BCR::*ABL1* Minor Quantification Programme

All Participant Report

Distribution - 232401 Participant ID -

Date Issued - 24 July 2023 Closing Date - 01 September 2023

Trial Comments

For this trial, 120 (95.2%) participants returned results. Six participants did not submit results for this trial with two laboratories pre-notifying us of this. 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.

Sample Comments

Two vials of lyophilised cell line material, samples mBCRQ 136 and mBCRQ 137, were issued to 126 participants for quantitative minor (e1a2) BCR::ABL1 (p190) analysis. Samples mBCRQ 136 and 137 were manufactured 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 136	0.396	Rearrangement Detected	Rearrangement Detected
Sample 137	0.272	Rearrangement Detected	Rearrangement Detected

All Participants Qualitative Results	Rearrangement Detected	No Rearrangement Detected
Sample 136	119	1
Sample 137	119	1

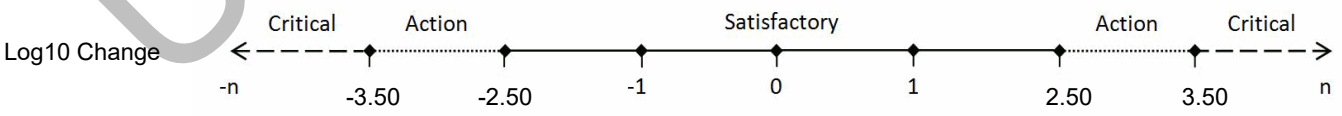
Result Type	Log10 Change Between Samples	Robust Mean Log10 Change	Robust SD Log10 Change
% ratio *BCR*::*ABL1* Minor/Reference Gene	-0.16	-0.21	0.09

z score*	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period		
		Satisfactory	Action	Critical
0.56	Satisfactory	1	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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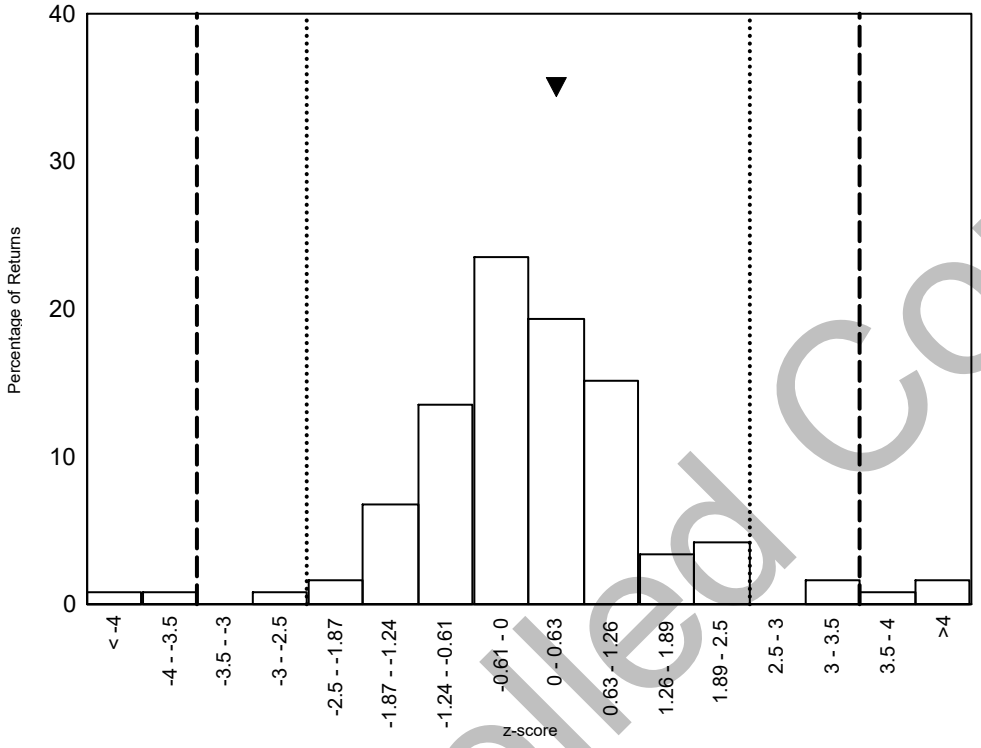
Histograms of Participant z scores

