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

Distribution – 252601

Participant –

Date Issued – 10 Jul 2025

Closing Date – 15 Aug 2025

Trial Comments

Two vials of lyophilised cell line material (samples KDV(M) 164 and 165) were distributed to 97 participants for *BCR*::*ABL1* kinase domain variant (mutation) (KDV(M)) analysis. Overall, 92 (94.8%) participants returned results for this trial. Of the five laboratories not submitting results, two 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) 164 and KDV(M) 165 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) fusion transcript and *BCR*::*ABL1* p210 positive cell lines expressing a specific kinase domain variant (point mutation) of the 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) 164

Your Results

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

Performance status feedback comments (as applicable):

Sample KDV(M) 165

Your Results

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

Performance status feedback comments (as applicable):

IMPORTANT: Scoring criteria for this trial have been informed by Human Genome Variation Society (HGVS) Nomenclature version v21.1.3[1-3]. Please refer to our website for further details regarding the performance monitoring system.

Your Performance

Sample Score(s)		Performance Status Classification (over a six sample period)
Sample KDV(M) 164	**Sample KDV(M) 165**	**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.

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 (MANE Select transcript) unless otherwise stated. Percentage values quoted have been subjected to rounding up/down to 1 decimal place. Descriptions of the consensus variant(s) which are fully compliant with the current Human Genome Variation Society (HGVS) Nomenclature specification are highlighted green.

Sample KDV(M) 164

Sequence change	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.756G>C[a]	85	92.4
c.(756G>C)[b]	1	1.1
756G>C[c]	1	1.1
c.755G>C[d]	1	1.1
c.756C>G[e]	1	1.1
c.947C>T[f]	1	1.1
c.944C>T[f]	1	1.1
Variant not detected[g]	1	1.1
Amino Acid change (protein)		
p.Gln252His[a]	80	87.0
p.(Gln252His)[h]	5	5.4
Gln252His[c]	1	1.1
p.Glu252His[i]	1	1.1
p.(Glu252His)[h,i]	1	1.1
p.Q252H[j]	1	1.1
p.Thr315Ile[f]	1	1.1
Thr315Ile[c,f]	1	1.1
Variant not detected[g]	1	1.1

Sample KDV(M) 165

Sequence change 1	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.944C>T[a]	87	94.6
c.(944C>T)[b]	1	1.1
944C>T[c]	1	1.1
c.759G>C[f]	1	1.1
c.756G>C[f]	1	1.1
Variant not detected	1	1.1
Amino Acid change (protein)		
p.Thr315Ile[a]	82	89.1
p.(Thr315Ile)[h]	6	6.5
Thr315Ile[c]	1	1.1
Gln252His[c,f]	1	1.1
p.Gln252His[f]	1	1.1
Variant not detected	1	1.1

[a] Description fully compliant with current HGVS Nomenclature when RNA/cDNA is used as the assay input material. When gDNA is utilised, parentheses (brackets) are required to indicate uncertainty (predicted protein description).
[b] Minor symbol/syntax irregularity - parentheses (brackets) at the DNA level are not required for this description.
[c] Absent symbol.
[d] Erroneous nucleotide position.
[e] Erroneous nucleotide type.
[f] Out of consensus variant (suspected sample transposition).
[g] Variant outside scope of dPCR assay.
[h] Parentheses (brackets) used erroneously for this nomenclature description if RNA (or cDNA produced from extracted RNA) was stated as the assay input material.
[i] Erroneous amino acid.
[j] Three letter amino acid code preferred.

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	87
RNA[a]	2
gDNA[b]	2
Not known[b]	1

[a] Next generation sequencing (NGS).
[b] Next generation sequencing (NGS) assay without *BCR*::*ABL1* enrichment/specific targeting.

PCR method approach to target region of interest

	Returns
Nested PCR (2 rounds of PCR)	38
Semi-nested PCR (2 rounds of PCR)	25
Single PCR	18
Single long range PCR	5
NGS commercial assay - Not known by user	5
Not known/Other	4

NGS = Next generation sequencing.

Ultimate target(s) of assay strategy

	Returns
BCR::*ABL1* fusion (targeting *ABL1* on the translocated region of chromosome 9 only)	79
Both *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9	13

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	63
Illumina MiSeq (NGS)	12
Illumina NextSeq (NGS)	7
Illumina Novaseq (NGS)	5
Ion Torrent S5 (NGS)	5
Allele specific PCR	3
Illumina MiniSeq (NGS)	2
Digital PCR	1

NGS = Next generation sequencing.

