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

Distribution – 252603 | Participant –

Date Issued – 27 Feb 2026 | Closing Date – 27 Mar 2026

Trial Comments

Three vials of lyophilised cell line material (scheduled scored trial samples KDV(M) 168 and 169, plus an additional optional educational sample KDV(M) Edu E), were distributed to 97 participants for *BCR*::*ABL1* kinase domain variant (mutation) (KDV(M)) analysis. Overall, 90 (92.8%) participants returned results. Of the seven laboratories not submitting results, six pre-notified us of their non return due to extenuating circumstances or current absence of a clinical service.

Sample Comments

In order to best mimic clinical material, samples were formulated from a mixture of cell lines (approximately $9x10^6$ total cells/sample). Samples KDV(M) 168, 169 and Edu E 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 line(s) expressing a specific kinase domain variant (point mutation) of the fusion transcript. Sample KDV(M) Edu E featured an overall lower abundance of *BCR*::*ABL1* p210 (major) transcript (wildtype and that featuring a kinase domain variant), which equated to approximately 1.3% on the International Scale (IS)[1].

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 (Human Genome Variation Society) for previous guidance regarding nomenclature.

Sample KDV(M) 168 – Scored sample

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence	Performance status for this sample
Your result				
Consensus result	**c.951C>G**	**p.Phe317Leu**	**NM_005157.6**	

Performance status feedback comments (as applicable):

Sample KDV(M) 169 – Scored sample

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[2-4]. Please refer to our website for further details regarding the performance monitoring system*.

***Any laboratory assigned unsatisfactory performance status for this trial (over a six sample period) 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.

Due to the specific nature of sample scoring for this programme, it has not yet been possible to automate running performance monitoring and embed this function within the trial report using our current system. We apologise for this limitation and any inconvenience caused. If you have any queries, please do not hesitate to contact us at admin@ukneqasli.co.uk

Additional optional educational sample KDV(M) Edu E – Not subject to scoring or performance monitoring

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence
Your result			
Consensus result	**c.944C>T**	**p.Thr315Ile**	**NM_005157.6**
	BCR::*ABL1* approximately 1.3% IS		
Feedback comments (as 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) 168 – Scored sample

Sequence change(s)	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.951C>G[a]	87	96.7
c.951c>g[b]	1	1.1
c.951C>T[c]	1	1.1
c.949T>C[c,d]	1	1.1
Amino Acid change (protein)		
p.Phe317Leu[a]	83	92.2
p.(Phe317Leu)[e]	6	6.7
(p.Phe317Leu)[b,e]	1	1.1

Sample KDV(M) 169 – Scored sample

Sequence change(s)	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.944C>T[a]	89	98.9
c.944c>t[b]	1	1.1
Amino Acid change (protein)		
p.Thr315Ile[a]	83	92.2
p.(Thr315Ile)[e]	6	6.7
c.Thr315Ile[f]	1	1.1

Sample KDV(M) Edu E – Additional optional educational sample

Sequence change(s)	Returns	Percentage of returns (%)
DNA sequence change (cDNA)		
c.944C>T[a]	71	78.9
c944C>T[b]	1	1.1
c.994C>T[d]	1	1.1
No variant detected	6	6.7
Not tested	11	12.2
Amino Acid change (protein)		
p.Thr315Ile[a]	68	75.6
p.(Thr315Ile)[e]	3	3.3
p.Thr.315Ile[b]	1	1.1
p.Thr315Ileq[g]	1	1.1
No variant detected	6	6.7
Not tested	11	12.2

[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.
[c] Erroneous nucleotide.
[d] Erroneous nucleotide position.
[e] Parentheses (brackets) used erroneously for this nomenclature description if RNA (or cDNA produced from extracted RNA) was stated as the assay input material.
[f] Erroneous prefix.
[g] Erroneous amino acid notation.

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]	1

[a] Next generation sequencing users (Archer Pan-Heme FusionPlex assay and Archer FusionPlex Custom assay).
[b] Next generation sequencing assay targeting selected *ABL1* coding exons.

PCR method approach to target region of interest

	Returns
Nested PCR (2 rounds of PCR)	38
Semi-nested PCR (2 rounds of PCR)	22
Single PCR	20
Single long range PCR	6
NGS – not stated	2
Commercial assay - not known by user	2
Nested reverse transcriptase PCR	1
Other	3

NGS = Next generation sequencing.

