BCR::*ABL1* Kinase Domain Variant (Mutation) Status (Accredited)

Distribution – 232401

Participant

Date Issued – 26 Jun 2023

Closing Date – 28 Jul 2023

Trial Comments

Two vials of lyophilised cell line material (samples KDV(M) 152 and 153) were distributed to 86 participants for *BCR*::*ABL1* kinase domain variant (mutation) (KDV(M)) analysis. Overall, 82 (95.3%) participants returned results for this trial. At the time of trial reporting, four participants have not submitted results; two laboratories pre-notified us of their non return.

Sample Comments

In order to best mimic clinical material, samples were formulated from a mixture of cell lines (approximately 9 x10^6 total cells/sample). Samples KDV(M) 152 and 153 were composed of a cell line lacking the *BCR*::*ABL1* p210 (major) transcript, a *BCR*::*ABL1* p210 positive cell line expressing 'wildtype' (non-point mutated) *BCR*::*ABL1* fusion transcript and a *BCR*::*ABL1* p210 positive cell line expressing a specific kinase domain variant (point mutation) of the *BCR*::*ABL1* fusion transcript.

We would like to acknowledge Dr. Paul La Rosée (University of Jena), who kindly donated the material for this programme. We are also grateful to Novartis who initially supported this pilot EQA scheme through an educational grant and Prof. Johan den Dunnen (Leiden University and Human Genome Variation Society) for previous guidance regarding nomenclature.

Sample KDV(M) 152

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence	Performance status for this sample
Your result	c.756G>C	p.Gln252His	NM_005157.6	**Satisfactory**
Consensus result	**c.756G>C**	**p.Gln252His**	**NM_005157.6**	

Performance status feedback comments (as applicable):

Sample KDV(M) 153

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence	Performance status for this sample
Your result	c.944C>T	p.Thr315Ile	NM_005157.6	**Satisfactory**
Consensus result	**c.944C>T**	**p.Thr315Ile**	**NM_005157.6**	

Performance status feedback comments (as applicable):

IMPORTANT: Scoring criteria have been informed by Human Genome Variation Society (HGVS) sequence variant nomenclature recommendations version 19.01 and subsequently version 20.05[1,2]. Please refer to our website for further details regarding the performance scoring system.

Your Performance

Sample Score(s)		Performance Status Classification (over a six sample period)
Sample KDV(M) 152	**Sample KDV(M) 153**	**Any laboratory assigned unsatisfactory performance status for this trial will be sent an alert email to the default participant contact with details of the classification. Additionally, please refer to the notifications panel in your Participant Hub area of the UK NEQAS LI website for further information.** UK NEQAS LI aim to notify relevant laboratories of persistent unsatisfactory and unsatisfactory performance within 20 working days following the issue of the trial report.
Satisfactory	**Satisfactory**	

N/A: Not applicable

All Participant Results

Detailed Results Breakdown

Nomenclature provided by participants in relation to an *ABL1* NM_005157 based or equivalent reference sequence unless otherwise stated. Percentage values quoted have been subjected to rounding up/down to 1 decimal place. Descriptions fully compliant with the current Human Genome Variation Society (HGVS) nomenclature recommendations are highlighted green.

Sample KDV(M) 152

Sequence change	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.756G>C[a]	77	93.9
c(756G>C)[b]	1	1.2
756G>C[c]	1	1.2
c.1120G>C[d]	1	1.2
c.759G>C[d]	1	1.2
No variant detected	1	1.2
Amino Acid change (protein)		
p.Gln252His[a]	70	85.4
p.(Gln252His)[e]	6	7.3
p.Q252H[f]	2	2.4
(p.Gln252His)[b,e]	1	1.2
Gln252His[c]	1	1.2
p.Glu252His[g]	1	1.2
No variant detected	1	1.2

Sample KDV(M) 153

Sequence change	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.944C>T[a]	74	91.4
c.(944C>T)[b]	1	1.2
c.944G>CT[h]	1	1.2
c.947C>T[d]	1	1.2
c.944C[h]	1	1.2
c.1308C>T[d]	1	1.2
No variant detected	2	2.5
Amino Acid change (protein)		
p.Thr315Ile[a]	69	85.2
p.(Thr315Ile)[e]	6	7.4
p.T315I[f]	2	2.5
(p.Thr315Ile)[b,e]	1	1.2
Thr315Ile[c]	1	1.2
No variant detected	2	2.5

