BCR::*ABL1* Minor Quantification Programme

All Participant Report

Distribution - 242501 Participant ID -

Date Issued - 06 June 2024 Closing Date - 05 July 2024

Trial Comments

This trial was issued to 141 participants. Overall, 136 (96.5%) participants returned results. Five participants did not submit results with one laboratory pre-notifying us of their non return. 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 as soon as possible.

PLEASE NOTE: As part of our transition to the ISO/IEC 17043:2023 standards all UK NEQAS LI quantitative reports for accredited programmes now include the uncertainty of the assigned value (robust mean).

Sample Comments

Two vials of lyophilised cell line material, samples mBCRQ 142 and mBCRQ 143, were issued to 141 participants for quantitative minor (e1a2) BCR::ABL1 (p190) analysis. Samples mBCRQ 142 and 143 were both manufactured (as a single batch) to be positive for the minor BCR::ABL1 transcript, mimicking measurable (minimal) residual disease (MRD) levels seen following treatment in chronic myeloid leukaemia (CML) or acute lymphoblastic leukaemia (ALL).

Results and Performance

QUANTITATIVE SCORING HAS BEEN APPLIED TO THIS TRIAL

% ratio *BCR*::*ABL1* Minor/ Reference Gene	Your Quantitative Results	Your Qualitative Results	Consensus Qualitative Results
Sample 142	0.048	Rearrangement Detected	Rearrangement Detected
Sample 143	0.047	Rearrangement Detected	Rearrangement Detected

All Participants Qualitative Results	Rearrangement Detected	No Rearrangement Detected
Sample 142	135	1
Sample 143	135	1

Result Type	Log10 Change Between Samples	Robust Mean Log10 Change	Robust SD Log10 Change	Uncertainty of the Assigned Value (Robust Mean)
% ratio *BCR*::*ABL1* Minor/Reference Gene	-0.01	0.00	0.12	± 0.01

z score*	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period		
		Satisfactory	Action	Critical
-0.08	Satisfactory	3	0	0

N/A = Not Applicable

***z score Limits Definitions**

Please note the scale below is applicable to the tables above and to the z-score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.

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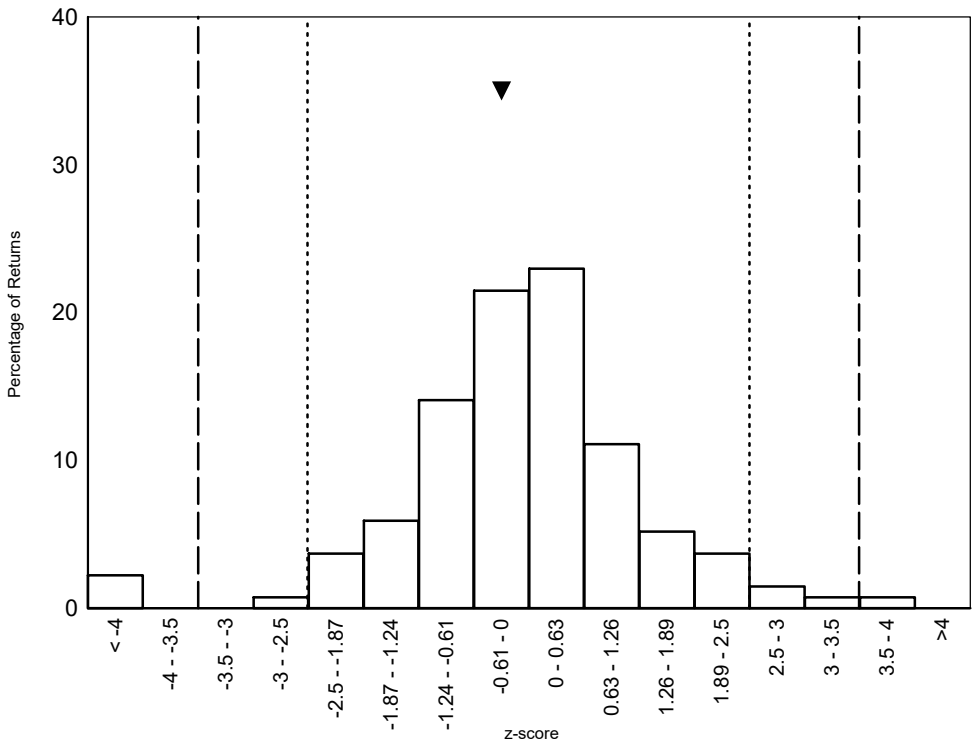
Histograms of Participant z scores

