

Pilot Minor *BCR-ABL1* Quantification Programme

Participant No:

Trial No: mBCRQ 151602

Date Issued: 27th Jan 2016

Date Closed: 26th Feb 2016

Two vials of lyophilised cell lines samples, mBCRQ 106 and mBCRQ 107, were issued to 53 participants for quantitative minor (e1a2) *BCR-ABL1* analysis.

Sample mBCRQ 106 was manufactured to carry no *BCR-ABL1* positive cells and sample mBCRQ 107 was manufactured carry a moderate number of *BCR-ABL1* positive cells.

In this trial, fifty-one (96.2%) participants returned results. Of the two participants who did not return results one pre notified us of their non return.

Table 1: Your Results

	Sample mBCRQ 106	Sample mBCRQ 107
Your <i>BCR-ABL1</i>/Control Gene %		
Median <i>BCR-ABL1</i>/<i>ABL1</i>	-	0.15
Lower Quartile	-	0.08
Upper Quartile	-	0.22
Inter Quartile Range (IQR)	-	0.14

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.

Having a negative sample in this trial (mBCRQ 106) means log reduction between the two trial samples has not been possible. Additionally a % *BCR-ABL1*/Control Gene histogram is not possible for sample mBCRQ 106.

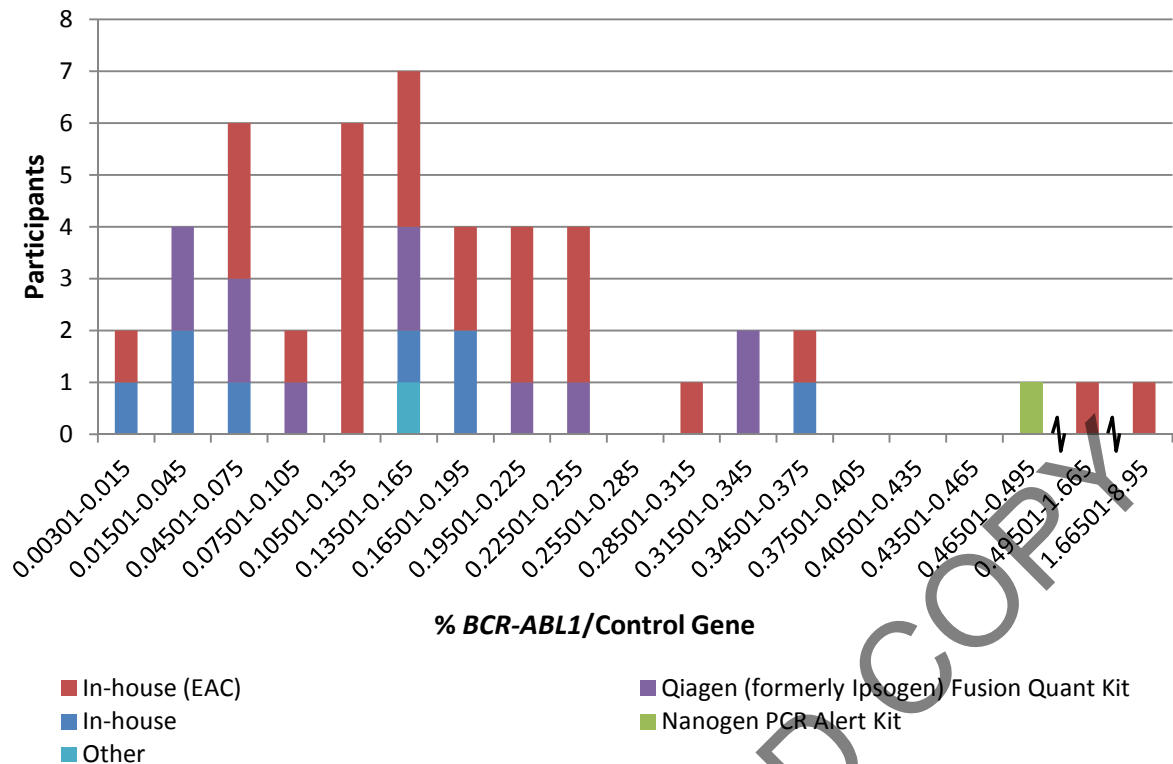


Figure 1: Frequency distribution histogram of % *BCR-ABL1*/Control Gene results for sample mBCRQ 107, by assay type.

