UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

BCR::ABL1 Major Quantification Programme

All Participant Report

Distribution - 232401 Participant ID -

Date Issued - 30 May 2023 Closing Date - 30 June 2023

Trial Comments

The trial was issued to 311 participants; 286 (92.0%) participants returned results. Of the 25 laboratories that did not return results, 10 pre-notified us of their intention to not return results and five participants requested an extension to results submission. Twelve participants returned non-International Scale (IS) data only, 113 participants returned IS data only and 161 participants returned both non-IS and IS data.

Sample Comments

Two vials of lyophilised material (BCRQ 185 and BCRQ 186) were issued for quantitative BCR::ABL1 analysis.

Results and Performance - Performance monitoring based on Single Sample z-score

	Your Unconverted Results (*BCR*::*ABL1*/Reference Gene %)	Your IS Results (%)	Your Log Transformed IS Result	Log Transformed Robust Mean
BCRQ 185	0.0152	0.0103	-1.99	-1.92
BCRQ 186	0.6053	0.408	-0.39	-0.32

Result Type	Log Change Between Samples	Robust Mean Log Change	Robust SD Log Change
Unconverted	-1.60	-1.56	0.23
IS	-1.60	-1.58	0.23
The Result on which you chose to be scored	Both % and IS		

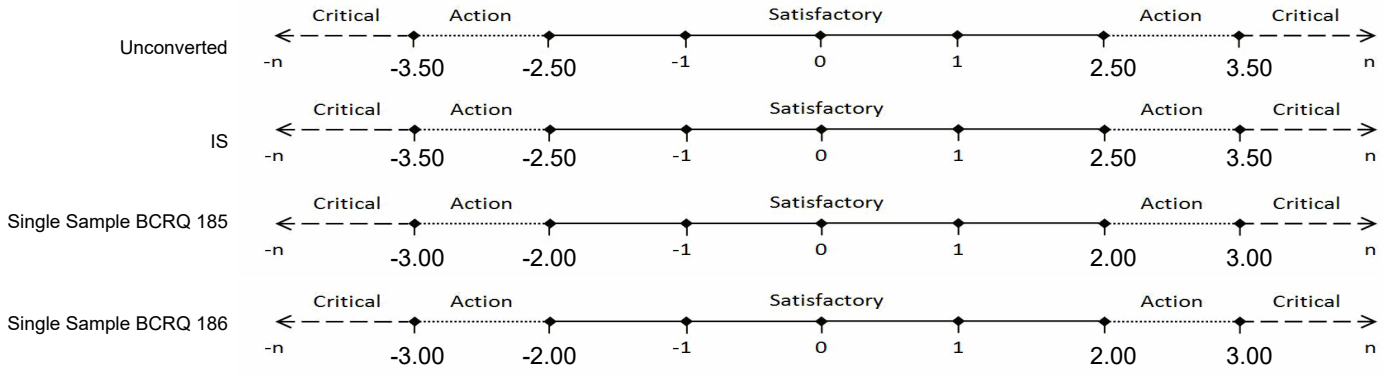
Sample	Single Sample z-score**	Performance Status for this Trial	Performance Status Classification Over 6 Sample Period		
			Satisfactory	Action	Critical
BCRQ 185	-0.29	Satisfactory	5	0	0
BCRQ 186	-0.29	Satisfactory	6	0	0

Reported Log Change	Log Change z-score**	Performance Status for this Trial *	Performance Status Classification Over 3 Trial Period		
			Satisfactory	Action	Critical
Unconverted	-0.17	Satisfactory	3	0	0
IS	-0.09	Satisfactory	3	0	0

* Please note, the information in this table is for information only. Performance monitoring is based on Single Sample data only