Log10 change between samples - % ratio *BCR*::*ABL1* Minor/Reference Gene

Please note ▼ denotes your result



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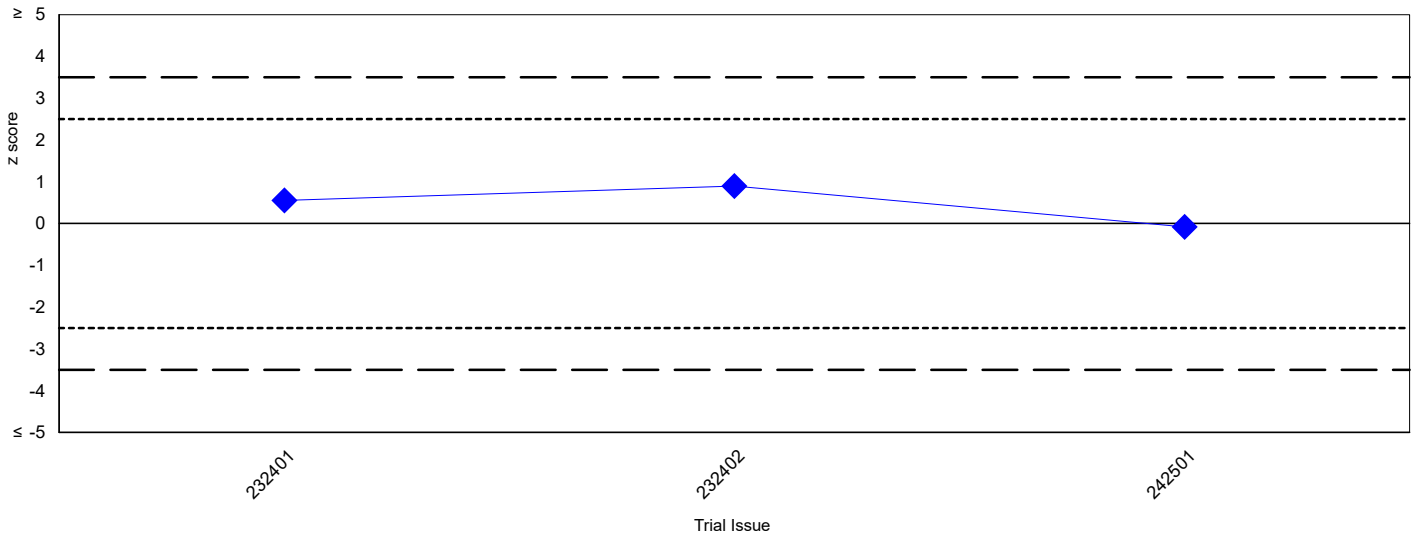
Shewhart Control Charts

(Please note each data point represents a single trial)