Method Breakdown:

Table 2: Control gene data summary

Control Gene	Number of Participants
<i>ABL1</i>	47 (92.2%)
<i>GUSB</i>	2 (3.9%)
<i>ABL1 & GUSB</i>	1 (2.0%)
<i>HMBS</i>	1 (2.0%)

Table 3: *ABL1* copy number

Sample	mBCRQ 106	mBCRQ 107
Median	84,331	78,944
Lower Quartile	55,304	42,328
Upper Quartile	160,000	125,400
Inter Quartile Range	104,696	83,072
Min	6,989	2312
Max	546,100	560,000
Number of participants reporting <10000 copy number (%)	1 (2.1%)	1 (2.1%)

Table 4: Instrument manufacturer and model

Assay Protocol	Number of Participants
ABI 7500	11 (21.6%)
ABI 7900HT	8 (15.7%)
Qiagen Rotorgene	7 (13.7%)
Roche LC 480	7 (13.7%)
ABI 7500HT	3 (5.9%)
Roche LC 1.5	3 (5.9%)
ABI ViiA 7	2 (3.9%)
Corbett RotorGene	2 (3.9%)
ABI 7900	1 (2.0%)
ABI 7700	1 (2.0%)
ABI 7300	1 (2.0%)
Corbett RotorGene	1 (2.0%)
Quantstudio Dx	1 (2.0%)
Biorad CFX96	1 (2.0%)
Roche LC 96	1 (2.0%)
Roche LC 2.0	1 (2.0%)
Roche LC 1.2	1 (2.0%)

Table 5: Assay protocol data summary

Assay Protocol	Number of Participants
In-house (EAC)	28 (54.9%)
Qiagen (formerly Ipsogen) Fusion Quant Kit	12 (23.5%)
In-house	10 (19.6%)
Nanogen PCR Alert Kit	1 (2%)

Table 6: Material used for standard curve

Material used for standard curve	Number of Participants
Qiagen (formerly Ipsogen) Fusion Quant standards	35 (68.6%)
In-house standards	11 (21.6%)
Nanogen Alert standards	2 (3.9%)
In-house standards calibrated to Qiagen (formerly Ipsogen) standards	1 (2.0%)
AB Analytica (for P190) / ERM-623 (for GUSB)	1 (2.0%)
Other	1 (2.0%)

Table 7: Assay references data summary

Assay references	Number of Participants
Gabert et al (2003) Leukemia 17, 2318-2357	37
Beillard et al (2003) Leukemia 17, 2474-2486	12
Foroni et al (2011) BJH 153 (2), 179-190	6
Van Dongen et al (1999) Leukemia 13, 1901-1928	6
Baccarani et al (2013) Blood 122 (6), 872-84	4
In-house (no published reference available)	2
Emig et al (1999) Leukemia 13 (11), 1825-1832	2
Foroni et al (2009) Am J Hema 84, 517-522	1
A Hochhaus et al (2002) Leukemia 16, 2190-2196	1

Trial Summary:

- Sample mBCRQ 106 was manufactured to contain only *BCR-ABL1* negative cells and sample mBCRQ 107 was manufactured to carry a moderate number of *BCR-ABL1* positive cells.
- In line with sample formulation, 46 out of 51 (90.2%) participants reported sample mBCRQ 106 as negative for *BCR-ABL1*. Of the 5 false positive results, the median % *BCR-ABL1*/Control Gene detected was 0.0012, the range of was 0.00085-0.008. Of the 5 participants that detected the *BCR-ABL1* transcript, 3 used the Qiagen assay and 2 used an in-house assay. There was no correlation between these participants with regards to which instrument was used.
- The median % *BCR-ABL1*/*ABL1* calculated from participant returns for sample mBCRQ 107 was 0.151 with an inter quartile range of 0.15.
- The median *BCR-ABL1*/*GUSB* (n=2) calculated from participant returns for sample mBCRQ 107 was 0.019%. With only 2 participants using *GUSB* we acknowledge the statistical limitations of this small data set.
- For those participants that detected a transcript in sample mBCRQ 106, the median *BCR-ABL1* Ct value was 39.6. The median *BCR-ABL1* Ct value for sample mBCRQ 107 was 32.9, with an inter quartile range of 3.2.
- Median *ABL1* control gene levels were 84,331 for sample mBCRQ 106, and 78,944 for sample mBCRQ 107.
- One participant had an *ABL1* level of <10,000 for sample mBCRQ 106 and sample mBCRQ 107.
- Median *GUSB* control gene levels were 962,000 for sample mBCRQ 106 and 748,180 for sample mBCRQ 107. With only 2 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/