Pilot Minor *BCR-ABL1* Quantification Programme (not accredited)

Participant No: **Trial No:** **mBCRQ 171801**

Date Issued: 20th Sep 2017 **Date Closed: 20th Oct 2017**

Two vials of lyophilised cell lines samples, mBCRQ 112 and mBCRQ 113, were issued to 58 participants for quantitative minor (e1a2) *BCR-ABL1* analysis.

mBCRQ 112 and mBCRQ 113 were duplicate samples manufactured to be positive for the minor *BCR-ABL1* transcript mimicking MRD levels seen following treatment in Chronic Myeloid Leukaemia and Acute Lymphoblastic Leukaemia.

In this trial, fifty-seven (98.3%) participants returned results.

Table 1: Your Results

	Sample mBCRQ 112	**Sample mBCRQ 113**
Your *BCR-ABL1*/Control Gene %		
Median *BCR-ABL1/ABL1*	**0.023**	**0.020**
Lower Quartile	**0.016**	**0.014**
Upper Quartile	**0.030**	**0.030**
Inter Quartile Range (IQR)	**0.014**	**0.017**
Your Log Reduction between sample mBCRQ 112 and mBCRQ 113		
Robust Mean Log Reduction between sample mBCRQ 112 and mBCRQ 113	**-0.01**	
Robust SD Log Reduction between sample mBCRQ 112 and mBCRQ 113	**0.15**	

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.

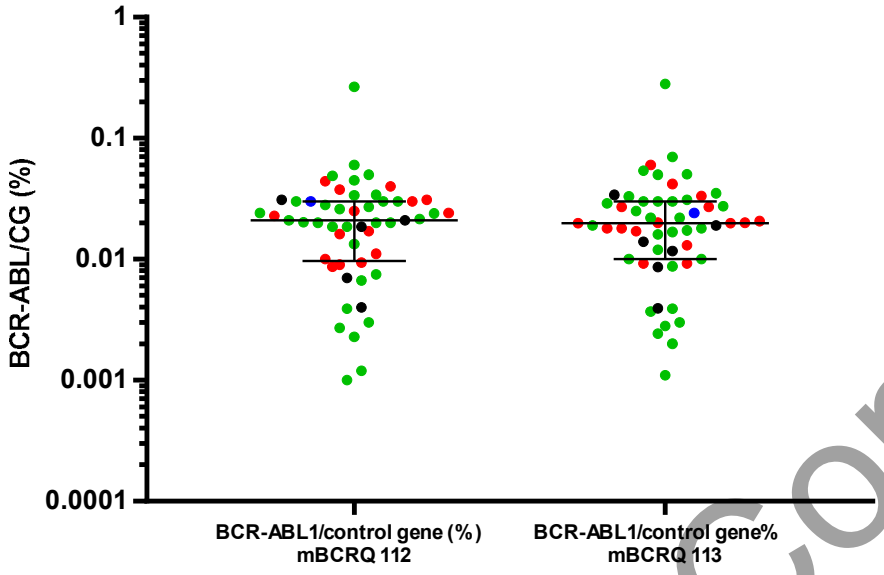

Figure 1: Scatter plot showing all participants % *BCR-ABL1/*Control Gene results for samples mBCRQ 112 and 113, by assay type. Larger horizontal black lines represent the median; smaller horizontal black lines represent the interquartile range..Green = In-house (EAC); Red = Qiagen Fusion Quant Kit; Black = In-house. Blue = Onestep BCR-ABL p190 Elite MGB KIT.

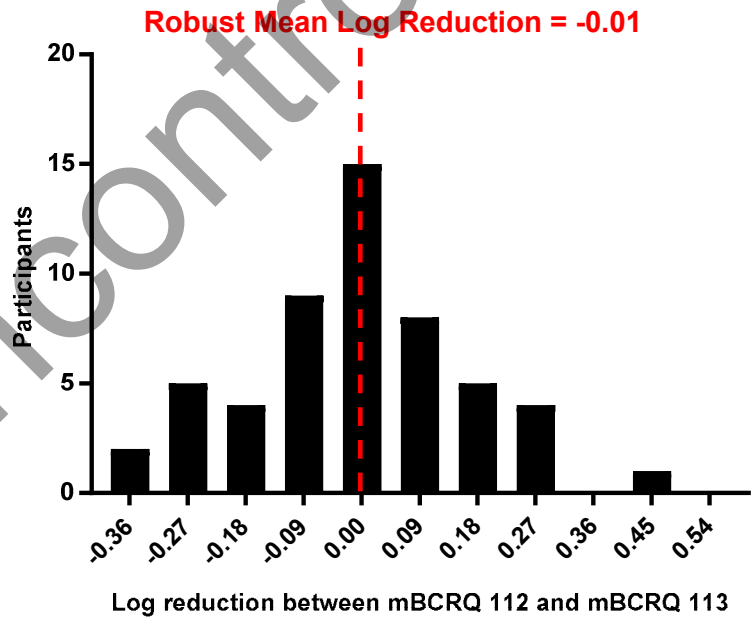

Figure 2: Frequency distribution histogram of log reduction between samples mBCRQ 112 and mBCRQ 113.