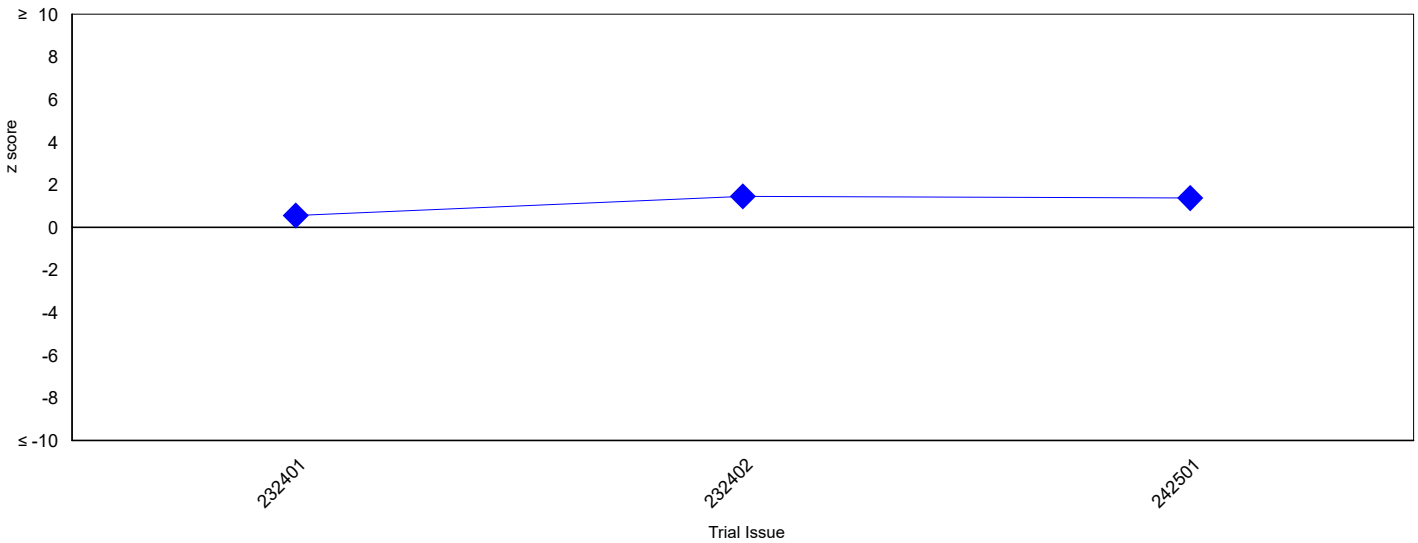
Log10 change between samples % ratio *BCR*::*ABL1* Minor/Reference Gene



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current trial and the two previous trials)

Log10 change between samples % ratio *BCR*::*ABL1* Minor/Reference Gene



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Please note, only methods/instruments used by ≥2 participants are included in the tables.

Instrument Summary

Method	Returns
Roche LC 480	24
Cepheid GeneXpert	23
Qiagen Rotorgene	18
ABI QuantStudio 5	13
Biorad QX200 Droplet Digital PCR	9
ABI 7500	8
ABI 7500 FAST	8
Biorad CFX96	6
ABI Step One Plus	5
ABI Vii A7	5
Roche LC 2.0	4
ABI QuantStudio 7	4
ABI 7500 FastDx	3
ABI QuantStudio 6	2
Diatech Pharmacogenetics Easy PGX	2

Kit/Method Summary

Method	Returns
In-house protocol (EAC)	43
Qiagen (formerly Ipsogen) Fusion Quant Kit	33
Cepheid Xpert BCR-ABL Ultra p190	23
In-house protocol	22
Onestep BCR-ABL p190 Elite MGB	4
OneStep SensiQuant BCR-ABL p190 BIOCLARMA	4
REALQUALITY RQ-BCR-ABL p190 One-Step kit	2
Diatech Pharmacogenetics Easy PGX p190	2

Material used for *BCR*::*ABL1* Minor Standard Dilutions Summary

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	63
Not Applicable	37
In-house standards	19
SensiQuant p190 Standard BIOCLARMA	4
Onestep BCR-ABL p190 Elite MGB Standards	3
AB Analitica standards	3
ERM-AD623 Certified Reference Material	2
EasyPGX Ready BCR-ABL p190	2

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Material used for Reference Gene Standard Dilutions Summary

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	57
Not Applicable	37
In-house standards	16
ERM-AD623 Certified Reference Material	13
SensiQuant p190 Standard BIOCLARMA	4
Onestep BCR-ABL p190 Elite MGB Standards	2
AB Analitica standards	2
EasyPGX Ready BCR-ABL p190	2

