BCR-ABL1 Kinase Domain Variant (Mutation) Status (Accredited)

Distribution – 202101

Participant –

Date Issued – 19 Aug 2020

Closing Date – 25 Sep 2020

Trial Comments

FINAL REPORT

Two vials of lyophilised cell line material (sample refs KDM 136 and KDM 137) were distributed to 74 participants for *BCR-ABL1* kinase domain variant (mutation) (KDV/M) analysis. Overall, 70 (94.6%) participants returned results for this trial at the time of reporting. Of the four laboratories that did not submit results, one pre-notified us of their non return and we await results from a further laboratory (following an agreed extension due to COVID 19 pandemic operational pressures).

This trial is the first distribution following ISO:17043 accreditation for the *BCR-ABL1* Kinase Domain Variant (Mutation) Status programme and is subject to formal performance monitoring.

Sample Comments

In order to best mimic clinical material, samples were formulated from a mixture of cell lines. Samples KDM 136 featured a cell line lacking the *BCR-ABL1* p210 (major) transcript, a *BCR-ABL1* p210 positive cell line expressing 'wild-type' (non-point mutated) *BCR-ABL1* fusion transcript and two *BCR-ABL1* p210 positive cell lines expressing a specific kinase domain variant (point mutation) of the *BCR-ABL1* fusion transcript (polyclonal). Samples KDM 137 featured a cell line lacking the *BCR-ABL1* p210 (major) transcript and a *BCR-ABL1* p210 positive cell line expressing 'wild-type' (non-point mutated) *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 KDM 136

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence	Performance status for this sample
Your result				
Consensus result	**c.763G>A c.944C>T**	**p.Glu255Lys p.Thr315Ile**	**NM_005157.5***	This programme is now ISO:17043 accredited

Performance status feedback comments (as applicable):

Sample KDM 137

Your Results

	DNA sequence change(s)	Protein sequence change(s)	*ABL1* Reference sequence	Performance status for this sample
Your result				
Consensus result	**No variant detected**	**No variant detected**	**NM_005157.5***	This programme is now ISO:17043 accredited
Performance status feedback comments (as applicable):				

* NM_005157.6 reference sequence is available (https://www.ncbi.nlm.nih.gov/nuccore/NM_005157)

IMPORTANT: This programme has now achieved UKAS ISO:17043 accreditation. 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 (Three Trial Period)		
Sample KDM 136	**Sample KDM 137**	**Satisfactory**	**Action**	**Critical**
		N/A	N/A	N/A

* PLEASE NOTE: *BCR-ABL1* Kinase Domain Variant (Mutation) Status 202101 is the first trial of this programme to be distributed with ISO:17043 accreditation. As such, running performance status (over a rolling 6 sample period) is not yet fully implemented.

N/A: Not applicable

All Participant Results

Detailed Results Breakdown

Nomenclature provided by participants in relation to an *ABL1*:NM_005157 based reference sequence unless otherwise stated.
Percentage values quoted have been subjected to rounding up/down to 1 decimal place.

Sample KDM 136

Sequence change 1	Returns	Percentage of returns
DNA sequence change (cDNA)		
c.763G>A [a]	63	90.0
c.736G>A [b]	1	1.4
Variant (mutation) not detected [c]	6	8.6
Amino Acid change (protein)		
p.Glu255Lys [a]	49	70.0
p.(Glu255Lys) [d]	6	8.6
(p.Glu255Lys) [d, e]	1	1.4
Glu255Lys [e]	2	2.9
p.E255K [f]	4	5.7
E255K [e, f]	2	2.9
Variant (mutation) not detected [b]	6	8.6

[a] Nomenclature fully compliant with current HGVS recommendations (RNA/cDNA assay input material).
[b] Positional numbering error at the cDNA level.
[c] Includes 2 laboratories concluding 'no variant detected' for sample KDM 136.
[d] Parenthesis used erroneously for this nomenclature description; cDNA produced from extracted RNA was stated as the assay input material.
[e] Nomenclature is not compliant with current HGVS recommendations.
[f] HGVS recommendations advocate use of three letter amino acid code.

Sequence change 2	Returns	Percentage of returns
DNA sequence change (cDNA)		
c.944C>T [a]	66	94.3
Variant (mutation) not detected [b]	4	5.7
Amino Acid change (protein)		
p.Thr315Ile [a]	50	71.4
p.(Thr315Ile) [c]	6	8.6
(p.Thr315Ile) [c, d]	1	1.4
p.315Thr>Ile [d]	1	1.4
Thr315Ile [d]	2	2.9
p.T315I [e]	4	5.7
T315I [d, e]	2	2.9
Variant (mutation) not detected [b]	4	5.7

[a] Nomenclature fully compliant with current HGVS recommendations (RNA/cDNA assay input material).
[b] Includes 2 laboratories concluding 'no variant detected' for sample KDM 136.
[c] Parenthesis used erroneously for this nomenclature description; cDNA produced from extracted RNA was stated as the assay input material.
[d] Nomenclature is not compliant with current HGVS recommendations.
[e] HGVS recommendations advocate use of three letter amino acid code.

