UK NEQAS LI *BCR*::*ABL1* Kinase Domain Variant (Mutation) Status KDV(M) - Performance Monitoring System

Aim

The scoring system is a rolling scheme that will identify unsatisfactory performance or persistent unsatisfactory performance of any participant. This is in order that UK NEQAS LI can provide support and guidance where needed and ensure that the Genetics NQAAP are informed, as appropriate. Please note that each programme will be scored independently.

Outline

Two samples are issued at each trial distribution that may or may not harbour a *BCR*::*ABL1* kinase domain variant(s) (point mutation(s)). There are three trials per annum.

The *BCR*::*ABL1* Kinase Domain Variant Status trial requires a qualitative response from participants. Therefore, participants are asked if, using their normal laboratory technique, they have detected a clinically reportable variant(s), and if so to describe that variant(s) using standardised sequence variant nomenclature at both the cDNA and protein levels (Den Dunnen *et al*., 2016 - Human Genome Variation Society (HGVS) nomenclature version 21.0 https://hgvs-nomenclature.org/stable/). Accordingly, participants are also asked to provide an accession number (and version) for an applicable Ensembl (EMBL-EBI) or RefSeq (NCBI-NIH) transcript reference sequence underpinning the variant description.

The presence/absence of a *BCR*::*ABL1* kinase domain variant(s) (point mutation) is determined by consensus (modal result) from participant data. Each participant response is then compared against the consensus result. If a consensus variant falls outside a laboratory's assay scope, the participant will be excluded from scoring in relation to that variant for a given sample.

With regard to nomenclature, variant descriptions are scored with reference to the Human Genome Variation Society (HGVS) nomenclature version 21.0 https://hgvs-nomenclature.org/stable/). Participant results for individual samples are classified as 'Critical', 'Action' or 'Satisfactory' according to the sample scoring criteria outlined below. The option to exclude a sample from scoring for a given participant is available to UK NEQAS LI.

Quantitative data (% variant (expressed) allele frequency, 'mutation load') may be submitted by participants but will not be formally scored (for educational purposes only).

<table>
<tr><th colspan="4">Sample scoring criteria</th></tr>
<tr><th colspan="2">Satisfactory (in consensus)</th><th>Action</th><th colspan="2">Critical</th><th>Action</th></tr>
<tr><td colspan="2">In line with all participants' modal result

Sequence change(s) described with rational and unambiguous IUPAC+/HGVS* based nomenclature</td><td>Nomenclature related issue(s) obstructing unambiguous description of sequence change(s)</td><td>Gross analytical error</td><td>Major nomenclature error(s) with potential clinical implications</td><td>Non return of result for a sample</td></tr>
<tr><td>Accurate and comprehensive nomenclature fully compliant with current HGVS recommendations

Reference sequence as specified by the MANE project#</td><td>Minor nomenclature issue(s) precluding full compliance with current HGVS recommendations

EXAMPLE
• Out of date reference sequence (e.g. legacy transcript) or Locus Reference Genomic (LRG)^
• Reference sequence accession lacking version number, as applicable
• Use of single letter amino acid code only at the protein level
• Inconsequential symbol or syntax oversight (for example / in place of > or brackets used at protein level following cDNA analysis)</td><td>EXAMPLE
• No (or the wrong) reference sequence^ provided (for a positive result)
• (Potentially) misleading use of HGVS syntax/symbol(s)
• Protein nomenclature only provided
• Positional numbering error at DNA level (including inclusion of UTR) but protein description correct
• Nucleotide error at DNA level but protein description correct
• Absent symbol (for example p. or c.)</td><td>EXAMPLE
• False positive
• False negative
• Out of consensus variant</td><td>EXAMPLE
• Amino acid error at protein level
• Positional numbering error at protein level</td><td rowspan="2">NOTE
Non return for <u>both</u> scored samples in a trial = Critical (via 2 x Action score)</td></tr>
<tr><td colspan="4">At least one incidence, if multiple sequence changes (variants) detected in an EQA sample</td></tr>
</table>

+ https://iupac.org/what-we-do/nomenclature/

* Den Dunnen, J. T. *et al*. HGVS Recommendations for the Description of Sequence Variants: Update *Hum. Mutat.* **37,** 564–569 (2016). Human Genome Variation Society (HGVS) nomenclature version 21.0 https://hgvs-nomenclature.org/stable/

Morales, J. *et al*. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604: 310-315 (2022).

^ Locus Reference Genomic (LRG) https://www.lrg-sequence.org/ (accessed July 2024). Preferable reference sequence sources: RefSeq from NIH-NCBI (https://www.ncbi.nlm.nih.gov/refseq/) or Ensembl from EBI-EMBL (https://www.ensembl.org/Homo_sapiens/Info/Index) as specified by the MANE project.

Extent of sample scoring criteria (nomenclature/false negative results): Sequence variants relating to the following residue (amino acid) positions - p.Met244, p.Gly250, p.Gln252, p.Tyr253, p.Glu255, p.Val299, p.Thr315, p.Phe317, p.Met351, p.Phe359 (tyrosine-protein kinase ABL1 isoform a, NP_005148.2). During the sample scoring classification process reference will be made to a participant's stated assay scope (as provided a trial results submission).