Sequencing approach

	Returns
Bidirectional (forward AND reverse)	84
Not applicable	4
Unidirectional (forward OR reverse)	4

Reference sequence

	Returns
NM_005157.6[a]	71
NM_005157.5	9
NM_005157 – no version number provided	4
ENST00000318560.5	3
ENST00000318560.6[a]	2
NM_005157.4	1
NM_005157.3	1
Not provided	2

[a] Latest available version of the *ABL1* Matched Annotation from the NCBI and EMBL-EBI (MANE) Select transcript.

Assay journal references (as reported by participants)

	Returns
In house method (no reference)	39
Soverini S. *et al.* (2013). *Blood* 122:9, 1634-1648	12
Hochhaus A. *et al.* (2002). *Leukemia* 16:11, 2190-2196	9
Branford S. *et al.* (2002). *Blood* 99:9, 3472-3475	10
Branford S. and Hughes T. (2006). Myeloid Leukemia Methods and Protocols eISBN 1-59745-017-0, 93-106*	6
Ernst T. *et al.* (2008). *Haematologica* 93:2, 186-192	6
Alikian M. *et al.* (2012). *American Journal of Hematology* 87:3, 298-304	6
MODHEM (Network for Molecular Diagnostics of Hematologic Malignancies)	4
Kizilors A. *et al.* (2019). *Lancet Haematology* 6(5):e276-e284	2
Khorashad J. *et al.* (2006). *Leukemia* 20:4, 658-663	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	**All methods**	**Sanger Sequencing**	**Next Generation Sequencing (NGS)**
0.01	1[a]	-	-
0.1	2[b]	-	1
>0.1-0.5	2	1[c]	1
1	8	-	8
2	1	-	1
2.5	2	-	2
3	5	-	5
4	1	-	1
5	9	1	8
7	1	1	-
10	11	10	1
15	10	10	-
20	36	36	-
25	1	1	-
30	2	2	-

[a] Allele Specific PCR.
[b] Includes digital PCR (n=1).
[c] Suspected typographical error at data submission.

Quantification – For educational purposes only

For cDNA/RNA template input material assays 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 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) 164 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]	26	92.2	92.0	5.5
Other[b]	18			

NGS (n=18): median VAF = 91.8% (IQR = 3.6%)

Sanger sequencing (n=8): median VAF = 97.5% (IQR = 9.3%)

Sample KDV(M) 165 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]	26	78.9	76.5	6.5
Other[b]	19			

NGS (n=18): median VAF = 75.5% (IQR = 5.3%)

Sanger sequencing (n=8): median VAF = 81.0% (IQR = 12.5%)

[a] Assay method with *BCR*::*ABL1* enrichment/specific targeting.
[b] Various calculation approaches, may include assays stated as targeting both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9.

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

Comments

Sample KDV(M) 164

In line with sample formulation, 95.7% of participants (88/92) detected a variant equivalent to p.Gln252His in sample KDV(M) 164.

- The c.756G>C p.Gln252His variant was outside of the assay scope for a digital PCR user.
- Three participants returned an out of consensus variant:
    - One laboratory erroneously described the variant at the DNA and protein levels (c.755G>C p.Glu252His).
    - Two participants reported a variant consistent with p.Thr315Ile, indicative of a sample transposition.
- One centre described the variant incorrectly as p.(Glu252His) but was in consensus at the DNA level (c.756G>C). A further laboratory returned erroneous DNA nomenclature (c.756C>G) but described the consensus variant at the protein level (p.Gln252His). Please refer to the table and footnotes on pages 3-4 for a breakdown of nomenclature submitted for sample KDV(M) 164.

Sample KDV(M) 165

In line with sample formulation, 96.7% of participants (89/92) detected a variant equivalent to p.Thr315Ile in sample KDV(M) 165.

One NGS user (Oncomine Myeloid MRD kit, Ion Torrent S5 instrument with Ion Reporter software) failed to detect a reportable variant in this sample. Given the clinical actionability of the c.944C>T p.Thr315Ile *BCR*::*ABL1* change[4,5], this variant position is expected to be within the scope of their assay. Although not all methodological information was provided, the participant appears to have employed genomic DNA as assay input material for the sequencing of the missense variant position on both the oncogenic *BCR*::*ABL1* fusion and *ABL1* gene on the non-translocated chromosome 9. Assay analytical sensitivity is limited by the absence of *BCR*::*ABL1* fusion targeting. The Oncomine Myeloid MRD kit is currently marketed (for research use only) as a general MRD panel with an integrated bioinformatics and reporting (Ion Reporter software) option; it is not specifically designed for the specialised purpose of *BCR*::*ABL1* kinase domain variant (mutation) status testing.