Sample KDM 137

	Returns	Percentage of returns
DNA sequence change (cDNA)		
No variant (mutation) detected	69	98.6
c.955G>A, c.1136C>T, c.1261A>G [a, b]	1	1.4
Amino Acid change (protein)		
No variant (mutation) detected	69	98.6
p.Glu255Lys, p.Thr315Ile, p.Lys357Glu [b]	1	1.4

[a] Nucleotide positional numbering includes *ABL1*:NM_005157 5' UTR (192 bp). Please be aware the Human Genome Variation Society (HGVS) recommendations advocate nucleotide numbering beginning with the A of the ATG initiation codon.
[b] Suspected sample switch incident.

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	66
DNA	2
RNA [a]	2

[a] Next Generation Sequencing users.

PCR method approach

	Returns
Nested PCR	35
Semi-nested PCR	23
Single PCR	9
Single long range PCR	2
Multiplex PCR [a]	1

[a] No further information known.

Target(s) of PCR amplification strategy

	Returns
BCR-ABL1 fusion (targeting *ABL1* on the translocated chromosome 9)	62
Both	7

Please note that for *BCR-ABL1* positive CML/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 chromosome 9 is conventionally the amplification target for assays involving subsequent sequencing.

Analysis method

	Returns
Sanger sequencing	56
Ion Torrent PGM (NGS)	3
Illumina MiSeq (NGS)	7
Allele specific PCR	2
PACBIO Sequel (NGS)	1
Illumina NovaSeq (NGS)	1
Illumina NextSeq (NGS)	1
Illumina MiniSeq (NGS)	1
Digital PCR	1

NGS = Next Generation Sequencing

Sequencing approach

	Returns
Bidirectional (forward AND reverse)	65
Unidirectional (forward OR reverse)	1
Not applicable/provided	4

Reference sequence used

	Returns
NM_005157.6 [a]	15
NM_005157.5 [a]	39
NM_005157.4 [a]	5
NM_005157.3 [a]	2
NM_005157 (no version provided) [a]	1
NM_007313.2 [b]	1
ENST00000318560.5 [a]	4
ENST00000318560.6 [a]	2
NM_14752 [c]	1

[a] A*BL1* mRNA isoform a.
[b] *ABL1* mRNA isoform b.
[c] Suspected data entry error, M14752 historic source sequence for NM_005157.

Please note isoform b derived sequences (including NM_007313) are not the preferred *ABL1* reference sequence in this clinical context. The NCBI GenBank *ABL1* gene webpage (www.ncbi.nlm.nih.gov/gene/25) is a valuable resource for obtaining relevant reference sequences at both a protein and DNA level. Please be aware the Locus Reference Genomic (LRG) sequence for *ABL1* is still pending approval and should not yet be formally employed (http://ftp.ebi.ac.uk/pub/databases/lrgex/pending/LRG_769.xml).

Assay journal references (as reported by participants)

	Returns
In house method (no reference)	24
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	8
Hochhaus A. *et al.* (2002). *Leukemia* 16:11, 2190-2196	8
Ernst T. *et al.* (2008). *Haematologica* 93:2, 186-192	6
Alikian M. *et al.* (2012). *American Journal of Hematology* 87:3, 298-304	5
Polakova K. *et al.* (2008). *Leuk Res* 32:8, 1236-1243	3
MODHEM (Network for Molecular Diagnostics of Hematologic Malignancies)	3
Soverini S. *et al.* (2004). *Clinical Chemistry* 50:7, 1205-1213	2
Soverini S. *et al.* (2013). *Blood* 122:9, 1634–1648	3
Cavelier S. *et al.* (2015) *BMC Cancer* 15:45	1
Wang L. *et al.* (2006). *Haematologica* 91:2, 235-239	1
Gruber F. *et al.* (2005). *Leukemia* 19:12, 2159-2165	1
Polakova *et al.* (2015). *J Cancer Res Clin Oncol* 141, 887-899	1
Other	1
Not stated	5

Additional assay information

	Returns	
Percentage (%) variant	**Limit of Detection (LoD)**	**Limit of Quantitation (LoQ)**
<1	1	0
1	2	1
1.5	0	1
2	2	1
3	2	2
4	0	1
5	7	5
10	7	5
15	10	4
20	17	4
25	1	0

Quantification

Figures in the tables below have been compiled from quantification data submitted by participants with a defined calculation approach. Descriptive statistics are included for those returns stating a PCR strategy (cDNA/RNA template input material) which targets the *BCR-ABL1* fusion (*ABL1* on the translocated chromosome 9) prior to analysis with >3 quantification data points available. Values quoted have been subjected to rounding up/down to 1 decimal place.

