Assessment of standardization and IS alignment efforts of BCR-ABL1 testing by the Chinese CML Alliance in an International External Quality Assessment Program

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INTRODUCTION

Sequential *BCR-ABL1* mRNA level measurement by reverse transcription quantitative polymerase chain reaction (RT-qPCR) is embedded in management of chronic myeloid leukaemia (CML) patients.

United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping (UK NEQAS LI) has been providing External Quality Assessment (EQA)/Proficiency Testing (PT) to 200 laboratories undertaking *BCR-ABL1* RT-qPCR analysis since 2007 (Scott et al., 2017).

The Chinese CML Alliance (CCA), established in 2011 for the purpose of the standardization of *BCR-ABL1* measurement includes 58 laboratories to date. Peking University People's Hospital (PUPH), the Chinese regional reference centre, has acquired conversion factors (CFs) for reporting BCR-ABL^{IS} through sample exchanges with the Adelaide IMVS reference laboratory. Other members acquired their laboratory specific CFs thorough sample exchange with PUPH, and their CFs were regularly revalidated through testing distributed samples by PUPH.

We reviewed performance of CCA laboratories in UK NEQAS LI's *BCR-ABL1* Quantification program to evaluate if technical standardization initiatives and local result alignment to the International Scale (IS) (defined as BCR-ABL^{IS}) increased accuracy and precision of *BCR-ABL1* measurement by CCA labs.

METHODS

5 CCA laboratories were included in 3 EQA/PT send-outs provided by UK NEQAS LI, comprising a total of six standard samples and one additional educational sample . The samples were tested by CCA laboratories, alongside up to 207 other international participants. The samples comprising of two lyophilised cell line samples containing a mix of BCR-ABL1 (e14a2) positive K562 cells in a background of BCR-ABL1 negative HL60 cells. K562 and HL60 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). Cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (both from Gibco ThermoFisher, Waltham, MA, USA). Pre-defined dilutions of K562 cells in HL60 cells were then prepared and freeze dried in 3-ml glass ampoules to contain 10–20 x 10^6 cells. Samples were dried for 24 h. Prior to distribution to ensure sample quality and homogeneity, a minimum of three selected samples were subjected to (phenol chloroform) RNA extraction, cDNA synthesis and BCR-ABL1 quantification. This was performed using the Ipsogen BCR-ABL1 Mbcr kit (Qiagen, Hilden, Germany) and the 7500 RQ-PCR machine (Applied Biosystems, Waltham, MA, USA). Analysis was performed in-line with UK recommendations (Foroni et al, 2011). Sample quality was defined as RNA optical density (OD) 260/280 ratio of between 1.8 and 2.2 and *ABL1* levels >10,000 per replicate. Replicate values must be within 1.2-fold of each other (Cross, 2009). Stability of trial samples was ensured by measuring ABL1 levels on a further three vials at trial closure.

CCA laboratories were asked to measure the % BCR-ABL1/reference gene levels in each sample using their normal laboratory protocols and report results obtained (either converted to the IS, unconverted or both) as well as certain methodological details. Laboratories were assessed against the IS robust mean BCR-ABL^{IS} for each sample tested and their proximity to the robust mean log reduction between two standard trial samples tested, providing information on CCA laboratory IS alignment and assay linearity.

RESULTS

Median BCR-ABL^{IS} sample levels in this study (calculated from all participant data) ranged from 6.1% to 0.002% (Figure 1). All five CCA laboratories used in-house based methods to measure *BCR-ABL1*; All 5 CCA labs used ABL1 as reference gene; 3 based their in-house method on the Europe Against Cancer protocols2; 2 used in-house protocols. CCA laboratories used either in-house (n=3) or commercially available standards (n=2) (Yuanqi Bio-Pharmaceutical, Shanghai, China).