Participants employing an NGS commercial panel with an assay design that does not incorporate the specific targeted enrichment of the *BCR*::*ABL1* transcript are strongly encouraged to review the suitability of this approach in the clinical context of Ph+ leukaemia.

The recommended input material for *BCR*::*ABL1* kinase domain variant (mutation) analysis is RNA (this may be processed as cDNA in the utilised assay) from peripheral blood or bone marrow leukocytes. Analysis of genomic DNA is not currently recommended for routine testing[5].

Two participants reported identification of a variant equivalent to p.Gln252His in sample KDV(M) 165, indicative of a sample transposition.

Consensus variant nomenclature for this sample was generally well handled. Please refer to the table and footnotes on page 4 for a breakdown of nomenclature submissions for sample KDV(M) 165.

In addition to the consensus c.756G>C p.Gln252His variant, a single laboratory reported identification of the c.845A>T p.Glu282Val change (Sanger sequencing, no VAF provided). This variant has not previously been identified specifically in KDV(M) EQA samples formulated from the same cell line harbouring the clinically significant c.756G>C p.Gln252His variant but it has been noted as a variant of unknown clinical significance and/or cell line artefact in other trials for this programme using similar EQA material. The c.845A>T p.Glu282Val additional variant was not subject to scoring for this trial.

Quantification

Overall, 47/92 (51.1%) of returning laboratories provided quantitative information for at least one of the consensus variants featured in this trial distribution.

The median expressed variant load calculated from the available equivalent quantitative data (RNA/cDNA based assays) for the c.756G>C p.Gln252His and c.944C>T p.Thr351Ile variants was 92.0% and 76.5% (n=26), respectively. Please refer to the tables on pages 8, which summarise equivalent quantification results (from comparable assay approaches) submitted by participants.

Of note, two participants stated the use of gDNA as assay input material. A further laboratory is also likely to have employed gDNA (based on the limited methodological information provided). All three centres submitted quantitative results for at least one of the two consensus variants; however, all values were gross outliers (low %VAF) due to the ultimate target of their assay encompassing both *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9.

Reference sequences and nomenclature

All returning laboratories providing a reference sequence stated the use of an *ABL1* (isoform a) MANE transcript based reference sequence (n=90). Two participants failed to provide any meaningful reference sequence information. We strongly encourage laboratories to always provide a version number with an accession reference to unequivocally identify the reference sequence. Human Genome Variation Society (HGVS) Nomenclature[1-3] includes a recommendation to use the transcript reference

sequence(s) specified by the Matched Annotation from the NCBI and the EMBL-EBI (MANE) collaboration project[6].

The current performance monitoring system for this programme references Human Genome Variation Society (HGVS) Nomenclature v21.1.3 (released 4 June 2025)[3].

Methodology

Please note, the recommended input material for *BCR*::*ABL1* kinase domain variant (mutation) analysis is RNA (this may be processed as cDNA in the utilised assay) from peripheral blood or bone marrow leukocytes. Analysis of genomic DNA is not currently recommended for routine testing[5].

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. **Laboratories are encouraged to ensure the type of EQA material employed is compatible with their assay approach before participation in this programme.**

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 (2025/26), 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.

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. Hart R. K. *et al.* HGVS Nomenclature 2024: improvements to community engagement, usability, and computability. *Genome Med.* 16(1):149 (2024).
3. https://hgvs-nomenclature.org/stable/
4. Smith, G. *et al.* A British Society for Haematology Guideline on the diagnosis and management of chronic myeloid leukaemia. *Br J Haematol.*191(2):171-193 (2020).
5. Cross, N.C.P. *et al.* European LeukemiaNet laboratory recommendations for the diagnosis and management of chronic myeloid leukemia. *Leukemia* 37:2150–2167 (2023).
6. Morales, J. *et al*. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604:310-315 (2022).

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 coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.

4.8.2 d) 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; no other activities in relation to this EQA exercise were externally provided.

4.8.2 d) 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.

4.8.2 g) 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 is informed when a UK participant is identified as having performance issues.

4.8.2 i) 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. 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 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.

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/