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

Please note ▼ denotes vour result



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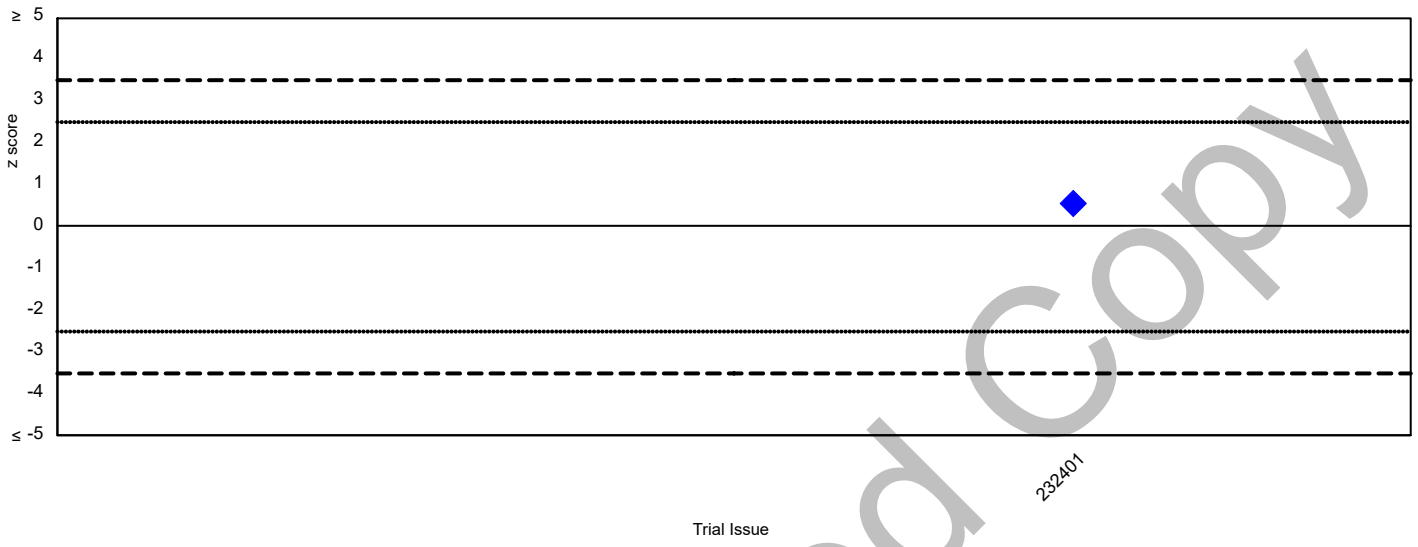
Shewhart Control Charts

(Please note each data point represents a single trial)

