Pilot *BCR-ABL1* Minor Quantification Programme (Not Accredited)

Participant No: **Trial No: mBCRQ 192002**

Date Issued: 30 Jan 2020 **Date Closed: 28 Feb 2020**

FINAL REPORT

Two vials of lyophilised cell line material, samples mBCRQ 122 and mBCRQ 123, were issued to 73 participants for quantitative minor (e1a2) *BCR-ABL1* (p190) analysis. Samples mBCRQ 122 and 123 were manufactured to be positive for the minor *BCR-ABL1* transcript, mimicking minimal residual disease (MRD) levels seen following treatment in chronic myeloid leukaemia (CML) or acute lymphoblastic leukaemia (ALL).

For this trial, 69 (94.5%) participants submitted results. Four participants failed to return any results with one laboratory pre-notifying us of this. Additionally, two laboratories were only able to submit results for a single sample. **Please note repeat samples are available for all programmes. In the event that your local quality control (QC) criteria are not met please contact us.**

Table 1: Your Results

	Sample mBCRQ 122	Sample mBCRQ 123
Your % *BCR-ABL1*/Control Gene		
Median % *BCR-ABL1/ABL1**	4.7	0.025
Lower Quartile*	3.1	0.014
Upper Quartile*	6.5	0.031
Inter Quartile Range (IQR)*	3.4	0.017
Your Log Reduction between sample mBCRQ 122 and mBCRQ 123		
Robust Mean Log Reduction between sample mBCRQ 122 and mBCRQ 123	2.33	
Robust SD Log Reduction between sample mBCRQ 122 and mBCRQ 123	0.17	

* Due to the differences in expression levels of the range of control genes used, results from different control genes cannot be meaningfully compared. As such, we have only calculated median sample results for participants using *ABL1* as a control gene.

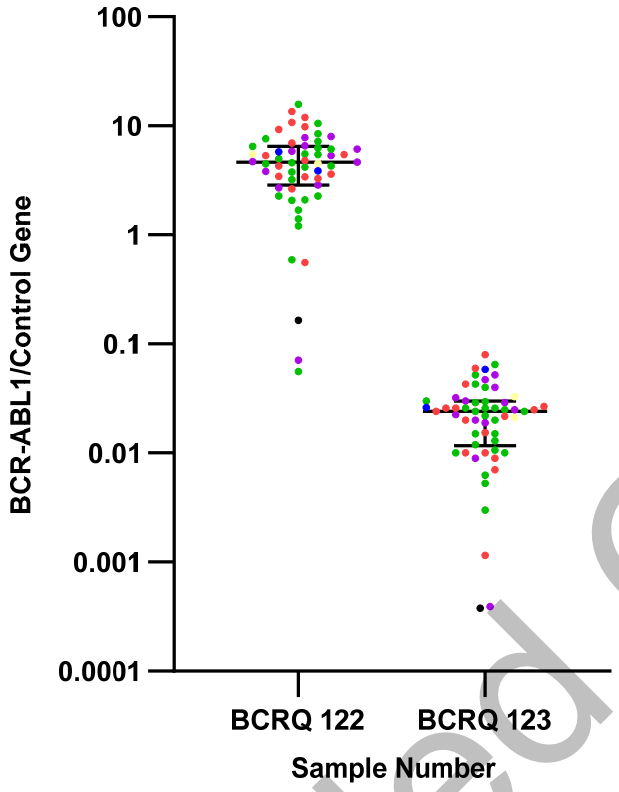

Figure 1: Scatter plot of % *BCR-ABL1*/Control Gene results for samples mBCRQ 122 and mBCRQ 123 by assay type. In-house (purple); In-house (EAC) (green); Onestep BCR-ABL p190 Elite MGB kit (blue); Qiagen (formerly Ipsogen) Fusion Quant kit (red); 3B BlackBio TRUPCR BCR-ABL1 Kit (black); Asuragen QuantideX qPCR BCRABL minor Kit (yellow). Long horizontal line represents the median; short horizontal lines represent the interquartile range.