****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.

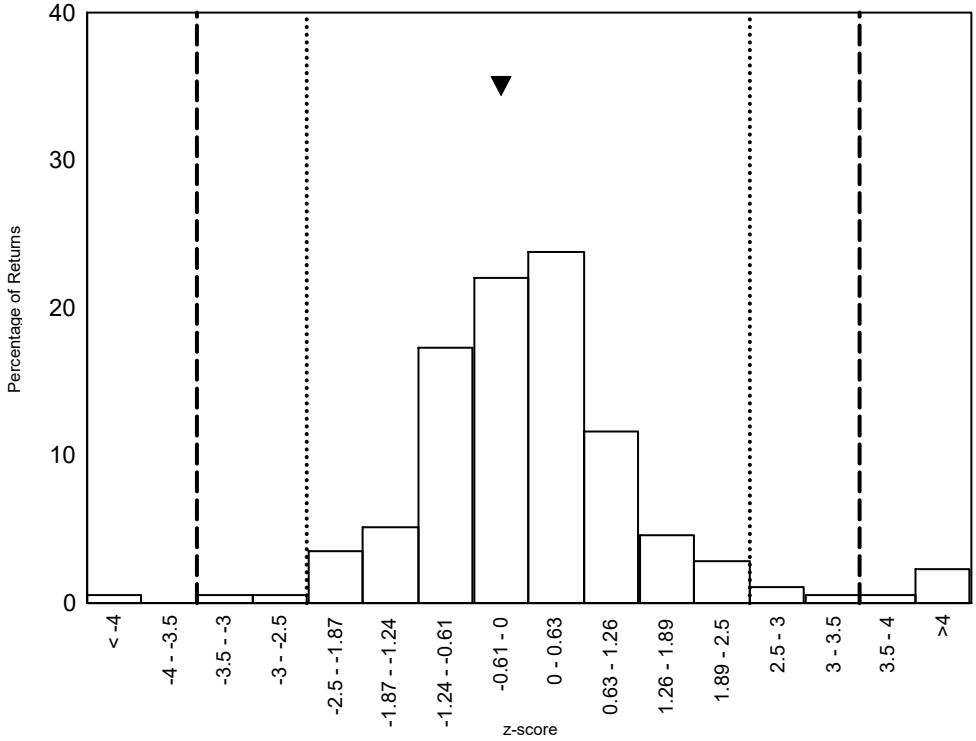


BCR::ABL1 Major Quantification Programme

Histograms of Participant z-scores

Log Change between samples - Unconverted *BCR*::*ABL1*/Reference Gene %

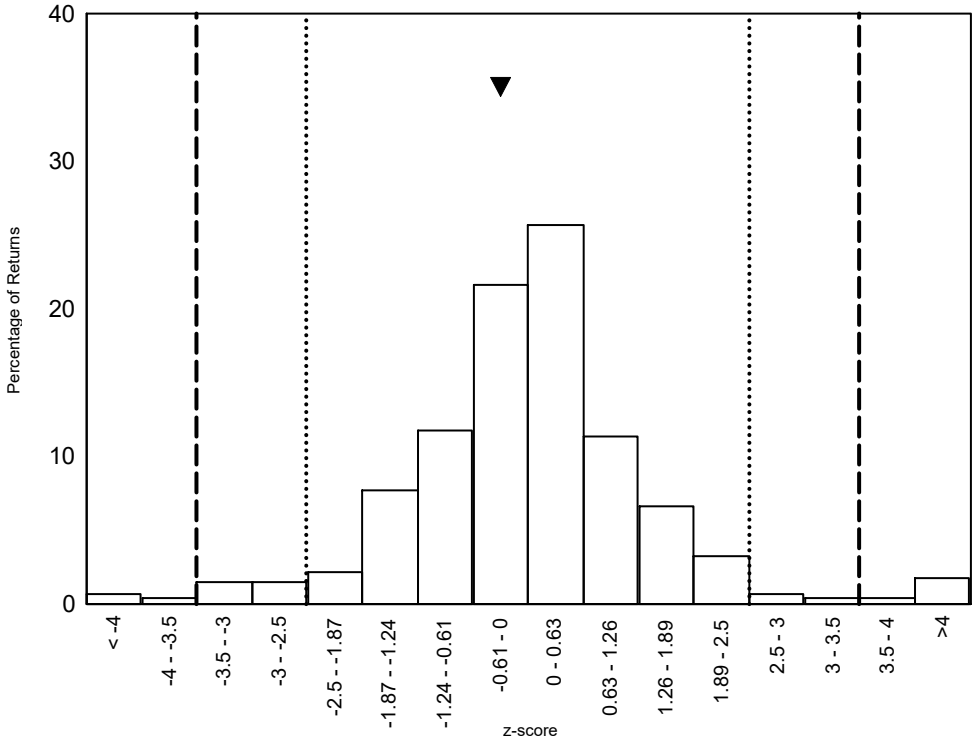
Please note ▼ denotes vour result



Histograms of Participant z-scores

Log Change between samples - IS *BCR*::*ABL1*/Reference Gene %

Please note ▼ denotes your result



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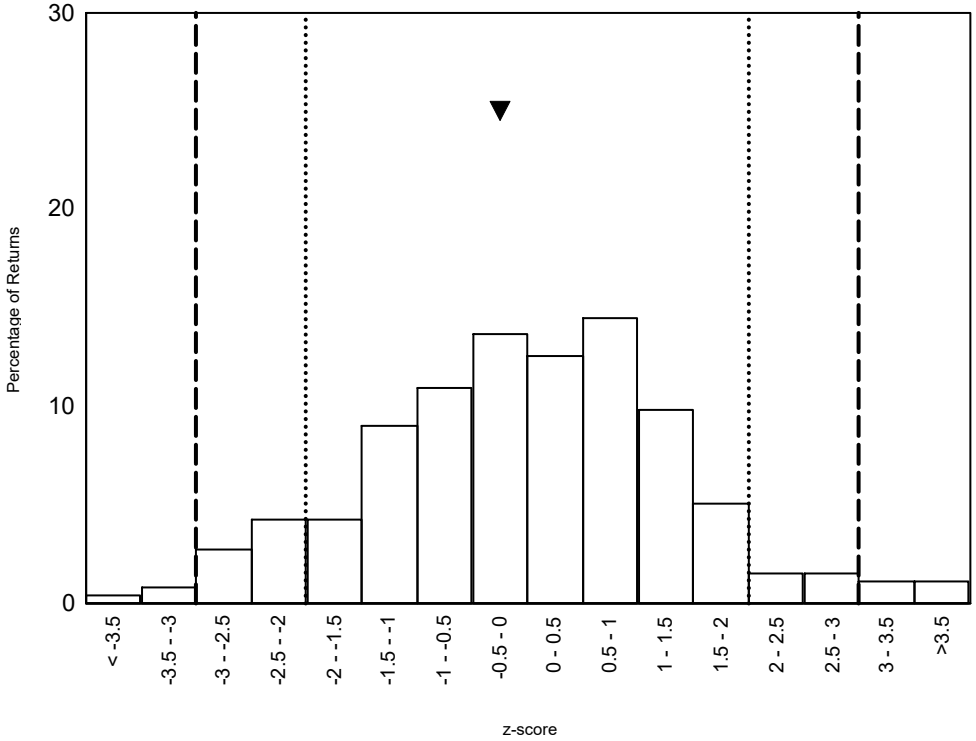
Histograms of Participant z-scores