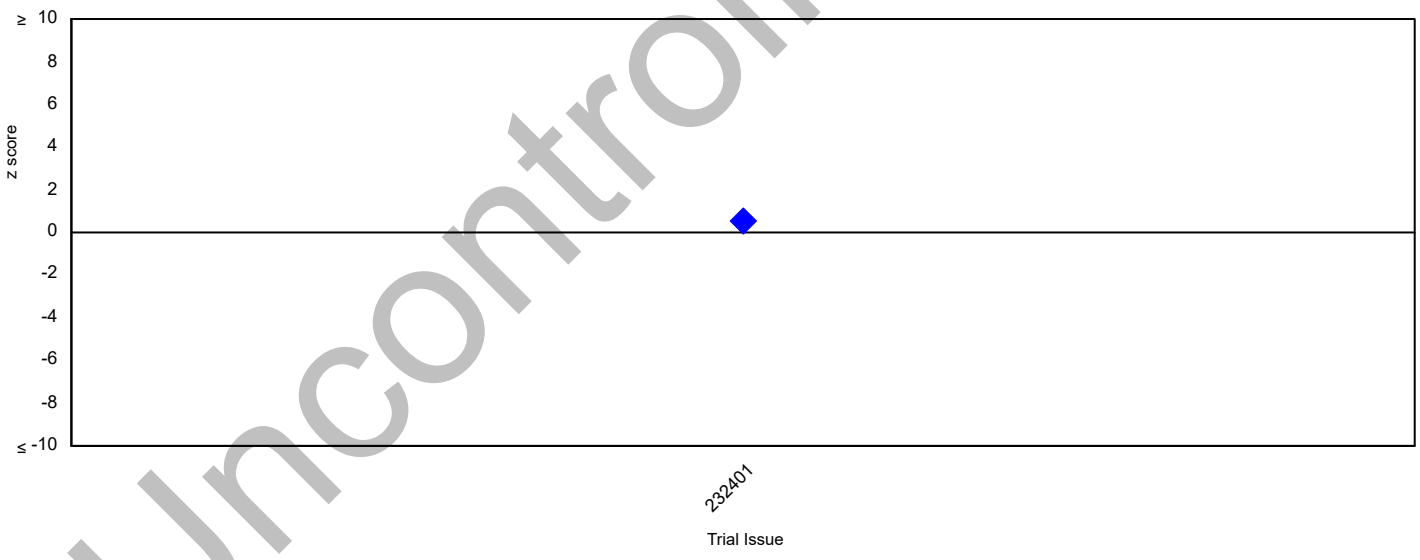
Log10 change between samples % ratio *BCR*::*ABL1* Minor/Reference Gene



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	25
Qiagen Rotorgene	16
Cepheid GeneXpert	14
Biorad QX200 Droplet Digital PCR	10
ABI QuantStudio 5	9
ABI 7500	7
ABI Step One Plus	5
Roche LC 2.0	5
ABI Vii A7	5
ABI 7500 FAST	5
Biorad CFX96	4
ABI QuantStudio 7	3
ABI 7500 FastDx	3
ABI QuantStudio 6	3
ABI 7900HT	2
Agilent AriaDx	2

Kit/Method Summary

Method	Returns
In-house protocol (EAC)	43
Qiagen (formerly Ipsogen) Fusion Quant Kit	31
In-house protocol	21
Cepheid Xpert BCR-ABL Ultra p190	14
Onestep BCR-ABL p190 Elite MGB	4
BioRad Expert Design Assay BCR-ABL p190	2
OneStep SensiQuant BCR-ABL p190 BIOCLARMA	2

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

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	60
Not Applicable	27
In-house standards	23
Onestep BCR-ABL p190 Elite MGB Standards	3
Mannheim standards	2
SensiQuant p190 Standard BIOCLARMA	2

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Material used for Reference Gene Standard Dilutions Summary

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	53
Not Applicable	29
In-house standards	20
ERM-AD623 Certified Reference Material	13
Mannheim standards	2
SensiQuant p190 Standard BIOCLARMA	2

