BCR::*ABL1* Minor Quantification Programme

All Participant Report

Distribution - 252602

Participant ID -

Date Issued - 30 October 2025

Closing Date - 28 November 2025

Trial Comments

This trial was issued to 142 participants. Overall, 132 (93.0%) participants returned results. Ten participants did not submit results with three laboratories notifying us of extenuating circumstances. 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 150 and mBCRQ 151, were issued to 142 participants for quantitative minor (e1a2) BCR::ABL1 (p190) analysis. Samples mBCRQ 150 and 151 were both 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 150	0.55	Rearrangement Detected	Rearrangement Detected
Sample 151	0.094	Rearrangement Detected	Rearrangement Detected

All Participants Qualitative Results	Rearrangement Detected	No Rearrangement Detected
Sample 150	132	0
Sample 151	132	0

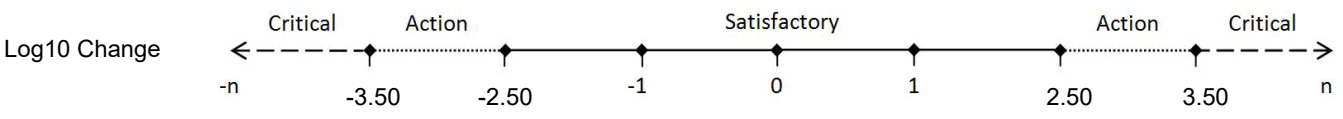
Result Type	Log10 Change Between Samples	Robust Mean Log10 Change	Robust SD Log10 Change	Uncertainty of the Assigned Value (Robust Mean)
% ratio *BCR*::*ABL1* Minor/Reference Gene	-0.77	-0.56	0.14	± 0.02

z score*	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period		
		Satisfactory	Action	Critical
-1.50	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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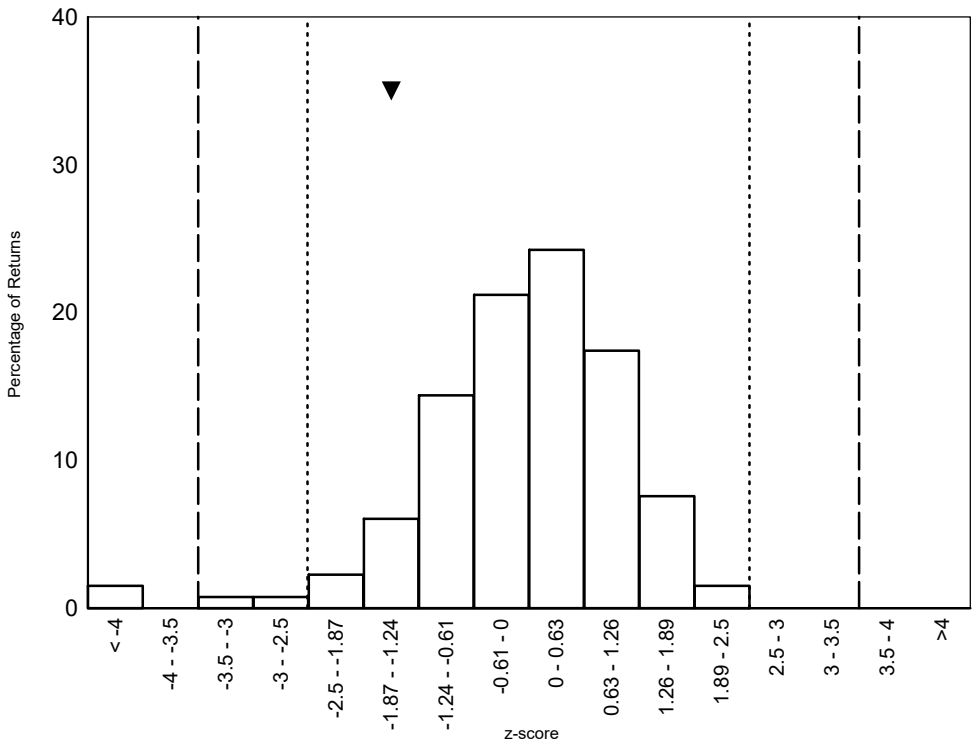
Histograms of Participant z scores