BCRQ 185 Single Sample % *BCR*::*ABL1*^{IS} z-score

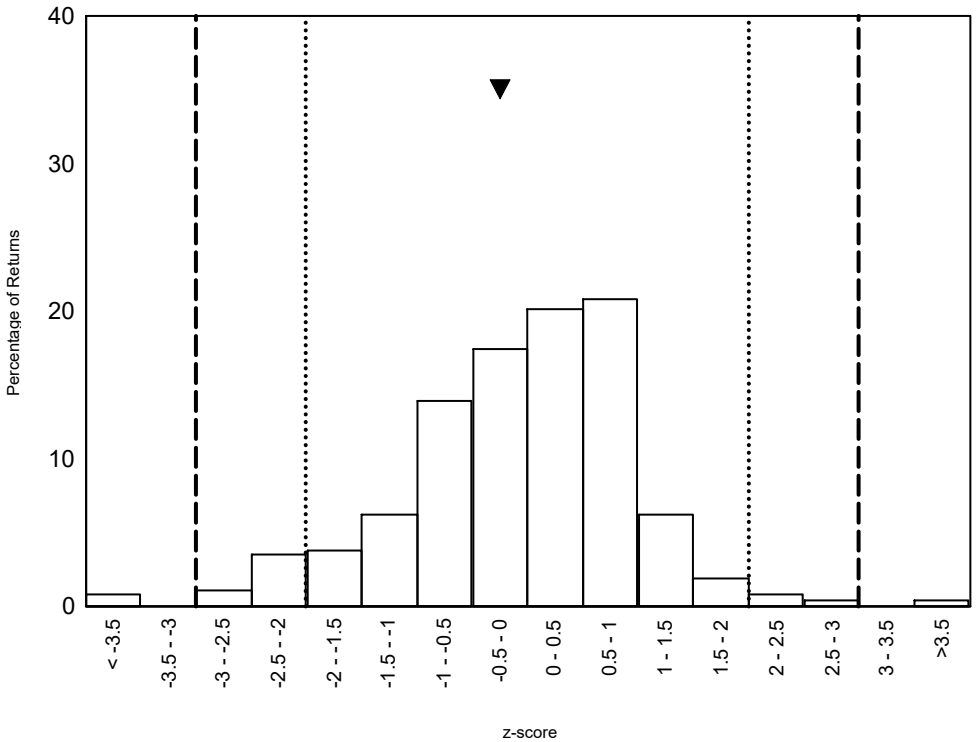
Please note ▼ denotes your result



Histograms of Participant z-scores

BCRQ 186 Single Sample % *BCR*::*ABL1*^{IS} z-score

Please note ▼ denotes your result



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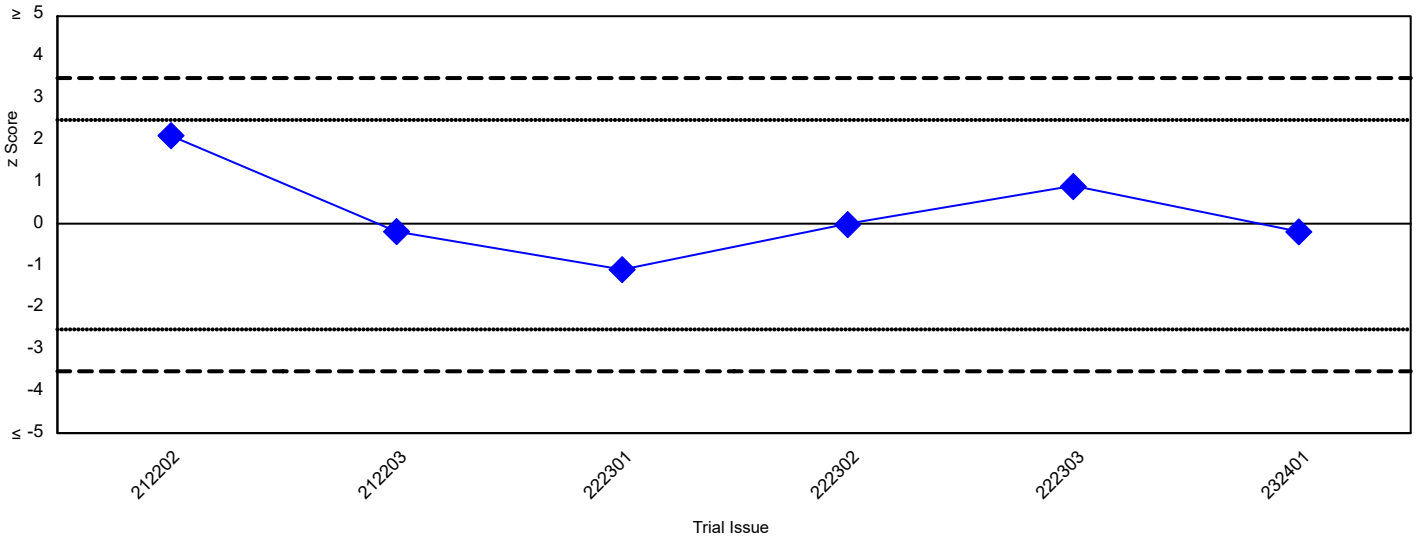
Shewhart Control Charts

(Please note each data point represents a single trial)

