CAP and NEQAS Proficiency Schemes for *BCR-ABL1* Minimal Residual Disease Analyzed Using the QXDx BCR-ABL %IS Kit

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Introduction

Chronic myelogenous leukemia (CML) is defined at the chromosomal level by the presence of a t9:22 translocation, resulting in Philadelphia chromosome generation. The junction of the *BCR-ABL1* fusion gene typically occurs between *BCR* exons 13 or 14 and *ABL* exon 2, resulting in potentially two alternate fusion transcripts (*e13a2* or *e14a2*). The Philadelphia chromosome's presence is used to diagnose CML and some types of acute lymphoblastic leukemia (ALL) mainly by in situ hybridization. After diagnosis and initiation of treatment, monitoring residual disease (MRD) is performed using PCR of the *BCR-ABL1* mRNA fusion gene transcripts. For most of the last 25 years, this has been performed primarily using fluorescent probe chemistries and quantitative PCR (qPCR) systems. This method relies on relative quantification of clinical samples, with unknown samples extrapolated off a standard curve run in parallel to achieve absolute quantification for control genes and fusion genes prior to conversion to the international scale (IS).

Over the last 20 years, there have been several developments in MRD monitoring in CML that have improved quantification at the molecular level and monitoring and treatment at the clinical level. Development of standardized assays by the European Leukemia Network (Gabert et al. 2003), the establishment of an IS via reciprocal sample analysis, and individual laboratory conversion factors (CF) (Hughes et al. 2006) have all dramatically improved patient data, clinical definition, and interlaboratory comparison of results for CML.

Use of the IS became more accessible for clinical testing laboratories with the release of the First World Health Organization (WHO) International Genetic Reference Panel for the quantification of *BCR-ABL1* translocation (White et al. 2010). With primary reference standards available to kit manufacturers and standards manufacturers globally, individual laboratories were able to generate a laboratory-specific conversion factor to align to the IS using secondary standards or complete *BCR-ABL1* test kits, without the need for complex reciprocal sample transfers.

After aligning to the IS by defining a laboratory conversion factor, individual laboratories must also show regular compliance with a nationally established or internationally recognized proficiency testing (PT) or external quality assurance (EQA) scheme to maintain their clinical testing accreditation. The dual purpose of such schemes is to allow an individual laboratory's performance to be compared to those of other laboratories performing the same test using contrived samples and for laboratories that vary too far from the collective mean to be guided back to an acceptable position by the PT or EQA organizations.

The QXDx BCR-ABL %IS Kit from Bio-Rad is a Droplet Digital PCR (ddPCR) Kit to monitor the *e13a2* and *e14a2* mRNA fusion gene transcripts in patients diagnosed with CML. The kit utilizes proprietary probe and primer chemistry and proprietary emulsion chemistry to facilitate the quantification of mRNA transcripts present in clinical samples by Droplet Digital PCR.

Unlike qPCR, Droplet Digital PCR does not require the use of a standard curve to generate absolute copy numbers. This reduces variability caused by, for example, inhibitors, which produce differences between the standard curve materials and the patient samples. Droplet counts generated on the QX200 Droplet Digital PCR System convert directly to copies per µl for the *BCR-ABL1* transcripts and allow for direct normalization to a multiplexed reference gene (*ABL1*). Inclusion of kit controls, specifically those for 10 and 0.1% IS, and a kit-specific conversion factor facilitates the direct generation of %IS and molecular response (MR) values for samples through the use of either the QXDx IVD Software (USA) or the semi-automated BCR-ABL Reporter Macro (Europe). The presence of calibrators in the kit ensures alignment of absolute values to the First WHO International Genetic Reference Panel. In particular, the 0.1% IS calibrator signifies a major molecular response, an important milestone in managing CML and tyrosine kinase inhibitor therapy response.

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Data presented in this white paper describe the use of the QXDx BCR-ABL %IS Kit for analysis of control materials as part of a proficiency testing scheme from the College of American Pathologists (CAP) and an external quality assurance scheme from UK NEQAS for Leucocyte Immunophenotyping (NEQAS). Samples were run on the QX200 ddPCR System with CAP samples acquired on the QXDx Software and NEQAS samples on the QuantaSoft Software 1.7.4. Consistent, high-quality data were obtained showing excellent reproducibility and linearity to collective mean values generated from both schemes across 5 years.

Results

The College of American Pathologists of the USA is the leading organization of board-certified pathologists, serving patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide (cap.org/about-the-cap). CAP's minimal residual disease *BCR-ABL1* p210 (MRD p210) scheme is a biannual test, which reference laboratories perform in April and October every year. Typically, three vials of purified cell-line RNA samples are shipped together (MRD 01-03 in April and MRD 04-06 in October). The reference laboratory is required to treat the samples exactly as they treat their patient samples. One of the three samples is a diagnostic sample, which is often a high positive. A log reduction calculation for the other two samples is done with respect to the diagnostic sample. The reference laboratory is blinded to the three samples provided. Postsubmission, CAP returns a participant summary with mean and median values for all laboratory participants for the three samples, allowing each reference lab to evaluate their performance. The QXDx BCR-ABL %IS Kit has been used to test the MRD p210 test samples since 2016.