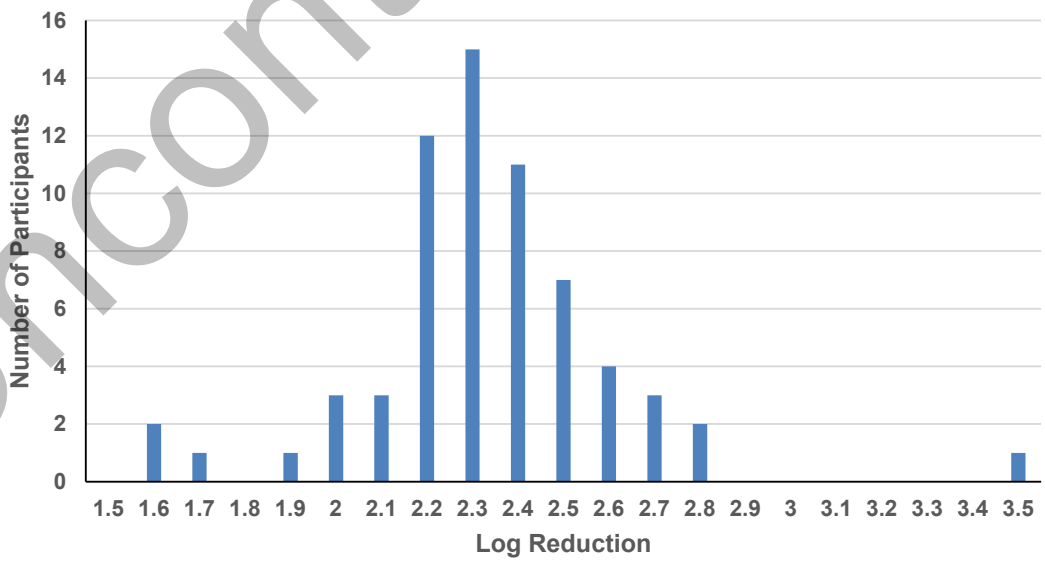

Figure 2: Frequency distribution histogram of log reduction between samples mBCRQ 122 and mBCRQ 123.

Method Breakdown

Table 2: Control gene summary

	Number of Participants
*ABL1**	61
*GUSB**	6
*ABL1** and *GUSB**	1
HMBS (PBGD)*	1

* HUGO Gene Nomenclature Committee (HGNC) approved gene names (www.genenames.org/)

Table 3: *ABL1* copy number

	Sample mBCRQ 122	Sample mBCRQ 123
n	57	56
Median	117,818	106,655
Lower Quartile	65,450	59,138
Upper Quartile	117,818	106,655
Inter Quartile Range (IQR)	52,368	47,517
Min	4,939	6,830
Max	828,000	584,000
Number of participants reporting <10,000 copy number	2 (3.5%)	1 (1.8%)

Not all participants stating *ABL1* as the control gene provided information regarding copy number achieved.

Table 4: *GUSB* copy number

	Sample mBCRQ 122	Sample mBCRQ 123
n	6	6
Median	542,872	595,619
Lower Quartile	306,180	418,769
Upper Quartile	703,510	755,819
Inter Quartile Range (IQR)	397,330	337,049
Min	106,815	45,825
Max	799,672	894,678
Number of participants reporting <24,000 copy number	0	0

We acknowledge the limitations of this small dataset.

Table 5: Instrument manufacturer and model

	Number of Participants
Roche LC 480	16
ABI 7500	13
Qiagen Rotorgene	8
ABI 7900HT	5
Quantstudio 5	3
ABI Viia7	3
Roche LC 2.0	2
ABI 7500HT	2
BioRad QX200 Droplet Digital PCR	2
ABI 7500 Fast Dx	2
ABI Step One Plus	1
Quantstudio 6	1
Roche LC 1.5	1
Biorad CFX96	1
Quantstudio Dx	1
ABI 7300	1
ABI Step One	1
Corbett RotorGene 3000	1
Quantstudio 3D	1
Cobas z480	1
croBEE RT PCR system	1
Not known	2

Table 6: Assay protocol

	Number of Participants
In-house (EAC)	33
Qiagen (formerly Ipsogen) Fusion Quant Kit	16
In-house	13
Onestep BCR-ABL p190 Elite MGB Kit	2
Asuragen QuantideX qPCR BCRABL minor Kit	2
3B BlackBio TRUPCR BCR-ABL1 Kit	1
Not known	2

Table 7: Material used for standard curve

	Number of Participants
Qiagen (formerly Ipsogen) Fusion Quant standards	39
In-house standards	11
In-house standards calibrated to Qiagen (formerly Ipsogen) standards	3
Onestep BCR-ABL p190 Elite MGB Standards	2
Asuragen QuantideX BCRABL minor calibrators	2
AB Analitica	2
Qiagen for mBCR-ABL1 and AD623 for ABL1	1
Ipsogen Fusion standards for p190 and ERM AD623 for GUSB	1
ERM AD623	1
Wessex plasmid	1
3B BlackBio TRUPCR BCR ABL1 standards	1
N/A - Digital PCR method (no standard curve)	2
N/A - DeltaCt method (no standard curve)	1
Not known	2