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

Please note ▼ denotes your result



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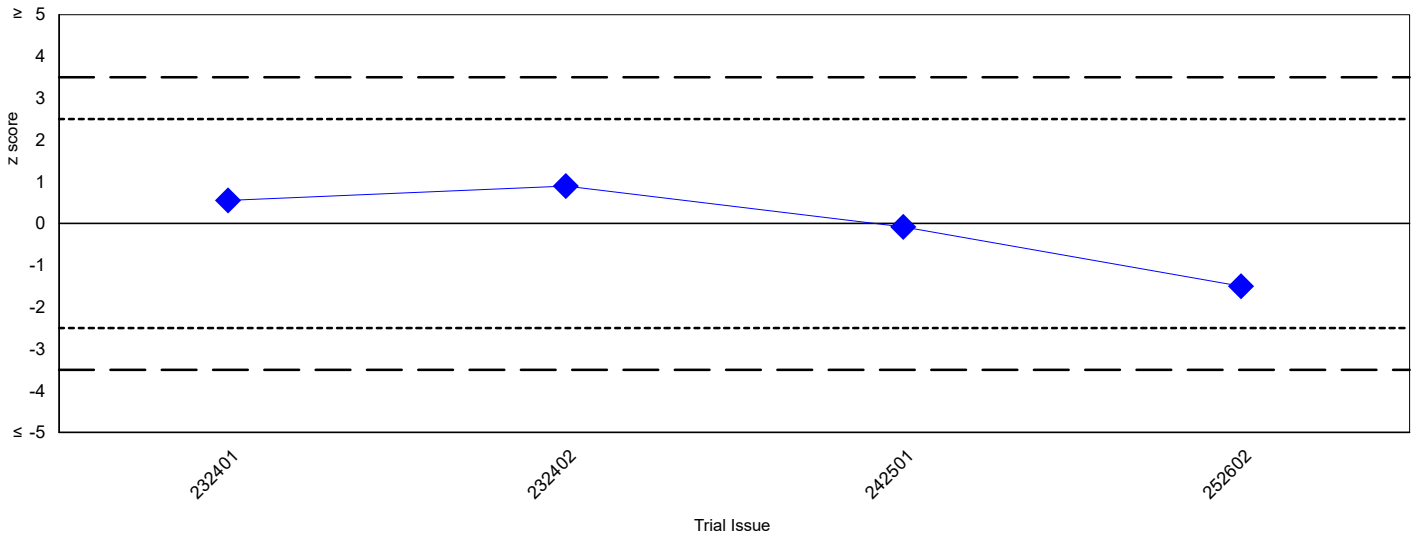
Shewhart Control Charts

(Please note each data point represents a single trial)

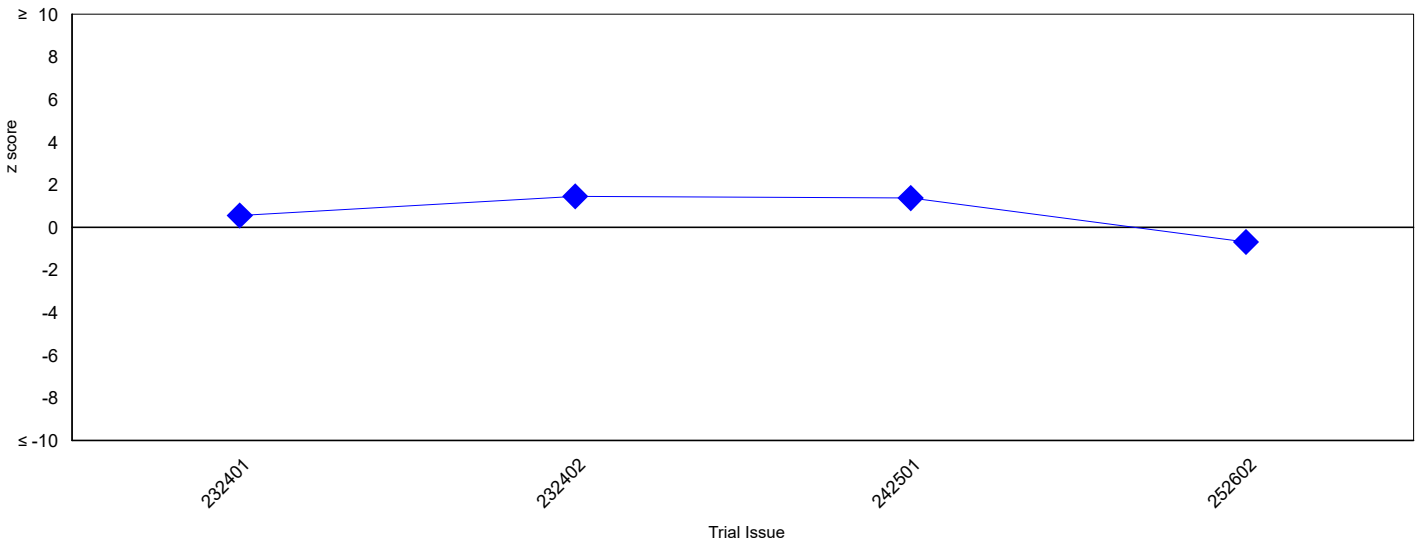
Log10 change between samples % ratio *BCR*::*ABL1* Minor/Reference Gene