UK NEQAS is a charitable consortium of external quality assessment laboratories, mostly evolved from NHS services in England, Scotland, Wales, and Northern Ireland (ukneqas.org.uk/). This paper shows data from the NEQAS — Major *BCR-ABL1* Quantification program that UK NEQAS offers triannually (January, May, and September). Unlike CAP, the NEQAS *BCR-ABL1* scheme samples are freeze-dried cell-line samples requiring extraction, and typically NEQAS provides the reference laboratory with two samples per testing period. The reference laboratories are required to extract and test RNA from these samples as if they were patient samples. Like CAP, NEQAS does not provide the reference laboratories a priori with the samples' expected values. Postsubmission, NEQAS provides the reference laboratories with a participant summary containing the compiled mean and median values of participant labs for each sample.

Bio-Rad research and manufacturing teams have been participating in CAP and NEQAS schemes for 5 years. Samples tested in this period are shown in Tables 1 and 2. Figure 1 is a consolidated bar chart of *BCR-ABL* %IS values with the CAP median and measured values in blue and orange, and NEQAS median and measured values in green and yellow, respectively. Note that for both CAP and NEQAS, the Bio-Rad measured value is very close to the median reported value even with samples that are <0.01% IS, which demonstrates the accuracy of the QXDx BCR-ABL %IS Kit. Four of the CAP samples shown in the bar graph have a median value of 0% IS with a high reference *ABL* load (>100,000 copies), to which the QXDx BCR-ABL %IS Kit has returned values of 0% IS, showing that the false-positive rate of the kit is 0.

Table 1. CAP proficiency tests 2016–2019.

Year	Trial	Median Reported %IS	Bio-Rad Measured %IS
2016	42491	0.002	0.002
		0.18	0.16
	42675	3.7	4.1
		0	0
2017	43040	27.1	26.1
		0.35	0.4
		0	0
	43221	0.0046	0.0033
		0.0046	0.0027
		27.3	27.4
		27.3	27.4
		0.39	0.39
		0.39	0.35
2018	43405	0	0
		0.025	0.02
		21.8	20.1
2019	43586	0.022	0.028
		20.8	22.6
		0	0
	43621	2.822	2.95
		0.00279	0.006
		21.99	22.91

Table 2. NEQAS proficiency tests 2018–2020.

Year	Trial	Median Reported %IS	Bio-Rad Measured %IS
2018	171803	0.08	0.082
		0.08	0.113
	181901	4.9	4.147
		0.027	0.017
	181902	3.6	3.67
		0.19	0.16
2019	181903	1.6	1.2
		0.05	0.05
	192001	0.02	0.03
		0.02	0.03
	192002	0.13	0.166
		0.05	0.047
2020	192003	3.3	4.35
		0.042	0.07
	202101	10	12.17
		0.0054	0.0026