Reference Gene Summary

Method	Returns
ABL1	122
GUSB	12

Assay Reference Summary

Method	Returns
Gabert et al. (2003) Leukemia 17(12):2318-2357	63
In house (no reference)	28
Baccarani et al. (2013) Blood 122(6):872-884	10
Cross et al. (2015) Leukemia 29(5):999-1003	8
Hochhaus et al. (2020) Leukemia 34(4):966-984	7
Foroni et al. (2011) Br J Haematol. 153(2):179-190	5
Pfeifer et al. (2019) Leukemia 33(8):1910-1922	5
Recommendations of the European Working Group for Adult AL	4
van Dongen et al. (1999) Leukemia 13(12):1901-1928	3
Commercial kit (reference not known)	3

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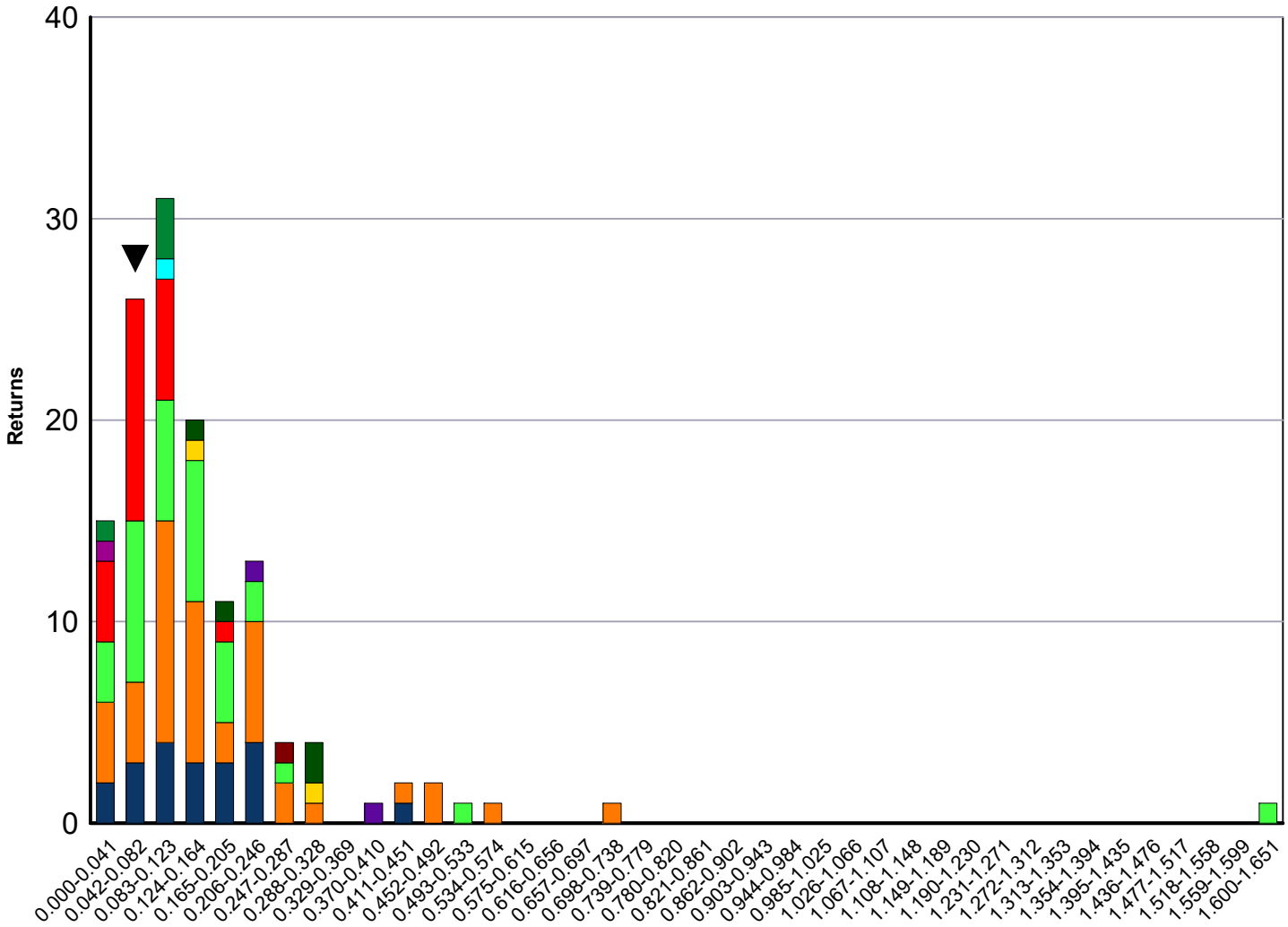
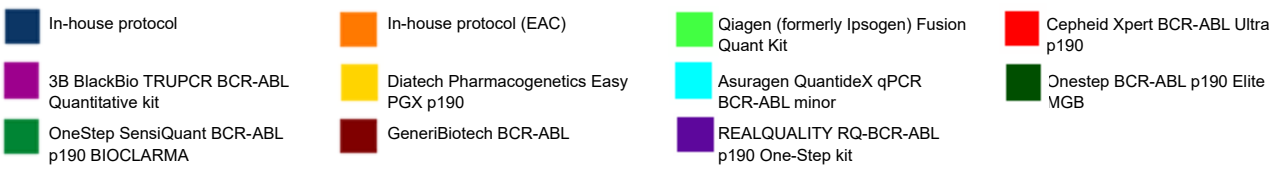
Frequency distribution histogram showing participant% ratio *BCR*::*ABL1* minor/Reference gene results, classified by kit or method for sample mBCRQ 142