[a] Nomenclature fully compliant with current HGVS recommendations when RNA/cDNA is used as the assay input material. When gDNA is utilised, parentheses (brackets) are required to indicate a predicted protein description.
[b] Minor symbol/syntax error.
[c] Absent prefix symbol.
[d] Nucleotide position error.
[e] Parentheses (brackets) used erroneously for this nomenclature description, RNA (or cDNA produced from extracted RNA) was stated as the assay input material (n=6 in total).
One laboratory using genomic DNA as assay input material applied parentheses appropriately.
[f] Three letter amino acid code preferable to single letter.
[g] Amino acid error.
[h] Nucleotide error.

Method Breakdown

Please note, figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Input (template) material for the detection/sequencing assay

	Returns
cDNA	78
RNA	3
gDNA	1

* Next generation sequencing (NGS) users (n=1), plus suspected typographical error by Sanger sequencing users (n=2).

PCR method approach

	Returns
Nested PCR (2 rounds of PCR)	38
Semi-nested PCR (2 rounds of PCR)	23
Single PCR	13
Single long range PCR	5
NGS - commercial assay	3
Other/combination	3

Target(s) of PCR amplification strategy

	Returns
BCR::*ABL1* fusion (targeting *ABL1* on the translocated region of chromosome 9 only)	71
Both *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9	10

Please note that for *BCR*::*ABL1* positive chronic myeloid leukaemia (CML)/acute lymphoblastic leukaemia (ALL) cases, clinically relevant *ABL1* kinase domain variants (point mutations), potentially conferring tyrosine kinase inhibitor (TKI) resistance, are present on the disease associated *BCR*::*ABL1* fusion allele. Therefore, *ABL1* on the translocated (Philadelphia) chromosome is conventionally the amplification target for assays involving subsequent sequencing.

Analysis method

	Returns
Sanger sequencing	61
Illumina MiSeq (NGS)	13
Illumina NextSeq (NGS)	4
Ion Torrent S5 (NGS)	3
Illumina MiniSeq (NGS)	2
Allele specific PCR	2
Digital PCR	1
Illumina Novaseq (NGS)	1
Ion Torrent Genexus (NGS)	1

NGS = Next generation sequencing.

Sequencing approach

	Returns
Bidirectional (forward AND reverse)	74
Unidirectional (forward OR reverse)	4
Not applicable	3

Reference sequence

	Returns
NM_005157.6*	49
NM_005157.5	17
ENST00000318560.5	5
NM_005157.3	4
NM_005157.4	3
ENST00000318560.6*	1

* Latest available version of the *ABL1* Matched Annotation from the NCBI and EMBL-EBI (MANE) Select transcript.
https://www.ncbi.nlm.nih.gov/refseq/MANE
https://tark.ensembl.org/web/mane_project/

Assay journal references (as reported by participants)

In house method (no reference)	30
Branford S. and Hughes T. (2006). Myeloid Leukemia Methods and Protocols eISBN 1-59745-017-0, 93-106*	11
Soverini S. *et al*. (2013). Blood 122:9, 1634-1648	9
Hochhaus A. *et al*. (2002). Leukemia 16:11, 2190-2196	8
Branford S. *et al*. (2002). Blood 99:9, 3472-3475	8
Alikian M. *et al*. (2012). American Journal of Hematology 87:3, 298-304	5
Ernst T. *et al*. (2008). Haematologica 93:2, 186-192	4
Khorashad J. *et al*. (2006). Leukemia 20:4, 658-663	3
MODHEM (Network for Molecular Diagnostics of Hematologic Malignancies)	3
Kizilors A. *et al*. (2019). Lancet Haematol. 6(5):e276-e284	2

As stated by >1 participant.

* Branford S. and Hughes T. (2006) Detection of BCR-ABL Mutations and Resistance to Imatinib Mesylate. In: Iland H., Hertzberg M., Marlton P. (eds) Myeloid Leukemia. Methods In Molecular Medicine, vol 125. Humana Press. (ISBN 978-1-58829-485-2).