Example application of the sample scoring criteria				
	Satisfactory	**Satisfactory (minor comment required on the participant report)**	**Action**	**Critical**
Reference sequence (*ABL1*)	**NM_005157.6[1]** **NM_005157.5[1]** **ENST00000318560.6[1]**	**X16416.1[1]** **M14752[1]** **NM_007313.2[2]** **LRG_769 (t1) or (p1)[3]** **LRG_769 (t2) or (p2)[3]** **NM_005157[4]** **ENST00000318560[4]**	**NM_005154.4[1]** **ENSG00097007[2]** **No reference sequence provided[3]**	**N/A**
	[1] Ensembl or RefSeq transcript reference sequence accession with version number. As specified by the MANE project.	*[1] Historical GenBank CDS/mRNA sequences now superseded by NIH RefSeq project.* *[2] Alternative transcript not specified by the MANE project.* *[3] Locus Genomic Reference project is no longer maintained.* *[4] No version number provided.*	*[1] Reference sequence not ABL1 (different gene).* *[2] Gene based genomic reference only provided to support cDNA nomenclature.* *[3] For a positive result.*	
***BCR*::*ABL1* KDV(M) cDNA nomenclature**	**c.763G>A** **NM_005157.6:c.763G>A**	**c.763G/A[1]** **c.G763A[1]** **c.763G-A[1]**	**c.763G>GA[1]** **c.955G>A[2]** **c.736G>A[2]** **c.762G>A[2]** **c.703C>A[2,3]** **c.763G>T[3]**	**No variant (mutation) detected[1]**
	Note: Fully compliant with HGVS recommendations.	*[1] Inconsequential symbol or syntax oversight.*	*[1] Potentially misleading symbol or syntax error* *[2] Positional numbering error (in consensus at protein level)* *[3] Nucleotide error (in consensus at protein level)*	*[1] With reference to stated assay scope to confirm false negative.*
***BCR*::*ABL1* KDV(M) protein nomenclature**	**p.Phe317Leu** **NP_005148.2:p.Phe317Leu**	**p.(Phe317Leu)[1]** **p.F317L[2]**	**Phe317Leu[1]** **p.Phe317PheLeu[2]**	**No variant (mutation) detected[1]** **p.Phe310Leu[2]** **p.Phe317Phe[3]** **p.Phe317Ala[3]** **p.P317L[3]** **p.F317K[3]**
	Note: Fully compliant with HGVS recommendations (RNA/cDNA assay input material).	*[1] Brackets not required (cDNA was analysed).* *[2] Use of single amino acid code.*	*[1] Absent symbol.* *[2] Potentially misleading symbol or syntax error.*	*[1] With reference to stated assay scope to confirm false negative.* *[2] Positional numbering error.* *[3] Amino acid error.*

Two 'Action' scores in a period of 3 samples would result in classification as a 'Critical'. Please note that whilst each 'Action' will be considered in combination with any other 'Actions' within the rolling 3 sample window, the same pair of 'Action' scores will not be combined to result in a 'Critical' classification more than once.

Any laboratory failing to return a result by the closing date of the trial will be regarded as an 'Action' for each sample. As such any laboratories that do not return results for both samples within a trial distribution will be classified as 'Critical'. Please note, results should not be submitted if samples fail local quality control (QC) measures. Repeat samples are available for all trials, if required. If following repeat sample(s) processing, the results obtained still do not pass local internal QC please contact UK NEQAS LI. If trial results are submitted based on suboptimal extraction and/or assay results, they will be subjected to the same performance monitoring mechanisms as all other participants.

Unsatisfactory performance in this programme is defined as any occurrence of 'Critical' performance. Unsatisfactory performance will be initially communicated to participants via their trial report. This will be followed by a letter/email and UK NEQAS LI Participant Hub notification, highlighting that their performance was unsatisfactory on the last trial, and offering support and guidance. The support and guidance offered will be tailored to the particular needs of the participant but may include the provision of repeat/additional samples plus telephone, email and/or face-to-face communications.

If a participant amasses three 'Critical' performances within a 6 sample period, their status is elevated to persistent unsatisfactory performance; a further letter/email and UK NEQAS LI Participant Hub notification will be issued highlighting this and, for UK based laboratories, the Genetics NQAAP informed.

Participant's results will be reviewed by a UK NEQAS LI Scientist(s) and any UK participant may, at the discretion of the Director and Specialist Advisory Group chairperson, be referred to the Genetics NQAAP even if they have not met the criteria for persistent unsatisfactory performance in any individual EQA programme.

As with all scoring systems it is important to note that these will be constantly reviewed to determine if they are providing the information required. The Director of the scheme retains the discretion to determine if any individual trial should not be scored.

References

Den Dunnen, J. T. *et al*. HGVS Recommendations for the Description of Sequence Variants: Update *Hum. Mutat.* **37,** 564–569 (2016)

Human Genome Variation Society (HGVS) nomenclature version 21.0: https://hgvs-nomenclature.org/stable/ (accessed July 2024)

Morales, J. *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604: 310-315 (2022)