Method Breakdown:

Table 2: Control gene data summary

Control Gene	Number of Participants
ABL1	52
GUSB	3
ABL1 & *GUSB*	1
PBGD/HMBS	1

Table 3: *ABL1* copy number

Sample	mBCRQ 112	mBCRQ 113
Median	92,375	76,163
Lower Quartile	49,162	46,399
Upper Quartile	165,597	148,456
Inter Quartile Range	116,434	102,057
Min	10	30
Max	1,700,000	1,300,000
Number of participants reporting <10000 copy number	3 (6%)	2 (4%)

Table 4: Instrument manufacturer and model

Assay Protocol	Number of Participants
ABI 7500	15
Roche LC 480	12
Qiagen Rotorgene	6
ABI 7900HT	5
ABI 7500HT	4
ABI ViiA-7	3
Corbett RotorGene 6000	3
Roche LC 1.2	2
Biorad CFX96	2
ABI 7700	1
Roche LC 2.0	1
Roche LC 96	1
Quantstudio Dx	1
BioRad QX200 Droplet Digital PCR System	1

Table 5: Assay protocol data summary

Assay Protocol	Number of Participants
In-house (EAC)	32
Qiagen (formerly Ipsogen) Fusion Quant Kit	16
In-house	8
Onestep p190 elite mgb kit elitech	1

Table 6: Material used for standard curve

Material used for standard curve	Number of Participants
Qiagen (formerly Ipsogen) Fusion Quant standards	38
In-house standards	9
In-house standards calibrated to Qiagen (formerly Ipsogen) standards	4
Nanogen Alert standards	1
AB Analitica for p190, ERM-AD623 for GUSB	1
ERM AD623, ABL1	1
DeltaCt method, no standard curve is used	1
Onestep p190 elite std elitech	1
Other	1

Table 7: Assay references data summary

Assay references	Number of Participants
Gabert et al (2003) Leukemia 17, 2318-2357	42
Beillard et al (2003) Leukemia 17, 2474-2486	16
Baccarani et al (2013) Blood 122 (6), 872-84	10
Foroni et al (2011) BJH 153 (2), 179-190	7
In-house (no published reference available)	4
Emig et al (1999) Leukemia 13 (11), 1825-1832	3
Foroni et al (2009) Am J Hema 84, 517-522	3
Van Dongen et al (1999) Leukemia 13, 1901-1928	2
A Hochhaus et al (2002) Leukemia 16, 2190-2196	1
Mensink et al (1998)	1

Trial Summary:

- mBCRQ 112 and mBCRQ 113 were duplicate samples manufactured to be positive for the minor *BCR-ABL1* transcript mimicking MRD levels seen following treatment in Chronic Myeloid Leukaemia and Acute Lymphoblastic Leukaemia
- The median *BCR-ABL1/ABL1* calculated from participant returns for sample mBCRQ 112 was 0.023%, and for sample mBCRQ 113 was 0.020%.
- Two participants did not detect any *BCR-ABL1* transcript in mBCRQ 112 only. One participant did not detect any *BCR-ABL1* transcript in mBCRQ 113 only. One participant did not detect any *BCR-ABL1* transcript in both samples.
- The median delta between participants results for sample 112 and 113 was 0.005% (IQR = 0.008).
- The robust mean log reduction between sample mBCRQ 112 and mBCRQ 113, calculated from all participant returns was -0.01, with a robust SD 0.15.
- 56/57 (98.2%) participants were within 2.5 SDs of the robust mean. One laboratory was between 2.5 and 3.5 SDs.
- When data was analysed by assay type, in-house EAC results showed greater variation cf. the participants using the Qiagen Fusion Quant kit (see Figure 1).
- The median *BCR-ABL1* Ct value for sample mBCRQ 112 was 22.96, with an inter quartile range of 2.01. The median *BCR-ABL1* Ct value for sample mBCRQ 113 was 22.99, with an inter quartile range of 2.33.
- Median *ABL1* control gene levels were 92,375 for sample mBCRQ 112, and 76,163 for sample mBCRQ 113.
- Three participants had *ABL1* levels of <10,000. Two of these participants had an *ABL1* level of <10,000 for both samples. One participant had an *ABL1* level of <10,000 for sample mBCRQ 112 only.
- Median *GUSB* control gene levels were 261,568 for sample mBCRQ 112 and 330,551 for sample mBCRQ 113. With only 3 participants using *GUSB* we acknowledge the limitations of this small data set.
- If our samples do not meet your local quality control criteria please contact us for a repeat sample, as you would for clinical samples.

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 Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations 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/