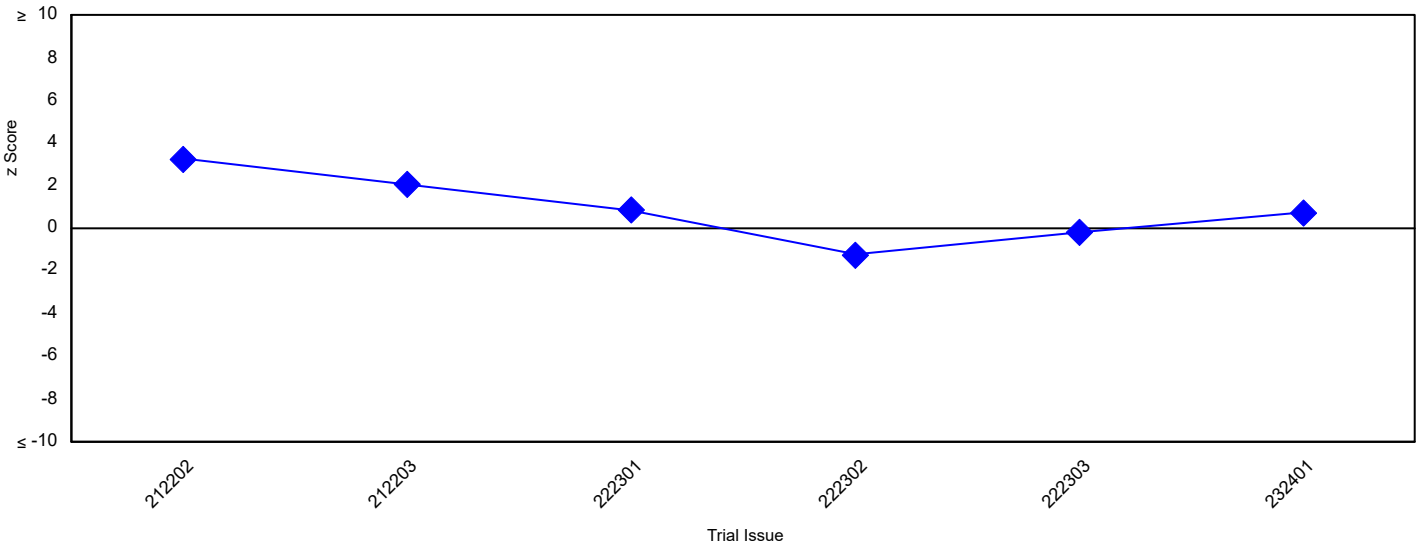
Log Change between samples Unconverted *BCR*::*ABL1*/Reference Gene %



Cusum Control Charts

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

Log Change between samples Unconverted *BCR*::*ABL1*/Reference Gene %

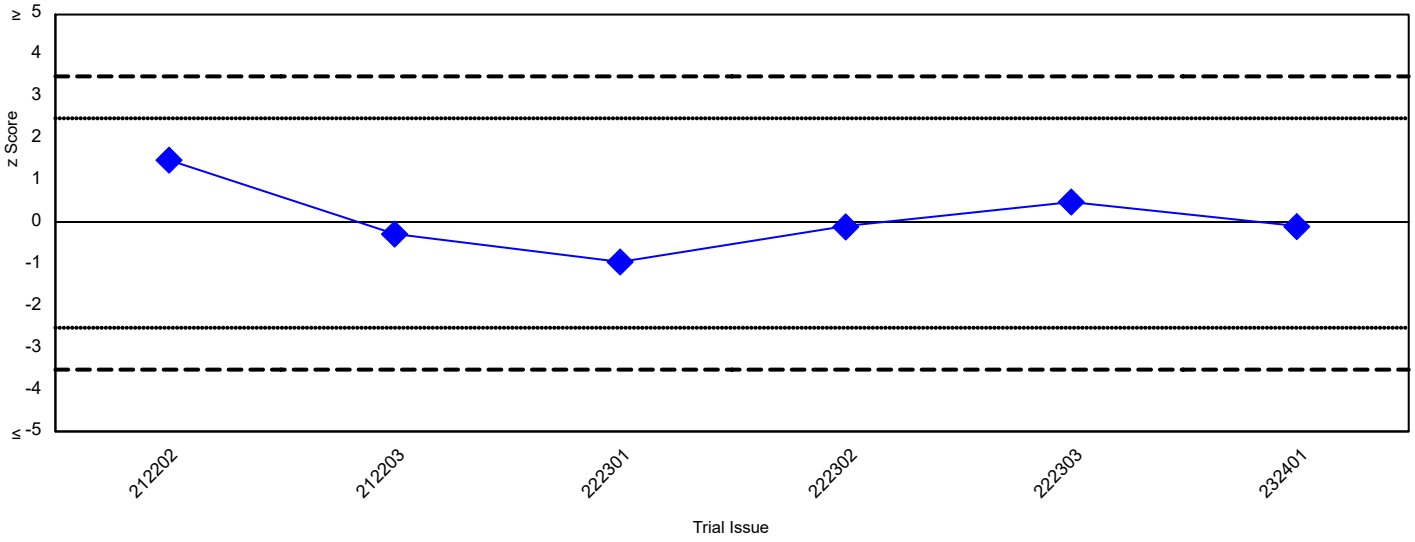


BCR::ABL1 Major Quantification Programme

Shewhart Control Charts

(Please note each data point represents a single trial)