Assay Limit of Detection (LoD)

	Returns	
Percentage (%) variant	**Sanger Sequencing**	**Next Generation Sequencing (NGS)**
<1	1*	1
1	2*	4
3	0	5
4	0	1
5	3	9
7	1	0
10	8	1
15	6	0
20	33	0
25	2	0
30	1	0

* Suspected typographical error.

For some participants using more than one method it may not have been possible to unequivocally identify which assay the stated LoD referred to. These laboratories have been excluded from the table above.

Quantification – For educational purposes only

Descriptive statistics are included in the tables below (when >5 quantification data points available) for those returns stating a PCR strategy which targets the *BCR*::*ABL1* fusion prior to analysis (cDNA/RNA template input material) and includes a defined calculation approach. Values quoted have been subjected to rounding up/down to 1 decimal place. We acknowledge the limitations of this small dataset.

Sample KDV(M) 152 variant (mutation) quantification: % variant allele frequency (VAF)

***ABL1* c.756G>C p.Gln252His**				
Calculation approach	**n**	**Mean**	**Median**	**IQR**
(Mut/(Mut+WT)) x 100[a]	23[b]	62.3	61.4	17.5
Other[c]	10			

a Assay method with *BCR*::*ABL1* enrichment.
b NGS (n=12): mean=61.4, median=59.7, IQR=12.3. Sanger (n=11): mean=63.3, median=64.0, IQR=24.9
c Includes various assay strategies and calculation approaches.

Mut = mutation (variant), WT = 'wildtype'. IQR = inter quartile range.

Sample KDV(M) 153 variant (mutation) quantification: % variant allele frequency (VAF)

***ABL1* c.944C>T p.Thr315Ile**				
Calculation approach	**n**	**Mean**	**Median**	**IQR**
(Mut/(Mut+WT)) x 100[a]	21[b]	80.2	80.0	7.8
Other[c]	10			

a Assay method with *BCR*::*ABL1* enrichment.
b NGS (n=11): mean=74.4, median=78.0 IQR=5.6. Sanger (n=10): mean=86.6, median=82.3, IQR=16.5
c Includes various assay strategies and calculation approaches.

Mut = mutation (variant), WT = 'wildtype'. IQR = inter quartile range.

Comments

Sample KDV(M) 152

In line with sample formulation, 78/82 (95.1%) of returning participants reported the c.756G>C p.Gln252His variant in sample KDV(M) 152.

- A single laboratory returned a false negative result for this sample (cDNA input material) using an in house NGS method (bidirectional sequencing, Illumina MiSeq). The centre stated their assay targets both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9 with a stated region of interest encompassing the c.756G>C p.Gln252His variant position.
- Additionally, two participants submitted a nucleotide position errors (c.1120G>C, c.759G>C); however, nomenclature was in consensus at the protein level (NM_005157 based reference sequences).
- One centre was in consensus at the DNA level but provided an erroneous amino acid (p. p.Glu252His).

- Overall, nomenclature was in good agreement. Two participants failed to provide the appropriate prefix (c. or p.). Two centres utilised the less preferable single letter amino acid code (p.Q252H)). Six laboratories inappropriately included parentheses in the protein description returned; cDNA/RNA was stated as the assay input material and therefore the protein description is not predicted.
- The median expressed variant load (variant allele frequency, VAF) calculated from the available equivalent quantitative data (all assay methods) for the c.756G>C p.Gln252His variant was 61.4% (n=23).

Sample KDV(M) 153

In line with sample formulation, 75/81 (92.6%) of returning participants reported the c.944C>T p.Thr315Ile variant in sample KDV(M) 153.