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
Cepheid GeneXpert	26
Roche LC 480	22
Qiagen Rotorgene	14
ABI QuantStudio 5	14
Biorad QX200 Droplet Digital PCR	11
ABI 7500	10
Biorad CFX96	6
ABI QuantStudio 7	5
ABI 7500 FastDx	5
ABI Step One Plus	4
ABI Vii A7	3
ABI 7500 FAST	3
Diatech Pharmacogenetics Easy PGX	3
ABI QuantStudio 6	2
Agilent AriaDx	2

Kit/Method Summary

Method	Returns
In-house protocol (EAC)	42
Qiagen (formerly Ipsogen) Fusion Quant Kit	28
Cepheid Xpert BCR-ABL Ultra p190	26
In-house protocol	19
OneStep SensiQuant BCR-ABL p190 BIOCLARMA	5
Onestep BCR-ABL p190 Elite MGB	4
Diatech Pharmacogenetics Easy PGX p190	4
REALQUALITY RQ-BCR-ABL p190 One-Step kit	2

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

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	55
Not Applicable	39
In-house standards	16
SensiQuant p190 Standard BIOCLARMA	5
Onestep BCR-ABL p190 Elite MGB Standards	4
AB Analitica standards	4
EasyPGX Ready BCR-ABL p190	4
ERM-AD623 Certified Reference Material	3

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Material used for Reference Gene Standard Dilutions Summary

Method	Returns
Qiagen (formerly Ipsogen) Fusion Quant standards	52
Not Applicable	38
In-house standards	14
ERM-AD623 Certified Reference Material	12
SensiQuant p190 Standard BIOCLARMA	5
EasyPGX Ready BCR-ABL p190	4
Onestep BCR-ABL p190 Elite MGB Standards	3
AB Analitica standards	2

Reference Gene Summary

Method	Returns
ABL1	115
GUSB	14

Assay Reference Summary

Method	Returns
Gabert et al. (2003) Leukemia 17(12):2318-2357	53
Commercial kit (reference not known)	35
In house (no reference)	15
Baccarani et al. (2013) Blood 122(6):872-884	7
Cross et al. (2015) Leukemia 29(5):999-1003	5
Hochhaus et al. (2020) Leukemia 34(4):966-984	4
Pfeifer et al. (2019) Leukemia 33(8):1910-1922	4
Foroni et al. (2011) Br J Haematol. 153(2):179-190	3
Recommendations of the European Working Group for Adult AL	3
van Dongen et al. (1999) Leukemia 13(12):1901-1928	2