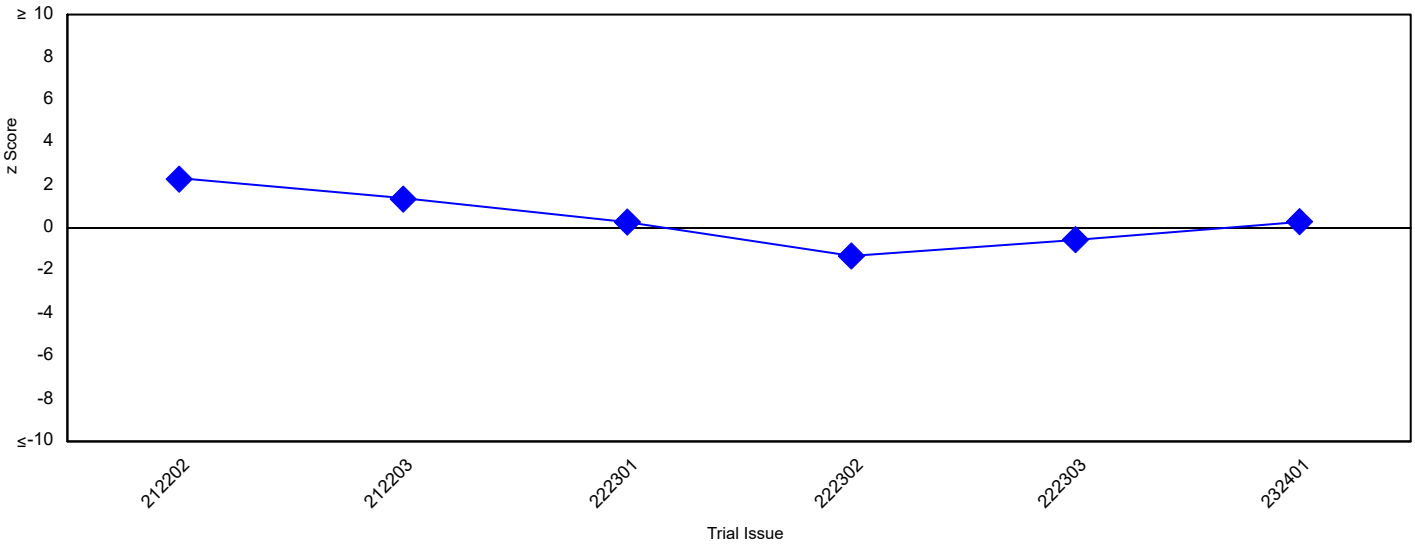
Log Reduction between samples IS *BCR*::*ABL1*/Reference Gene %



Cusum Control Charts

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

Log Reduction between samples IS *BCR*::*ABL1*/Reference Gene %

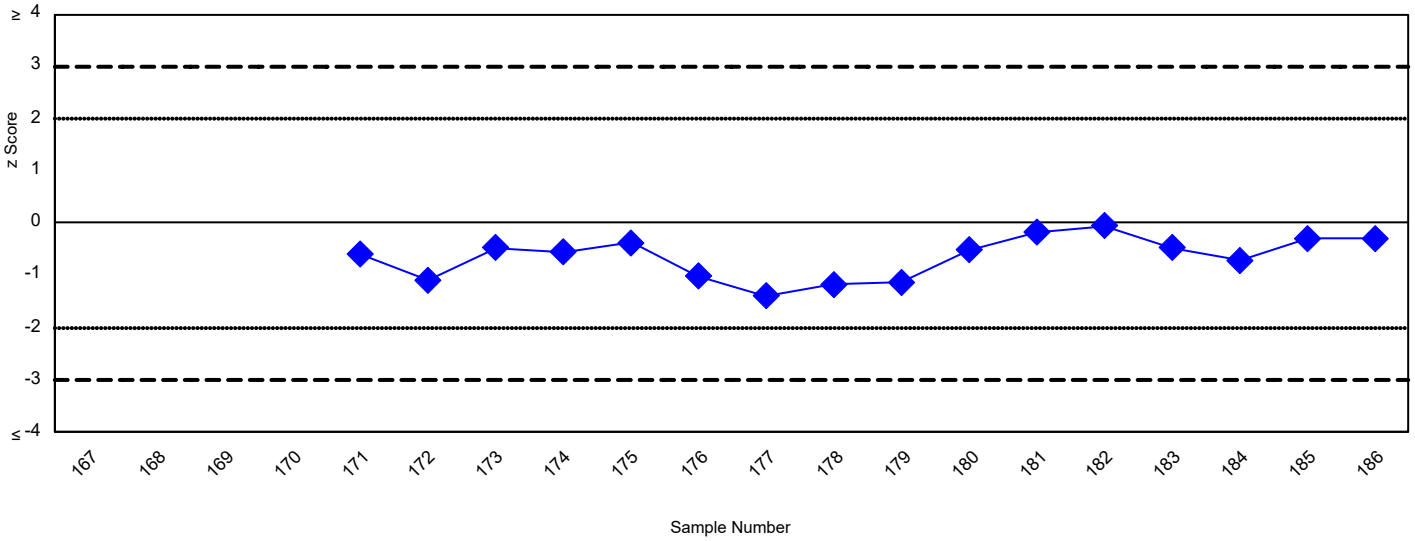


BCR::ABL1 Major Quantification Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