Sample KDM 136 mutation (variant) quantification

***ABL1* c.763G>A, p.Glu255Lys**				
Calculation approach	**n**	**Median**	**Mean**	**Range**
Mut/(Mut+WT) x 100	17*	30.0	33.2	25.0 - 50.0
Mut/WT x 100	4	35.0	36.5	26.0 - 50.0
Other	3			

* 9 Sanger sequencing, 8 NGS (various platforms)

***ABL1* c.944C>T, p.Thr315Ile**				
Calculation approach	**n**	**Median**	**Mean**	**Range**
Mut/(Mut+WT) x 100	18*	43.5	45.4	30.0 - 75.0
Mut/WT x 100	4	39.0	38.5	36.0 - 40.0
Other	3			

* 10 Sanger sequencing, 8 NGS (various platforms)

Mut = Mutation (variant), WT = 'Wild-type'

Comments

Sample KDM 136

In line with sample formulation 68 (97.1%) returning laboratories identified at least one missense sequence change for this sample.

- 61/68 (89.7%) participants reporting a sequence change detected both the c.763G>A, p.Glu255Lys and c.944C>T, p.Thr315Ile variants in sample KDM 136.
- Despite both variants being encompassed by their stated assay scope, 4 participants identified the c.944C>T, p.Thr315Ile variant only and two participants the c.763G>A, p.Glu255Lys variant only. All but one of these participants employed Sanger sequencing (nested PCR on cDNA (n=4) and DNA (n=1) templates). The remaining laboratory used RNA Next Generation Sequencing on the Illumina MiSeq platform.
- Two participants failed to detect any variants in sample KDM 136. The p.Glu255Lys and p.Thr315Ile variants are within the stated scope of their assays. Both laboratories used bidirectional Sanger sequencing targeting *ABL1* on the translocated chromosome 9. One of the participants stated use of a single PCR on a DNA template and the other nested PCR from cDNA input material.
- Nomenclature at the cDNA level was in good agreement with only a single laboratory making a positional numbering error (see tables pages 3 and 4).
- The approach to protein nomenclature demonstrated some variation. However, this was limited to minor syntax/symbol errors and/or use of the less preferable single letter amino acid code.
- Overall, 26 returning laboratories submitted optional accompanying quantification data (on detection of a consensus variant and with the required methodological supporting information) for sample KDM 136. Please refer to the tables on page 7 for further information.
- 50/70 (71.4%) returning participants, using various analysis methods, reported an additional variant (A to G substitution). 47/50 (94.0%) described the variant as c.1069A>G, p.Lys357Glu. Median reported VAF was 25.5% (n = 14).
    - Similar findings have been described in previous trials when the same p.Glu255Lys known positive cell line material was included in sample formulation (KDM 141501, KDM 161702). It is evident that some of the engineered cell lines employed by this programme harbour change(s) in addition to the target *BCR-ABL1* substitution variant.
    - It is important to evaluate, for any sample tested, whether unanticipated variants are clinically relevant. At the time of trial reporting, no reference in the literature or listing in an applicable cancer/general population database could be identified for the NM_005157.6(*ABL1*):c.1069A>G, p.Lys357Glu additional variant[3].
    - In accordance with the performance monitoring system for this programme, this variant is not subject to scoring. Please refer to our website for further details regarding the scoring system.

Sample KDM 137

In line with sample formulation 69 (98.6%) returning laboratories reported no variant detected for this sample.

- One participant reported identification of the c.955G>A, p.Glu255Lys and c.1136C>T, p.Thr315Ile (and additional c.1261A>G, p.Lys357Glu) variants in sample KDM 137 (cDNA positional numbering provided by the participant included the 5' untranslated region). The same laboratory failed to detect any sequence change in sample KDM 136. A sample switch event is therefore suspected.

Final Remarks

***BCR-ABL1* Kinase Domain Variant (Mutation) Status 202101 is the first trial of this programme to be distributed with ISO:17043 accreditation status. As such, it includes formal application of the scoring criteria and performance monitoring system.**

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 available 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), at the next trial issue participants will be reminded to return only results with clinical significance and relevance to the scoring system.

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.

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

Reference(s)

1. Den Dunnen, J. T. et al. HGVS Recommendations for the description of sequence variants: Update *Hum. Mutat.* **37,** 564–569 (2016).
2. http://varnomen.hgvs.org/
3. Alamut Visual (version 2.11.0) https://www.interactive-biosoftware.com/alamut-visual/

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: nicola.rose@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/