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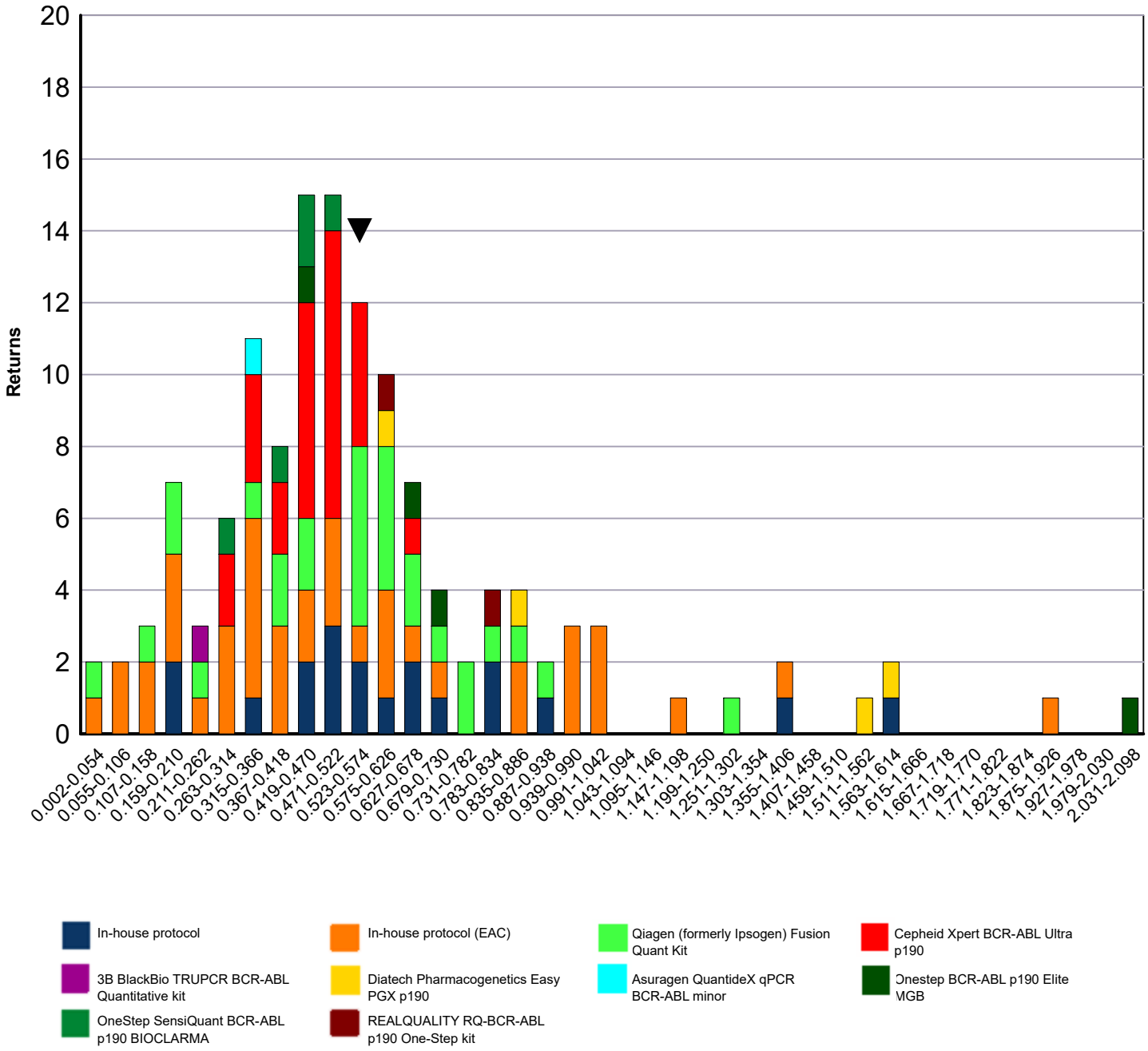
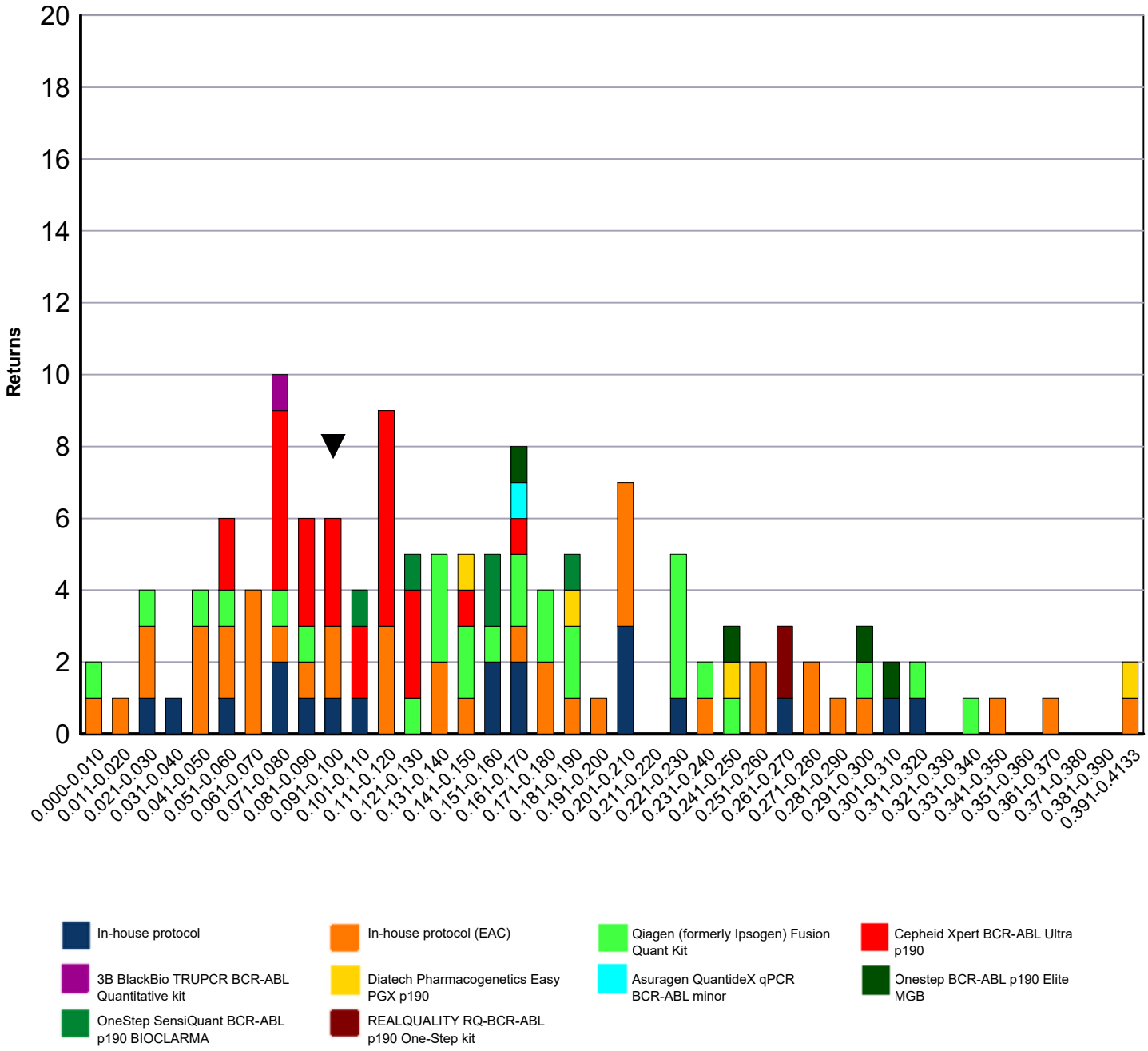
Frequency distribution histogram showing participant% ratio *BCR*::*ABL1* minor/Reference gene results, classified by kit or method for sample mBCRQ 150