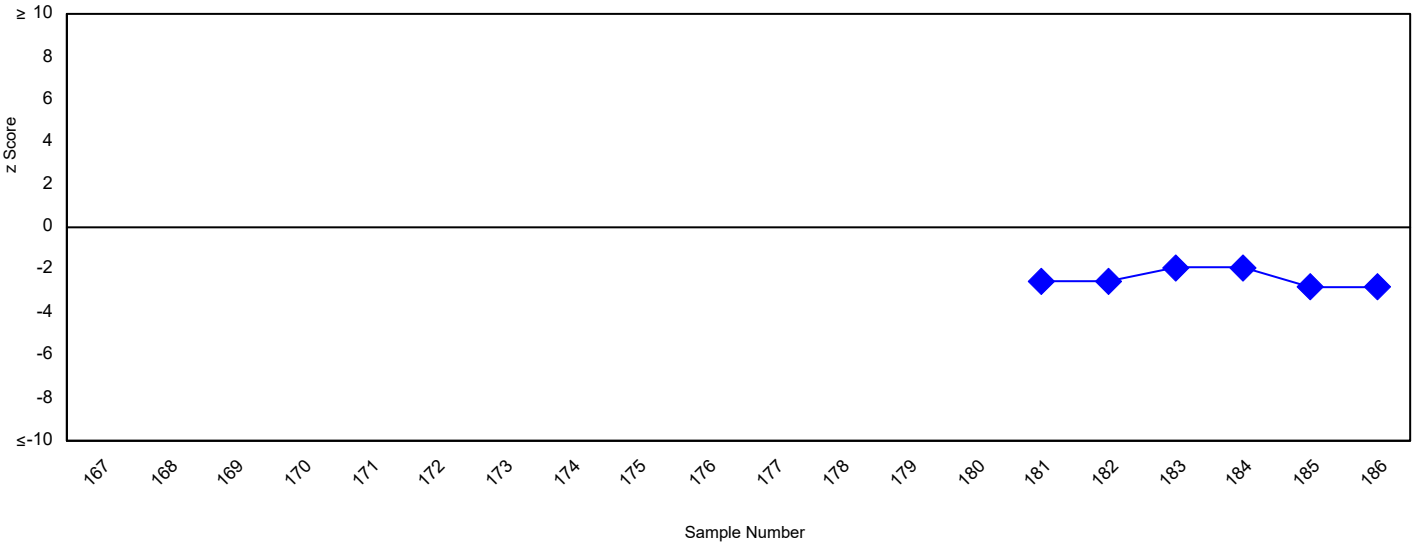
Single Sample % *BCR::ABL1*^{IS}



Cusum Control Charts

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

Single Sample % *BCR::ABL1*^{IS}



BCR::ABL1 Major Quantification Programme

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

Instrument Data Summary

Method	Returns
Cepheid GeneXpert	101
Qiagen Rotorgene	32
Roche LC 480	30
ABI 7500	24
ABI QuantStudio 5	13
Biorad QX200 Droplet Digital PCR	12
ABI QuantStudio 7	9
Biorad CFX96	8
ABI 7500 FAST	7
ABI 7300	6
ABI Step One Plus	6
ABI 7900HT	5
Roche LC 2.0	5
ABI Vii A7	5
Agilent AriaMX Real Time PCR System	4
ABI 7500 FastDx	3
ABI QuantStudio 12K	2
Roche LC96	2

Standard Dilution Data Summary

Method	Returns
Not Applicable	98
ERM-AD623 Certified Reference Material	40
Ipsogen Fusion Quant standards	23
Ipsogen BCR-ABL1 Mbcr IS-MMR Kits single plasmid	22
Ipsogen plasmids	20
Ipsogen IS MMR standards	14
None	12
In-house plasmids	8
BCR-ABL P210 ELITe Standard	7
Ipsogen BCRABL1 Mbcr RGQ RTPCR Single Plasmid	6
Asuragen QuantideX BCRABL IS Kit calibrators	5
Bioclarma SensiQuant P210 Standards	4
BlackBio TRUPCR Standards	4
K562 cDNA	2
In-house standards	2
Mannheim standards	2
In-house standards calibrated to Qiagen standards	2

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Kit/Method Data Summary

Method	Returns
Cepheid GeneXpert Ultra BCR-ABL assay	99
In-house protocol (EAC)	44
Qiagen (formerly Ipsogen) IS MMR Kit	33
In-house protocol	23
In-house (EAC-modified)	13
Qiagen (formerly Ipsogen) Fusion Quant Kit	13
QIAGEN Ipsogen BCR-ABL1 Mbcr RGQ RT-PCR	9
Biorad CE-IVD QXDx BCR-ABL IS Kit	8
Ipsogen kit	7
BCR-ABL P210 ELITe MGB Kit (Elitech Group)	7
Other	5
Bioclarma SensiQuant P210 Kit	4
Asuragen QuantideX qPCR BCRABL IS Kit	3
3B BlackBio TRUPCR BCR-ABL Quantitative kit	3
Cepheid GeneXpert Monitor BCR-ABL assay	2
Asuragen Quantidex qPCR BCR-ABL1 IS Kit	2

Reference Gene Data Summary

Method	Returns
ABL1	261
GUSB	16
BCR	3

Source of Conversion to the IS Data Summary

Method	Returns
Cepheid GeneXpert	87
QIAGEN Ipsogen IS Kits IS-MMR Calibrator	40
ELN/EUTOS 4 Standardization Project	30
Mannheim	15
Wessex	13
Napoli	11
Laboratory derived	11
EliTech Reference	8
Biorad QxDx BCR-ABL IS Kit	8
Adelaide	5
Asuragen ARQ IS Calibrator Panel	4
BlackBio TRUPCR	4
Molecular MD	4
Acrometrix	3
Qiagen (formerly Ipsogen) IS MMR	2
Bern	2
Aarhus	2
AB Analitica BCR-ABL p210 Reference	2