Reference Gene Summary

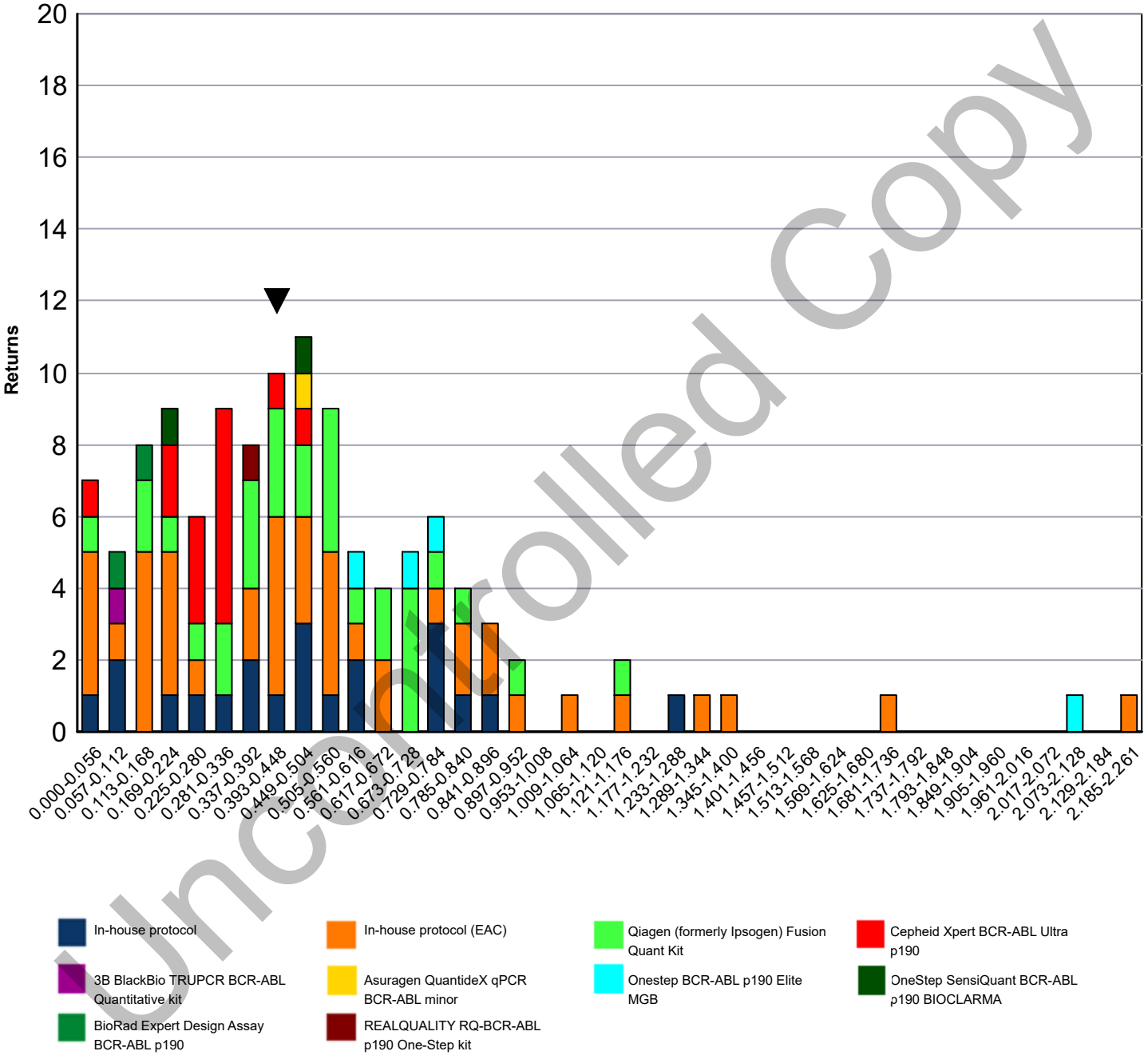
Method	Returns
ABL1	103
GUSB	13
HMBS (PGD)	3

Assay Reference Summary

Method	Returns
Gabert et al. (2003) Leukemia 17(12):2318-2357	56
In house (no reference)	27
Baccarani et al. (2013) Blood 122(6):872-884	11
Hochhaus et al. (2020) Leukemia 34(4):966-984	6
Cross et al. (2015) Leukemia 29(5):999-1003	5
Foroni et al. (2011) Br J Haematol. 153(2):179-190	4
van Dongen et al. (1999) Leukemia 13(12):1901-1928	4
Beillard et al. (2003) Leukemia 17(12):2474-2486	3
Recommendations of the European Working Group for Adult ALL	2