Ultimate target(s) of assay strategy

	Returns
BCR::*ABL1* fusion (targeting *ABL1* on the translocated region of chromosome 9 only)	77
Both *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9	12
Not known	1

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	65
Illumina MiSeq (NGS)	13
Illumina NextSeq (NGS)	5
Illumina Novaseq (NGS)	4
Ion Torrent S5 (NGS)	2
Allele specific PCR	2
Agarose gel	1
Illumina MiniSeq (NGS)	1
NGS – not specified	1

NGS = Next generation sequencing.

Sequencing approach

	Returns
Bidirectional (forward AND reverse)	83
Unidirectional (forward OR reverse)	5
Not applicable/not known	2

Reference sequence

	Returns
NM_005157.6[a]	74
NM_005157.5	6
ENST00000318560.6[a]	3
ENST00000318560.5	3
NM_005157.4	2
NM_005157 – no version provided	1
NM_005157.3	1
NM_0051157.6[b]	1

[a] Latest available version of the *ABL1* Matched Annotation from the NCBI and EMBL-EBI (MANE) Select transcript.
[b] Suspected typographical error at data entry.

Assay journal references (as reported by participants)

	Returns
In house method (no reference)	34
Soverini S. *et al.* (2013). *Blood* 122:9, 1634-1648	14
Branford S. *et al.* (2002). *Blood* 99:9, 3472-3475	10
Hochhaus A. *et al.* (2002). *Leukemia* 16:11, 2190-2196	9
Branford S. and Hughes T. (2006). Myeloid Leukemia Methods and Protocols eISBN 1-59745-017-0, 93-106*	9
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)	3
Khorashad J. *et al.* (2006). *Leukemia* 20:4, 658-663	2
Kizilors A. *et al.* (2019). *Lancet Haematology* 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	**All methods**	**Sanger Sequencing**	**Next Generation Sequencing (NGS)**
<1	3[a]	1[b]	1
1	7	1[b]	6
2.5	1	0	1
3	6	0	6
4	1	0	1
5	10	1	9
7	1	1	0
8	1	1	0
10	7	6	1
15	12	12	0
20	37	37	0
25	2	2	0
30	2	2	0

[a] Includes allele specific PCR (n=1).
[b] Suspected typographical error at data submission.

Quantification – For educational purposes only

For cDNA/RNA template input material assays only - expressed variant allele frequency (VAF)

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) 168 variant (mutation) quantification: % variant allele frequency (VAF)

***ABL1* NM_005157.6: c.951C>G p.Phe317Leu**				
Calculation approach	**n**	**Mean**	**Median**	**IQR**
(Mut/(Mut+WT)) x 100[a]	25	98.0	99.0	2.9
Other[b]	15			

NGS (n=18): median VAF=98.5% (IQR=2.0%)

Sanger sequencing (n=7): median VAF=100.0% (IQR=0.0%)

Sample KDV(M) 169 variant (mutation) quantification: % variant allele frequency (VAF)

***ABL1* NM_005157.6: c.944C>T p.Thr315Ile**				
Calculation approach	**n**	**Mean**	**Median**	**IQR**
(Mut/(Mut+WT)) x 100[a]	26	94.3	97.0	5.3
Other[b]	16			

NGS (n=18): median VAF=96.0% (IQR=2.3%)

Sanger sequencing (n=8): median VAF=100.0% (IQR=7.5%)

Sample KDV(M) Edu E – Additional optional educational sample

***ABL1* NM_005157.6: c.944C>T p.Thr315Ile**				
Calculation approach	**n**	**Mean**	**Median**	**IQR**
(Mut/(Mut+WT)) x 100[a]	23	91.4	99.4	4.0
Other[b]	11			

NGS (n=16): median VAF=99.0% (IQR=6.3%)

Sanger sequencing (n=7): median VAF=100.0% (IQR=0.0%)

[a] Assay methods with *BCR*::*ABL1* enrichment/specific targeting only.
[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) 168

In line with sample formulation, all returning participants (90/90) reported a result consistent with the detection of a variant equivalent to p.Phe317Leu in sample KDV(M) 168.

- Two participants provided erroneous DNA nomenclature (c.951C>T, c.949T>C) but were in consensus at the protein level.
- At the protein level, six laboratories inappropriately included parentheses in the nomenclature description provided (cDNA was stated as assay input material).
- Two laboratories noted detection of a low-level c.325G>A p.Gly109Ser variant. This additional variant is not within the scope of the programme and is not subject to scoring.

Sample KDV(M) 169

In line with sample formulation, all returning participants (90/90) reported detection of the c.944C>T p.Thr315Ile variant in sample KDV(M) 169.

- At the protein level, a single participant included a nomenclature prefix error (c.Thr315Ile) and five laboratories inappropriately included parentheses in the nomenclature description provided (cDNA was stated as assay input material).