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 151



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

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

Trial Comments

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

- In line with sample composition, a *BCR*::*ABL1* minor transcript was detected by all returning participants for samples mBCRQ 150 and mBCRQ 151 (n=132).

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 150 was 0.51 with an inter quartile range (IQR) of 0.32 (n=115).
- The median % ratio *BCR*::*ABL1*/*ABL1* for participants using *ABL1* as a reference gene for sample mBCRQ 151 was 0.15 with an inter quartile range (IQR) of 0.13 (n=115).
- Eighty two of the 115 participants utilising *ABL1* as the sole reference gene for *BCR*::*ABL1* minor quantification returned copy number information. Median *ABL1* control gene levels were 117,658 and 95,574 copies for samples mBCRQ 150 and mBCRQ 151, respectively.
- Four laboratories (4/82, 4.9%) 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

- Fourteen 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 150 was 0.21 with an inter quartile range (IQR) of 0.32 (n=14).
- The median % ratio *BCR*::*ABL1*/*GUS* for participants using *GUSB* as a reference gene for sample mBCRQ 151 was 0.070 with an inter quartile range (IQR) of 0.073 (n=14).
- Median *GUSB* reference gene levels were 469,611 and 514,527 copies for samples mBCRQ 150 and mBCRQ 151, respectively (n=13).
- No participant utilising *GUSB* as the reference gene reported levels <24,000 copies for sample mBCRQ 150 or mBCRQ 151.

Median returned *BCR*::*ABL1* minor (e1a2, p190) mean absolute level (all methods) for samples mBCRQ 150 and mBCRQ 151 was 727 copies (n=95) and 179 copies (n=95), respectively.

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Log_{10} change data

- The robust mean log_{10} change between sample mBCRQ 150 and mBCRQ 151 was -0.56 with a robust standard deviation (SD) of 0.14.
- Two participants incurred a critical trial score with a z score of <-3.5 or >3.5. Both laboratories utilised 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 for each 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 17043:2023

7.4.3.2 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: admin@ukneqasli.co.uk

7.4.3.2 b) Person(s) authorising this report: Mr Stuart Scott (Director) of UK NEQAS LI.

7.4.3.2 c) Administration and shipping for this programme is provided by EQA International Limited.

7.4.3.2 c) Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider. Aside from the activities mentioned above, no other activities in relation to this EQA exercise were externally provided.

7.4.3.2 c) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

7.4.3.2 f) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel (NQAAP) is informed when a UK participant is identified as having performance issues. Please note, the activities of the NQAAPs are currently paused, whilst alternative funding mechanisms are sought.

7.4.3.2 h) All EQA samples are prepared in accordance with strict standard operating procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue.

7.4.3.2 j), m), n), o) & r) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the design and implementation of the programme, example annotated reports including and the performance systems applied to assess performance (for BS EN ISO/IEC 17043:2023 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.

7.4.3.2 l) We do not assign values against reference materials or calibrants.

7.4.3.2 q) Details of the programme designs as authorised 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.

7.4.3.2 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/

7.4.3.2) 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/