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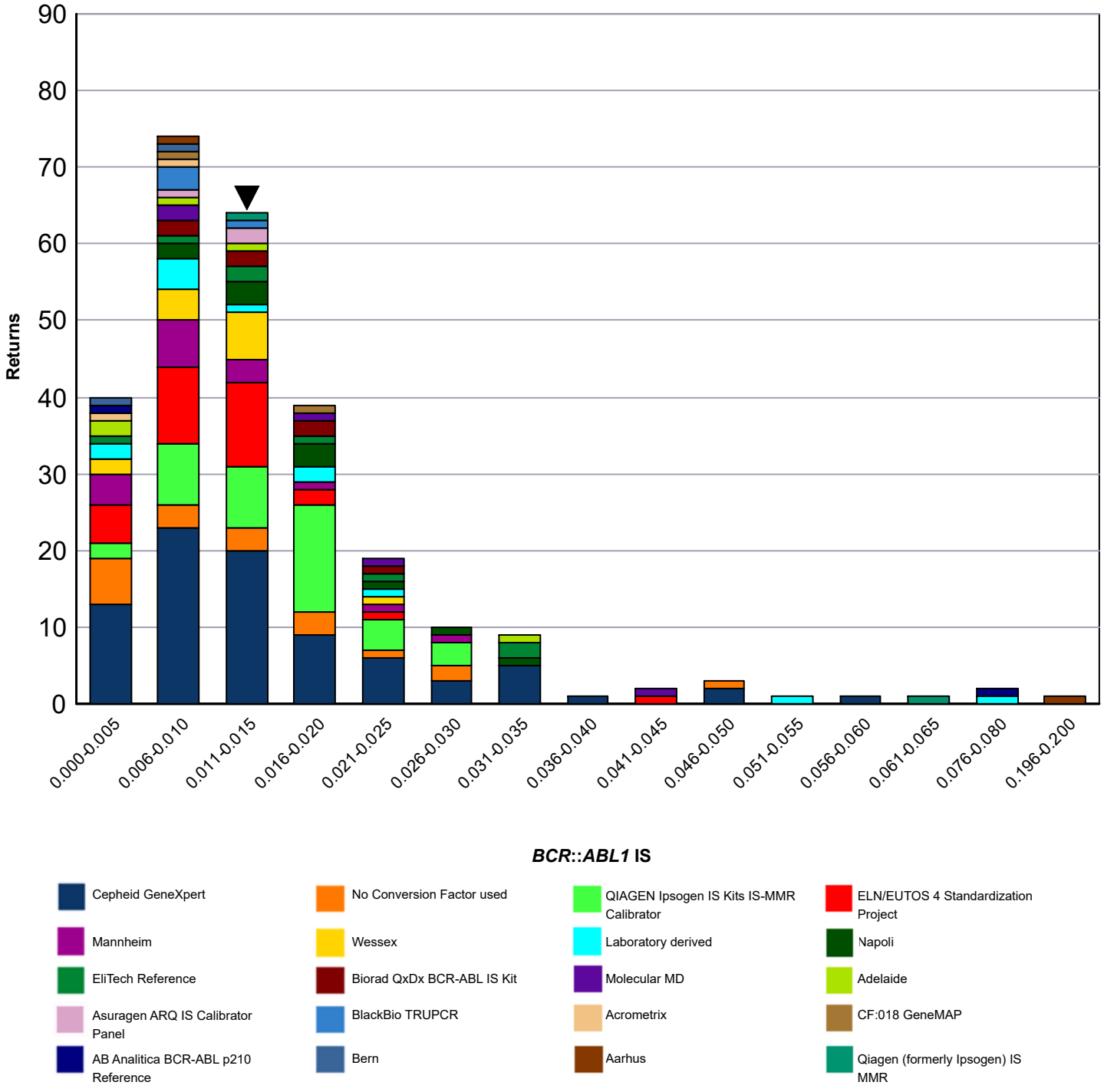
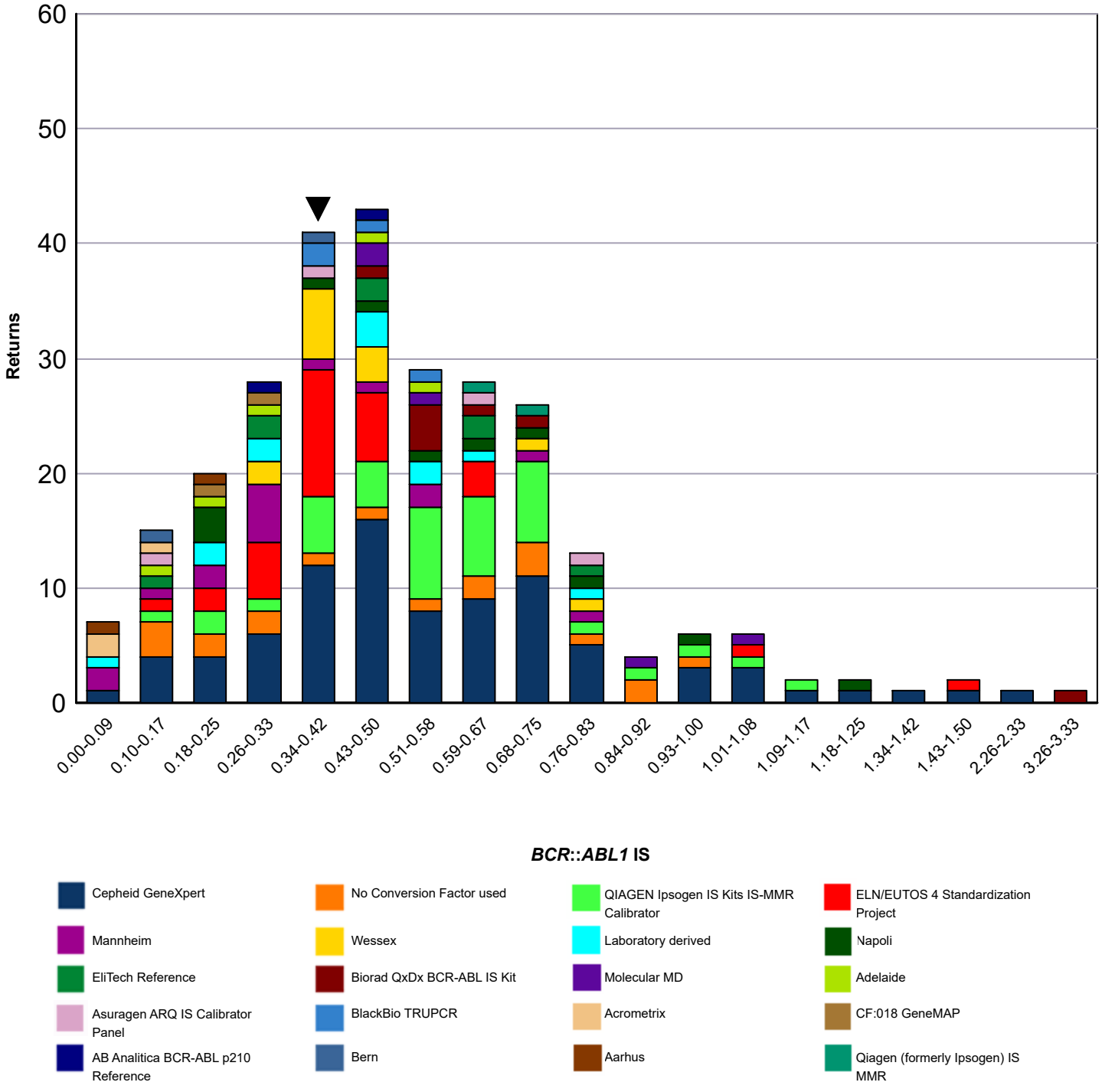
Frequency distribution histogram showing participant *BCR*::*ABL1*/Reference gene results, classified by IS conversion method for sample 185