Sample KDV(M) Edu E

Overall, 79/90 (87.8%) of returning laboratories also tested the additional optional KDV(M) Edu E sample. Of those centres submitting a result, 73/79 (92.4%) successfully detected the c.944C>T p.Thr315Ile variant. The sample was formulated to represent a total *BCR*::*ABL1* p210 transcript level equivalent to approximately 1.3% on the International Scale (IS)[1]. Historically, scored trial samples for this programme have been distributed featuring a much higher abundance of *BCR*::*ABL1* p210 transcript, which may be less clinically relevant.

Nomenclature issues observed were attributable to likely typographical mistakes and included a positional error at the DNA level (c.994C>T) and incorrect protein notation (p.Thr315Ileq).

Six returning participants failed to identify the p.Thr315Ile variant in the KDV(M) Edu E sample. This included both Sanger sequencing (n=4) and NGS (n=2) users.

There was no clear methodological correlation. The majority of laboratories (n=5) returning a false negative result appropriately quoted an assay targeting specifically the *BCR*::*ABL1* fusion (cDNA input, various PCR methodologies for enrichment). However, one Sanger sequencing user (LoD=10%) employed a single PCR to target both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9; the participant noted that they are transitioning to a more suitable assay which targets specifically the *BCR*::*ABL1* fusion. Sufficient *BCR*::*ABL1* specific transcript enrichment is required to permit the detection of potentially clinically actionable kinase domain variant(s) in a CML patient possibly relapsing from a previously achieved Complete Cytogenetic Response (CCyR) (1% *BCR*::*ABL1* on the IS). The single laboratory using gDNA as input material for an NGS based assay targeting both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9 did not test optional sample KDV(M) Edu E.

Of note, two participants utilising Archer FusionPlex based assays with RNA input material detected the c.944C>T p.Thr315Ile variant in sample KDV(M) Edu E with their NGS approach but returned gross outlier quantification values (VAF=2.5-3.0%). Consistent with an assay design with absent/limited *BCR*::*ABL1* targeted enrichment, one laboratory (Archer FusionPlex Pan Heme panel) stated their assay targets both the *BCR*::*ABL1* fusion and *ABL1* on the non-translocated chromosome 9. However,

the other laboratory (Archer FusionPlex Custom panel) noted their assay as targeting *BCR*::*ABL*1 only (no further details known).

A single laboratory reported detection of an additional unanticipated variant (c.1064A>G p.Glu355Gly, no VAF provided) in this educational sample. The p.Glu355Gly variant is noted as clinically significant in the literature and associated with resistance to asciminib[5].

Quantification

Overall, 43/90 (47.8%) of returning laboratories provided quantitative information (all methods) for at least one of the consensus variants featured in this trial distribution.

Please refer to the tables on page 8, which summarise equivalent quantification results (from comparable assay and calculation approaches) submitted by participants.

Reference sequences and nomenclature

All returning laboratories stated the use of an *ABL1* (isoform a) transcript-based reference sequence (n=90). However, a single laboratory failed to provide a version number, and one participant included a typographical mistake in the accession number submitted. 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[2-4] 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].

In accordance with HGVS Nomenclature, all laboratories utilised the three-letter amino acid notation.

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.**

Assay analytical sensitivity is limited by the absence of *BCR*::*ABL1* fusion targeting. 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.

Final Remarks

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

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 2026/27 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% expressed VAF). UK NEQAS LI will continue to aim to periodically offer an additional low-level variant(s) sample (5 - 20% target VAF and/or with total p210 *BCR*::*ABL1* transcript level approximately 0.1-1% IS) 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. Branford, S. *et al*. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. *Blood* 112(8):3330-3338 (2008).
2. den Dunnen, J.T. *et al.* HGVS recommendations for the description of sequence variants: 2016 Update *Hum. Mutat.* 37:564-569 (2016).
3. Hart, R.K. *et al.* HGVS Nomenclature 2024: improvements to community engagement, usability, and computability. *Genome Med.* 16(1):149 (2024).
4. https://hgvs-nomenclature.org/stable/
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 17043:2023

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/

Elements of this trial/report were produced with AI assistance, including data analysis. All AI-generated content was used in an augmented capacity, independently reviewed for scientific accuracy, and approved by the responsible scientific lead and a member of senior management prior to inclusion, in line with standard report-checking procedures. AI use is governed by the organisation's AI Acceptable Use Policy (QM 837), developed with reference to ISO/IEC 17043:2023 (clauses 4.4 impartiality, 5.2 confidentiality, and 7.11 reports), and supported by documented audit trails and mandatory human verification of all AI-generated outputs.