BCR::*ABL1* Minor Quantification Programme

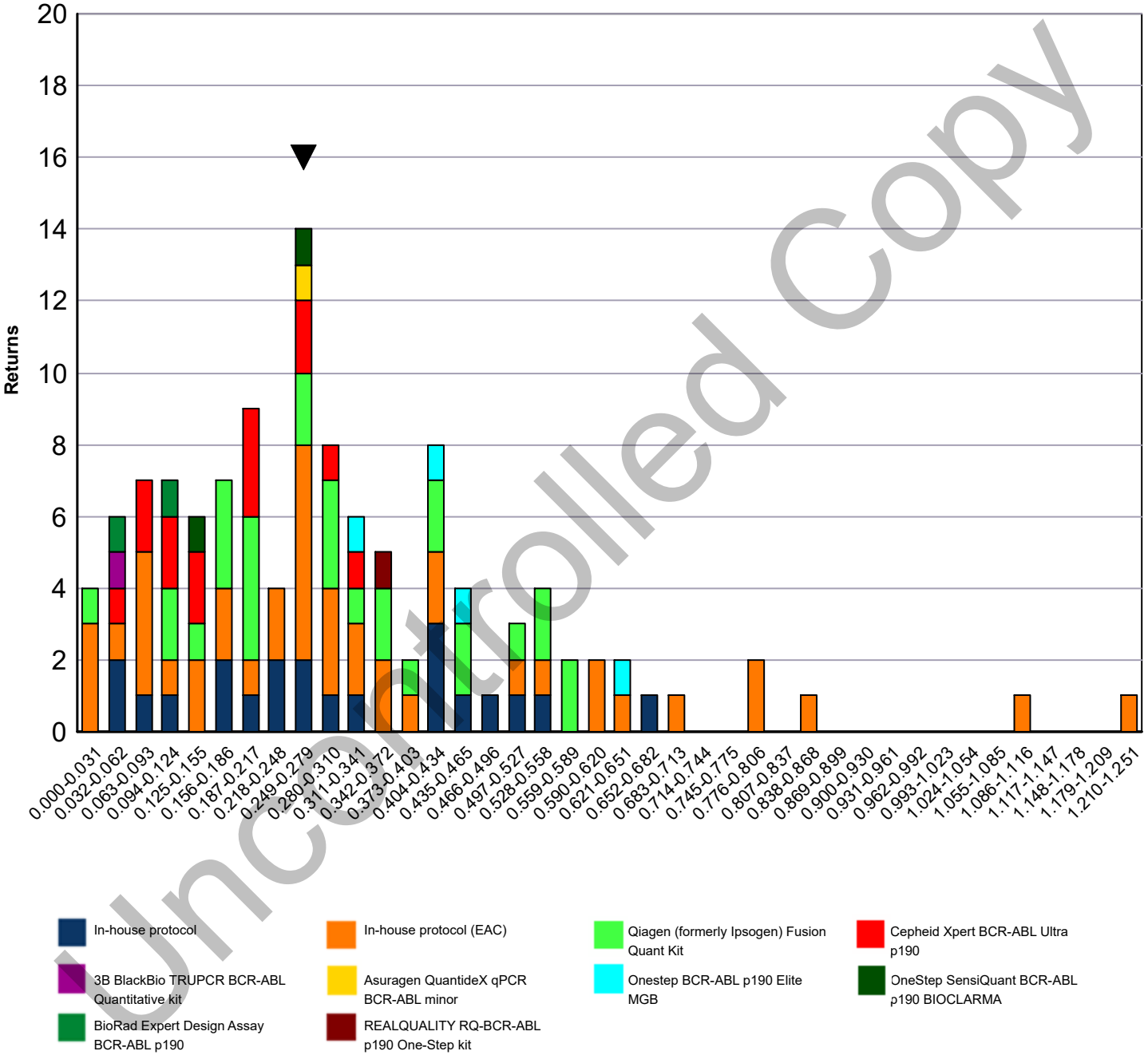
Frequency distribution histogram showing participant% ratio *BCR*::*ABL1* minor/Reference gene results, classified by kit or method for sample mBCRQ 136



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

BCR::*ABL1* Minor Quantification Programme

Frequency distribution histogram showing participant% ratio *BCR*::*ABL1* minor/Reference gene results, classified by kit or method for sample mBCRQ 137



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

***BCR*::*ABL1* Minor Quantification Programme**

Trial Comments

IMPORTANT: This programme is now ISO 17043:2010 accredited with formal scoring and performance monitoring applied.