Gross outliers may have been excluded from this plot to facilitate the display of data points.

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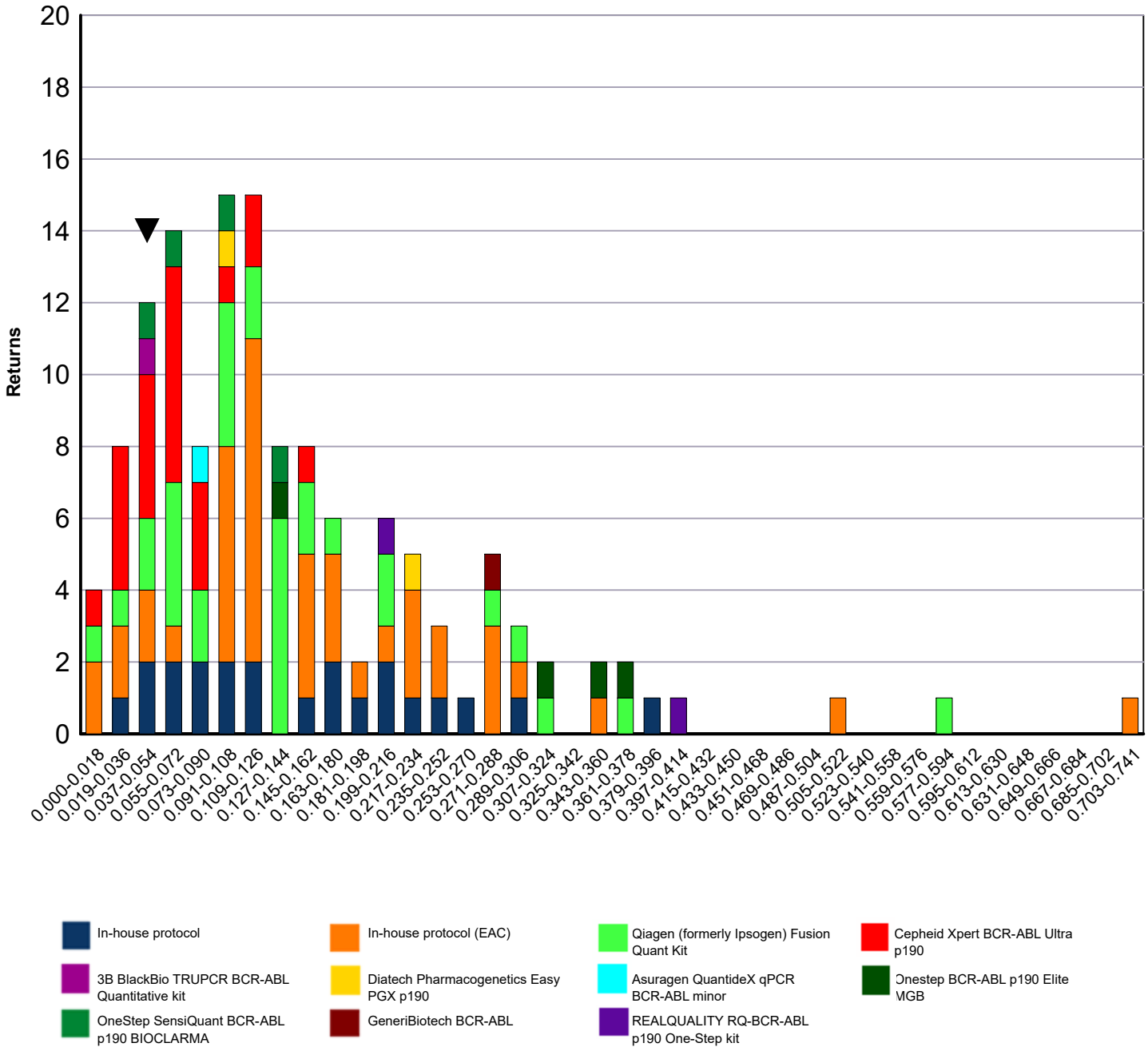
Frequency distribution histogram showing participant% ratio *BCR*::*ABL1* minor/Reference gene results, classified by kit or method for sample mBCRQ 143