Table 1: Table to show assigned values for samples in this study

Trial	Sample Number	Assigned Value BCR-ABL1/ABL1 Unconverted (Median)	Assigned Value Unconverted Log Reduction (Robust Mean)	Unconverted Robust SD	Assigned Value BCR-ABL^{IS} (Median)	Assigned Value IS Log Reduction (Robust Mean)	IS Robust SD
BCRQ 161702	BCRQ 142	9.70	1.81	0.15	7.8	1.82	0.15
	BCRQ 143	0.15			0.11		
BCRQ 161703	BCRQ 144	1.10	1.30	0.16	0.79	1.3	0.17
	BCRQ 145	0.04			0.03		
BCRQ 171801	BCRQ 146	0.51	0.82	0.11	0.39	0.84	0.14
	BCRQ 147	0.081			0.05		
	Edu D	0.0027	n/a	n/a	0.002	n/a	n/a

ABL1 was detected in all samples by the 5 centres with 4 achieving *ABL1* levels >100,000. Additionally, 4/5 detected *BCR-ABL1* transcript in all seven samples but 1/5 laboratories failed to detect any transcript in 1 sample (the sample that had the lowest level of transcript (median BCR-ABL^{IS} = 0.002%; ~MR $^{4.7}$)).

4/5 laboratories measured BCR-ABL^{IS} levels within +2 SD of the robust means for individual sample and log reduction analysis undertaken between samples for each send-out. One laboratory was -5.21 SD of the robust mean for sample 147 and +5.52 SD from the robust mean for sample 148 resulting in a log reduction -18.47 from the robust SD for this trial issue.

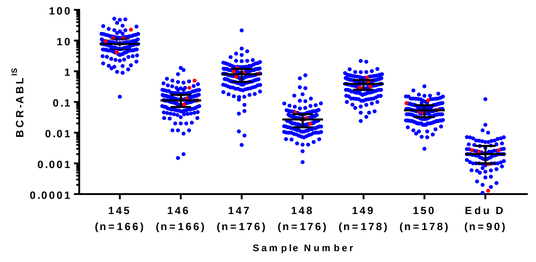

Figure 1: Scatter plot showing all participant BCR-ABL^{IS} data (blue) compared to CCA laboratory results (red) for the seven samples tested as part of the study. Larger horizontal black lines represent the median; smaller horizontal black lines represent the range. Figure shows CCA results with transpositions corrected. The real Edu D results of all 5 labs are shown.

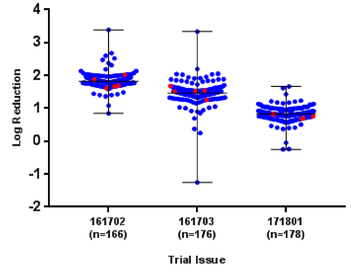

Figure 2: Scatter plot showing all participant log reduction data (blue) compared to CCA laboratory data(red) for the three trial issued as part of this project. Larger horizontal black lines represent the median; smaller horizontal black lines represent the range. Figure shows CCA results with transpositions corrected.

CONCLUSIONS

This study shows that efforts by the CCA to standardize *BCR-ABL1* measurement and align local results to the IS have been successful. Satisfactory results (within ±2SD of the robust mean) were obtained in 4/5 laboratories providing reassurance that patient testing in these laboratories is accurate and precise, down to MR$^{4.7.}$ A root cause analysis undertaken in the laboratory with results >±2SD from the robust mean found a pre-analytical sample transposition error that when reanalyzed was within the acceptable range for both individual sample and log reduction results. Pre analytical errors are known to account up to 70% of all mistakes made in laboratory diagnostics (Plebani et al., 2013) and laboratories should have robust procedures in place to prevent these errors. A root cause analysis undertaken in the laboratory who did not report any *BCR-ABL1* transcript in the lowest level sample (BCRQ Edu D; ~MR $^{4.7}$) identified that the laboratory did, in fact, detect the transcript, but did not report it due to over caution as the levels of transcript present in the sample was very low. However, with *the BCR-ABL1* and *ABL1* copy numbers identified by the laboratory they could have confident in reporting this sample as positive and providing a BCR-ABL^{IS} value in line with current recommendations (Cross et al., 2015).

The study showed that some CCA laboratories omitted the *BCR-ABL1* standard curve, something not recommended in technical guidelines (Foroni et al., 2011) as erroneous results may occur due to unrecognised assay drift or suboptimal amplification. Standard curves for both *ABL1* and *BCR-ABL1* should always be run; however it is acceptable to run a standard curve each day for multiple runs but these must use the same master mix used for the sample quantification (Foroni et al., 2011).

Future studies will include more laboratories in the CCA network to assess if the use of internationally recognized calibrants can achieve even greater standardization.

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