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

- The *BCR*::*ABL1* minor transcript was detected by 119/120 (99.2%) participants in samples mBCRQ 136 and mBCRQ 137. The same participant did not detect *BCR*:*ABL1* minor in either sample and used the RT-qPCR Qiagen (formerly Ipsogen) Fusion Quant Kit; reporting *ABL1* reference gene copy numbers of 253 and 356, respectively.
- Amplification resulting in <10,000 *ABL1* molecules per sample is considered sub-optimal and participants are reminded not to submit results based on suboptimal quality control[1]. Repeat samples are available for all trials. To request repeat samples, please contact repeatsamples@ukneqasli.co.uk

ABL1 reference gene data

- The median % ratio *BCR*::*ABL1*/*ABL1* for participants using *ABL1* as a reference gene for sample mBCRQ 136 was 0.46, with an inter quartile range (IQR) of 0.38.
- The median % ratio *BCR*::*ABL1*/*ABL1* for participants using *ABL1* as a reference gene for sample mBCRQ 137 was 0.28, with an IQR of 0.23.
- Eighty four out of 103 participants utilising *ABL1* as the sole reference gene for *BCR*::*ABL1* minor quantification returned copy number information. Median *ABL1* control gene levels were 109,694 and 119,148 for samples mBCRQ 136 and mBCRQ 137, respectively.
- For sample mBCRQ 136 there were 7 (8.3%) participants that reported *ABL1* levels <10,000. For sample mBCRQ 137, there were five (6.0%) participants that reported *ABL1* levels <10,000. Amplification resulting in <10,000 *ABL1* molecules per sample is considered sub-optimal[1] and participants are reminded that repeat samples are available for all trials. To request repeat samples, please contact repeatsamples@ukneqasli.co.uk

GUSB reference gene data

- Thirteen 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*/*GUSB* for participants using *GUSB* as a reference gene for sample mBCRQ 136 was 0.16, with an IQR of 0.30.
- The median % ratio *BCR*::*ABL1*/*GUSB* for participants using *GUSB* as a reference gene for sample mBCRQ 137 was 0.11 with an IQR of 0.22.
- Median *GUSB* reference gene levels were 326,033 and 300,786 for samples mBCRQ 136 and mBCRQ 137, respectively.

- One participant utilising *GUSB* as the reference gene reported levels <24,000 for both sample mBCRQ 136 and mBCRQ 137.

Log_{10} change data

- The robust mean log_{10} change between sample mBCRQ 136 and mBCRQ 137 was -0.21 with a robust standard deviation (SD) of 0.09.
- Five participants incurred a critical trial score with a z score of <-3.5 / >3.5.
- Of the five participants, three utilised the Cepheid GeneXpert Ultra BCR-ABL p190 assay, one utilised an in-house (EAC) RT-qPCR protocol and one used the Onestep BCR-ABL p190 Elite MGB assay.

Please note the use of a different method for the quantification of individual EQA/PT samples within the same trial distribution is not compatible with the log_{10} change approach 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 (p190) transcript, and the inherent variability of RNA extraction, cDNA synthesis and the RT-qPCR analysis methods. Please refer to our website for performance monitoring system information: https://www.uknegasli.co.uk/ega-pt-programmes/molecular-haemato-oncology-programmes/bcr-abl1-minor-quantification-accredited/

In the absence of an IS for the *BCR*::*ABL1* minor transcript, it is prudent that ongoing 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 methods run within the same laboratory.

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**, 1910–1922 (2019). Erratum in: *Leukemia* 34(7):1970 (2020).

BCR::*ABL1* Minor Quantification Programme

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: 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) 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/