In-house protocol
In-house protocol (EAC)
Qiagen (formerly Ipsogen) Fusion Quant Kit
Cepheid Xpert BCR-ABL Ultra p190
3B BlackBio TRUPCR BCR-ABL Quantitative kit
Diatech Pharmacogenetics Easy PGX p190
Asuragen QuantideX qPCR BCR-ABL minor
Onestep BCR-ABL p190 Elite MGB
OneStep SensiQuant BCR-ABL p190 BIOCLARMA
GeneriBiotech BCR-ABL
REALQUALITY RQ-BCR-ABL p190 One-Step kit

Gross outliers may have been excluded from this plot to facilitate the display of data points.

BCR::*ABL1* Minor Quantification Programme

PLEASE NOTE: As part of our transition to the ISO/IEC 17043:2023 standards all UK NEQAS LI quantitative reports for accredited programmes now include the uncertainty of the assigned value.

This report shows the uncertainty of the assigned value (robust mean) for the % ratio *BCR*::*ABL1*/Reference gene $\log_{10}$ change in accordance with the ISO 13528:2022 and ISO/IEC 17043:2023 standards.

Trial Comments

BCR::*ABL1* (e1a2) minor (p190) qualitative data

- In line with sample composition, a *BCR*::*ABL1* minor transcript was detected by 135/136 (99.3%) of returning participants in samples mBCRQ 142 and mBCRQ 143. The single laboratory failing to identify the rearrangement in both samples utilised the Cepheid Xpert BCR-ABL Ultra p190 kit.

BCR::*ABL1* (e1a2) minor (p190) quantitative data - *ABL1* reference gene

- The median % ratio *BCR*::*ABL1*/*ABL1* for participants using *ABL1* as a reference gene for sample mBCRQ 142 was 0.12 with an inter quartile range (IQR) of 0.12 (n=121).
- The median % ratio *BCR*::*ABL1*/*ABL1* for participants using *ABL1* as a reference gene for sample mBCRQ 143 was 0.12 with an inter quartile range (IQR) of 0.13 (n=121).
- Ninety three out of the 122 participants utilising *ABL1* as the sole reference gene for *BCR*::*ABL1* minor quantification returned copy number information. Median *ABL1* control gene levels were 98,000 and 91,558 copies for samples mBCRQ 142 and mBCRQ 143, respectively.
- Eight laboratories (8/93, 8.6%) reported *ABL1* levels <10,000 copies for at least one of the samples. Amplification resulting in <10,000 *ABL1* molecules per sample is considered sub-optimal[1] and participants are reminded that repeat samples are typically available for all trials. To request repeat samples, please contact repeatsamples@ukneqasli.co.uk. If you would like additional technical support regarding the processing of and/or nucleic acid extraction from of our lyophilised EQA sample material, please contact admin@ukneqasli.co.uk.