Table 8: Assay references summary

	Number of Participants
Gabert et al. (2003) Leukemia 17, 2318-2357	39
Beillard et al. (2003) Leukemia 17, 2474-2486	16
Baccarani et al. (2013) Blood 122 (6), 872-84	11
In-house (no published reference available)	7
Foroni et al. (2011) BJH 153 (2), 179-190	5
Van Dongen et al. (1999) Leukemia 13, 1901-1928	5
Foroni et al. (2009) Am J Hema 84, 517-522	4
Hochhaus et al. (2002) Leukemia 16, 2190-2196	3
Emig et al. (1999) Leukemia 13 (11), 1825-1832	1
Mensink et al. (1998) BJH 102 (3), 768-774	1
Other	1

Table 9: Reference(s) and trial protocol(s) utilised for the reporting of minor *BCR-ABL1* quantification results to clinicians

	Number of Participants
Gabert et al. (2003) Leukemia 17, 2318-2357	33
Baccarani et al. (2013) Blood 122(6), 872-84	19
Cross et al. (2015) Leukemia 29 (5), 999-1003	16
Beillard et al. (2003) Leukemia 17, 2474-2486	11
Foroni et al. (2011) BJH 153, 179-190	7
Recommendations of the European Working Group for Adult ALL (2011) UNI-MED	4
Cross et al. (2012) Leukemia 26, 2172-5	3
van der Velden et al. (2007) Leukemia 17, 604-611	2
van Dongen et al. (1999) Leukemia. 13(12):1901-28	2
Moppett et al .(2003) Leukemia 17, 268-270	1
Thomas (2007) Hematology Am. Soc. Hematol. Educ. Program 435-443	1
Other	2

Trial Summary:

Sample mBCRQ 122

- Sample mBCRQ 122 was manufactured to be positive for the minor *BCR-ABL1* transcript. All returning participants for sample mBCRQ 122 detected a minor *BCR-ABL1* transcript (n = 68).
- The median %*BCR-ABL1/ABL1* calculated from participant returns for sample mBCRQ 122 was 4.7.
- The median RT-qPCR *BCR-ABL1* Ct value for sample mBCRQ 122 was 26.9, with an inter quartile range of 2.5.

Sample mBCRQ 123

- Sample mBCRQ 123 was manufactured to be positive for the minor *BCR-ABL1* transcript. Two of the returning participants for this sample (n = 68) failed to detect a minor *BCR-ABL1* transcript. There was no methodological association. One of the participants returning a false negative result noted a single replicate was positive (ABI 7500 in house protocol, *HMBS* control gene). However, both laboratories omitted to supply control gene copy number information. **Please note repeat samples are available for all programmes. In the event that your local quality control (QC) criteria are not met please contact us.**
- The median %*BCR-ABL1/ABL1* calculated from participant returns for sample mBCRQ 123 was 0.025.
- The median *BCR-ABL1* RT-qPCR Ct value for sample mBCRQ 123 was 34.8, with an inter quartile range of 2.5.

Log Reduction

- The robust mean log reduction between sample mBCRQ 122 and mBCRQ 123, calculated from all participant returns was 2.33, with a robust SD = 0.17.

- 59/65 (90.8%) participants were within 2.5 SDs of the robust mean. Two laboratories yielded results between 2.5 and 3.5 SDs.
- Four participants returned results which generated a log reduction >3.5 SDs from the robust mean. Three of the laboratories used the Roche LC 480 instrument with various protocols/kits and standards. The other was a droplet digital PCR user (BioRad QX200 with in-house EAC protocol, *GUSB* control gene). One of the Roche LC 480 users stated *ABL1* control gene copy numbers of <8,000 for both samples mBCRQ 122 and 123.
- It was not possible to calculate a log reduction for four participants due to these laboratories submitting a zero % *BCR-ABL1*/CG result for a given sample or returning a result for only a single sample.

Control Genes

- Median *ABL1* control gene levels were 117,818 for sample mBCRQ 122, and 106,655 for sample mBCRQ 123.
- Two participants reported an *ABL1* copy number of <10,000. One laboratory (as previously discussed) submitted *ABL1* levels of <10,000 for both samples.
- Median *GUSB* control gene levels were 542,872 for sample mBCRQ 122 and 595,619 for sample mBCRQ 123. With only 6 participants using *GUSB* we acknowledge the limitations of this small data set.

Repeat samples are available for all programmes. In the event that your local quality control (QC) criteria are not met please contact us. Please do not submit results based on a suboptimal extraction.

We are beginning preparations to enable UKAS ISO:17043 accreditation for this programme. We would encourage participants to share any comments they may have that might help us to improve subsequent trials. Please do contact us (admin@ukneqasli.co.uk) with any related suggestions or comments. We plan to formally survey participants to gather feedback during the 2020/21 financial year.

Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Mr Liam Whitby (Director) and Mr Stuart Scott (Centre Manager).

4.8.2 c) Person(s) authorizing this report:
Mr Liam Whitby (Director) or Mr Stuart Scott (Centre Manager) of UK NEQAS LI.

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website:
http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/