTddPCR.

Table SVI. *BCR-ABL1* detection rate in samples A-J for participants using RTddPCR and RTqPCR.

Table SVII. *F*-test for two-sample variances to show statistical significance between RTddPCR and RTqPCR results.

Table SVIII. Linearity (R^2) for participants who performed both RTddPCR (A) and RTqPCR (B) for samples A–G.

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