- Two laboratories (cDNA input material) returned a false negative result for this sample, this included the same participant failing to detect the consensus variant in sample KDV(M) 152. The other centre utilised the Oncomine Myeloid/MRD assay (ThermoFisher Scientific) on the Ion Torrent S5/Genexus platforms targeting both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9 with a stated region of interest encompassing the c.944C>T p.Thr315Ile variant position.
- The same two participants submitting nomenclature errors for sample KDV(M) 152 also returned nucleotide position errors (c.947C>T, c.1308C>T) for sample KDV(M) 153; however, the accompanying protein descriptions were in consensus (NM_005157 based reference sequences).
- A further two laboratories provided descriptions at the DNA level with a missing or erroneous nucleotide (c.944C, c.944G>CT), again the accompanying protein descriptions were in consensus.
- Overall, nomenclature was in good agreement. One participant failed to provide the appropriate prefix (p.). Two centres utilised the less preferable single letter amino acid code (p.T315I). Six laboratories inappropriately included parentheses in the protein description returned; cDNA/RNA was stated as the assay input material and therefore the protein description is not predicted.
- The median expressed variant load (variant allele frequency, VAF) calculated from the available equivalent quantitative data (all assay methods) for the c.944C>T p.Thr315Ile variant was 80.0% (n=21).

Quantification

Overall, 50% (41/82) of returning laboratories provided quantitative information for at least one of the samples featured in this trial. Please refer to the tables on page 7, which summarise equivalent quantification results submitted by participants.

Reference sequences

All laboratories stated the use of an *ABL1* isoform a transcript based reference sequence. We strongly encourage laboratories to always provide a version number with an accession reference to unequivocally identify the reference sequence.

Moreover, the Human Genome Variation Society (HGVS) nomenclature guidelines[2] now include a recommendation to use the transcript reference sequence(s) specified by the Matched Annotation from the NCBI and the EMBL-EBI (MANE) collaboration project[3]. Locus Reference Genomic (LRG) reference sequences are no longer maintained and therefore, are not the most suitable reference sequence option.

IMPORTANT reference sequence related minor change to the programme scoring criteria for 2023/24. Please refer to our website to access the performance monitoring system: https://www.ukneqasli.co.uk/eqa-pt-programmes/molecular-haemato-oncology-programmes/bcr-abl1-kinase-domain-variant-mutation-status-accredited/

Final Remarks

To implement formal sample scoring classification and performance monitoring, a core list of clinically actionable *BCR*::*ABL1* kinase domain variants (point mutations) has been produced (informed by review of the literature and currently available EQA material). The core list of residue (amino acid) positions encompasses: p.Met244, p.Gly250, p.Gln252, p.Tyr253, p.Glu255, p.Val299, p.Thr315, p.Phe317, p.Met351 and p.Phe359 (tyrosine-protein kinase ABL1 isoform a, NP_005148.2). During the sample scoring classification process, reference is made to a participant's stated assay scope (as provided at trial results submission). **To mitigate the reporting of additional variants (i.e. those of unknown clinical significance and/or cell line artefacts), participants are reminded at trial data entry to return only results with clinical significance.**

For the financial year (2023/24), samples for this programme will continue to be formulated with the aim of suitability for analysis by several commonly employed techniques, including Sanger sequencing (variant(s) representing >20% VAF). UK NEQAS LI will continue to aim to periodically offer an additional low-level variant(s) sample (5 - 20% target VAF) as an educational exercise. Variants <5% VAF are beyond the scope of the current scheme.

The cell lines utilised in this programme have been pre-validated by The European Treatment Outcome Study (EUTOS) group. However, it is important to note they are cell lines stably transfected with cDNA constructs and therefore do not exactly reflect the genetic context of clinical samples. At this time no alternative suitable material exists for the purposes of *BCR*::*ABL1* kinase domain variant (mutation) testing EQA.

Please note that returning more than one set of trial results requires a secondary registration. If you use multiple methods and would like to submit more than one set of trial results, please contact admin@ukneqasli.co.uk for further information.

Repeat samples are available for all programmes. In the event that your local quality control criteria are not met please contact us. Please do not submit results based on a suboptimal nucleic acid extraction.

Reference(s)

1. den Dunnen, J. T. *et al.* HGVS Recommendations for the description of sequence variants: 2016 Update *Hum. Mutat.* 37, 564–569 (2016).
2. http://varnomen.hgvs.org/ (Version 20.05).
3. Morales, J. *et al*. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. Nature 604: 310-315 (2022).
4. https://www.lrg-sequence.org/ (accessed Dec 2023).

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 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/