BCR::*ABL1* (e1a2) minor (p190) quantitative data - *GUSB* reference gene

- Twelve participants reported utilising *GUSB* as the sole reference gene for *BCR*::*ABL1* minor quantification. We acknowledge the limitations of this small dataset.
- The median % ratio *BCR*::*ABL1*/*GUS* for participants using *GUSB* as a reference gene for sample mBCRQ 142 was 0.058 with an inter quartile range (IQR) of 0.061 (n=12).
- The median % ratio *BCR*::*ABL1*/*GUS* for participants using *GUSB* as a reference gene for sample mBCRQ 143 was 0.050 with an inter quartile range (IQR) of 0.068 (n=12).

- Median *GUSB* reference gene levels were 262,039 and 315,253 copies for samples mBCRQ 142 and mBCRQ 143, respectively (n=11).
- No participant utilising *GUSB* as the reference gene reported levels <24,000 copies for sample mBCRQ 142 or mBCRQ 143.

Median returned *BCR*::*ABL1* minor (e1a2, p190) mean absolute level (all methods) for samples mBCRQ 142 and mBCRQ 143 was 112 copies (n=103) and 108 copies (n=103), respectively.

Log_{10} change data

- In keeping with the sample formulation, the robust mean log_{10} change between sample mBCRQ 142 and mBCRQ 143 was 0.00 with a robust standard deviation (SD) of 0.12.
- Four participants incurred a critical trial score with a z score of <-3.5 or >3.5. Three of the laboratories utilised the Cepheid GeneXpert Ultra BCR-ABL p190 assay and the other an in-house RT-qPCR assay.

Final Remarks

Please note the use of a different method (assay approach or instrument type) for the quantification of individual EQA/PT samples within the same trial distribution is not compatible with the basis of the *BCR*::*ABL1* Minor Quantification programme as it undermines the application of z scores to the log_{10} change value, which underpins the scoring system utilised. The design of this EQA/PT programme is shaped by the absence of an International Scale (IS) for the *BCR*::*ABL1* minor (e1a2, p190) transcript, and the inherent variability of RNA extraction, cDNA synthesis and the RT-qPCR analysis methods. Please refer to our website for current performance monitoring system information.

For this programme there is currently no stipulation for the testing of EQA/PT samples to be undertaken on the same or separate assay runs. UK NEQAS LI plans to review the current online data entry pages for this programme with the aim of potentially adapting the design to permit the submission of RT-qPCR standard curve information from more than one run. This would better accommodate the return of results when the two EQA/PT samples provided in a given trial distribution are analysed on separate assay runs. We will also continue to monitor the uptake of digital PCR and, as appropriate, may be required to make further modifications to the online data entry pages and trial report format in the future.

In the absence of an IS for the *BCR*::*ABL1* minor transcript, it is prudent that MRD assessment for a given patient be conducted using the same method. Or an internal validation performed to account for the impact of potential bias between the different methodological approaches.

Reference(s)

1. Pfeifer, H. *et al.* Standardisation and consensus guidelines for minimal residual disease assessment in Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL) by real-time quantitative reverse transcriptase PCR of e1a2 BCR-ABL1. *Leukemia* 33(8):1910–1922 (2019). Erratum in: *Leukemia* 34(7):1970 (2020).

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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
e-mail: amanda.newbould@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) Pre issue and post closure testing of samples for this programme is subcontracted, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were subcontracted. Where subcontracting occurs, it is placed with a competent subcontractor and the EQA provider is responsible for this work.

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/