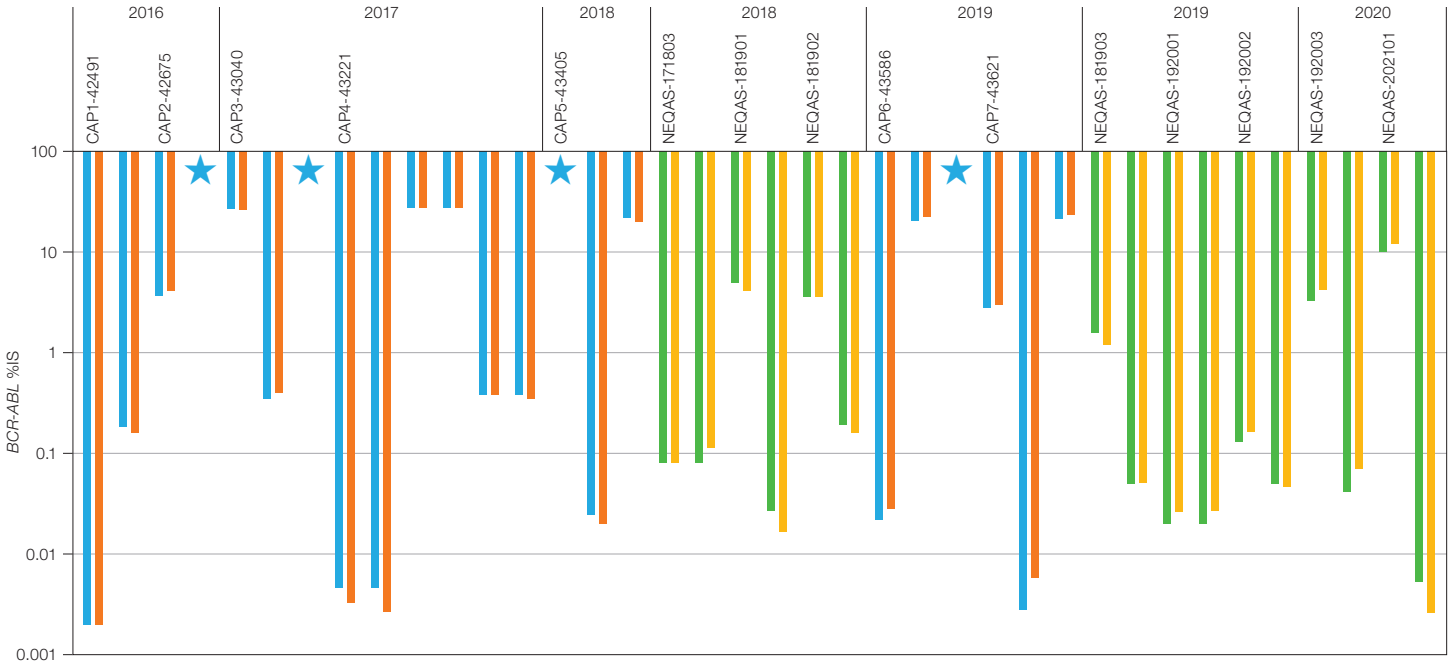

Fig. 1. Consolidated data from QXDx BCR-ABL %IS Kit for both CAP and NEQAS. 0% IS values are represented in the timeline by blue stars. Measured *BCR-ABL* %IS values are represented in orange for CAP and yellow for NEQAS. Median returned values for *BCR-ABL* %IS are blue for CAP and green for NEQAS.

Figure 2 shows a scatter plot of the median returned MR value vs. the measured MR value for both CAP and NEQAS. The 0% IS values were removed from the dataset to generate these graphs. The samples exhibit high linearity over the dynamic range (MR 0.5–MR 4.7) of Droplet Digital PCR as is evident from the R^2 values >0.97 for both CAP and NEQAS. The slopes are very close to 1 with a negligible bias for both proficiency tests.

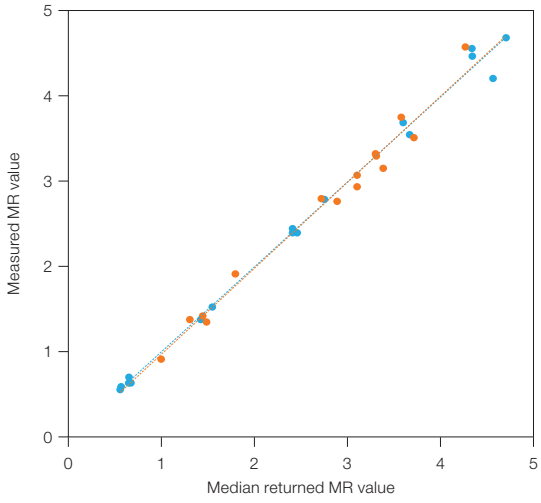

Fig. 2. Linear regression of QXDx BCR-ABL %IS Kit median returned MR value vs. the measured MR value for CAP and NEQAS. Measured MR CAP (●); measured MR NEQAS (●); linear measured MR CAP (----); linear measured MR NEQAS (----). CAP R^2 = 0.9947, y = 1.0019x – 0.0041; NEQAS R^2 = 0.9796, y= 1.0133x – 0.0515.

Discussion

Analysis of approximately 40 samples from both CAP and NEQAS across 5 years showed excellent comparability of measured values to scheme median returned values in the range %IS 27.1–0.002, MR 0.58–4.71. The data also showed excellent linearity across this range, with R^2 = 0.9947 for CAP, R^2 = 0.9796 for NEQAS, and collectively an R^2 = 0.9912. The data included four sets of negative samples from the CAP schemes, for which the QXDx BCR-ABL %IS Kit returned negative results with an associated *ABL* sensitivity >MR5 (Cross et al. 2015).

These data illustrate the capability of the QXDx BCR-ABL %IS Kit to reproducibly detect and monitor *BCR-ABL1* transcripts for CML MRD from levels expected immediately postdiagnosis to levels of *BCR-ABL1* transcripts commensurate with a deep molecular response.

Conclusions

The workflow for the QXDx BCR-ABL %IS Kit is simple and easy to use, without the need for standards or a standard curve. With a straightforward workflow, a short, approximately 7-hour turnaround time, and no standard curves requirement, Droplet Digital PCR is ideally suited for monitoring patients with CML. With the stand-alone absolute quantification capabilities of Droplet Digital PCR, each QXDx BCR-ABL %IS Kit is provided with a lot-specific conversion factor. This eliminates the need for lengthy laboratory-assigned conversion factor protocols, making the adoption of Droplet Digital PCR for CML monitoring even easier than traditional qPCR systems.

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