BCR::ABL1 Major Quantification Programme

Frequency distribution histogram showing participant *BCR*::*ABL1*/Reference gene results, classified by IS conversion method for sample 186



BCR::ABL1 Major Quantification Programme

Trial Comments

Performance monitoring for participants utilising *ABL1* as a reference gene and selecting to be assessed on the *BCR*::*ABL1*^{IS} is now based on single sample z-scores.

Please note, single sample scoring on the International Scale is only applicable to laboratories using *ABL1* as a reference gene as validation data has suggested that alternative control gene expression (e.g. *GUSB*, *BCR* and *B2M*) in the EQA material is significantly different compared to the WHO standards causing these laboratories to no longer be reporting on the IS. We will no longer be displaying single sample z-scores or performance classifications for non *ABL1* users for this reason. **The programme does not have a sufficient number of alternative reference gene users to robustly score them as individual user groups on single sample results therefore non *ABL1* users will continue to be scored using log change.**

IS single sample data

- The median *BCR*::*ABL*^{IS} for sample BCRQ 185 was 0.012%, with an IQR of 0.012%.
- For BCRQ 185, nine participants incurred a critical trial score with a z-score of <-3.0 / >3.0.
- Of the nine, four utilised the Cepheid GeneXpert Ultra BCR-ABL assay, one used the Biorad CE-IVD QXDX BCR-ABL1 IS kit, one reported use of the Qiagen (formerly Ipsogen) IS MMR Kit, one utilised the Molecular MD IS Kit, one used an in-house protocol and one an in-house (EAC) protocol.
- In addition, four participants did not detect any *BCR*::*ABL1* transcript in sample BCRQ 185.
- The median *BCR*::*ABL*^{IS} for sample BCRQ 186 was 0.49%, with an IQR of 0.31%.
- Three participants incurred a critical trial score with a z-score of <-3.0 / >3.0.
- All three participants incurring a critical result for BCRQ 185 also incurred a critical result for BCRQ 186.

Unconverted log change data

- The robust mean unconverted log change between sample BCRQ 185 and BCRQ 186 was -1.56 with a robust SD of 0.23.
- Six participants had a z-score of <-3.5 / >3.5.
- Of the six participants, two utilised an in-house (EAC) protocol, one used the Biorad CE-IVD QXDX BCR-ABL1 IS kit, one utilised the Molecular MD IS Kit, one utilised the Cepheid GeneXpert Ultra BCR-ABL assay and one reported use of an in-house (EAC-modified) protocol.
- In the unconverted dataset, four participants did not detect any *BCR*::*ABL1* transcript in sample BCRQ 185.
- The unconverted log change data is included for interest only.

IS log change data

- The robust mean IS log change between sample BCRQ 185 and BCRQ 186 was -1.58 with a robust SD of 0.23.

- Of the participants scored using IS log change, two incurred a critical trial score with a z-score of <-3.5 / >3.5.
- Of these, one used an in-house (EAC) protocol, and one utilised an in-house (EAC-modified) protocol.

ABL1 Data

- The unconverted median for participants using *ABL1* as a reference gene for sample BCRQ 185 was 0.017% *BCR*::*ABL1/ABL1,* with an IQR of 0.017%.
- The unconverted median for participants using *ABL1* as a reference gene for sample BCRQ 186 was 0.63% *BCR*::*ABL1/ABL1,* with an IQR of 0.33%.
- One hundred and fifty-eight out of 261 participants utilising *ABL1* as the sole reference gene for *BCR*::*ABL1* quantification returned control gene information. Median *ABL1* control gene levels were 117,087 and 129,994 for samples BCRQ 185 and BCRQ 186, respectively.
- For BCRQ 185 there were six (3.8%) participants that reported *ABL1* levels <10,000. For BCRQ 186, there were five (3.2%) 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@uknegasli.co.uk

GUSB Data

- Sixteen participants reported utilising *GUSB* as the sole reference gene for *BCR*::*ABL1* quantification.
- The unconverted median for participants using *GUSB* as a reference gene for sample BCRQ 185 was 0.0034% *BCR*::*ABL1/GUSB,* with an IQR of 0.004%.
- The unconverted median for participants using *GUSB* as a reference gene for sample BCRQ 186 was 0.15% *BCR*::*ABL1/GUSB,* with an IQR of 0.17%.
- Median *GUSB* control gene levels were 295,036 and 289,063 for samples BCRQ 185 and BCRQ 186, respectively.
- One participant utilising *GUSB* as the reference gene reported levels <24,000[2] for sample BCRQ 185.

Following discussions at the most recent UK NEQAS LI molecular specialist advisory group meeting, the decision has been made that from the trial issued in September 2023 (232402) all laboratories not submitting results on the International Scale will be an automatic critical result. Furthermore, laboratories will no longer be able to choose to be scored on unconverted data. If you have further questions, please contact admin@uknegasli.co.uk.

Reference(s)

1. Foroni, L. *et al.* Guidelines for the measurement of BCR-ABL1 transcripts in chronic myeloid leukaemia. *Br J Haematol* **153**, 179–90 (2011).

2. Cross, NCP. *et al*. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia **29**, 999-1003 (2015).

BCR::ABL1 Major 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/