BRAF p.Val600Glu (V600E) Mutation Status for Hairy Cell Leukaemia Programme

Distribution - 222302

Participant ID -

Date Issued - 26 July 2022

Closing Date - 02 September 2022

Trial Comments

This trial was issued to 71 participants, of which 70 (98.6%) returned results. The single laboratory not returning results prenotified us of their intention not to return data.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI . BRAF 152 was manufactured to be negative for the BRAF p.Val600Glu (V600E) variant, whilst BRAF 153 was manufactured to be positive for this variant.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 152		No Mutation Detected
Sample BRAF 153		Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 152	0	70
Sample BRAF 153	70	0

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical

N/A = Not Applicable

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Template Type

	Returns
DNA	69
cDNA	1

PCR Type

	Returns
Allele Specific PCR	18
Droplet Digital PCR	18
Real-Time PCR	10
Single PCR	10
PCR for Next generation Sequencing	9
Multiplex PCR	4

Protocol Type

	Returns
In-house Assay	44
BioRad PrimePCR ddPCR kit	14
Diatech Pharmacogenetics Easy Kit	3
Biocartis Idylla	2
Entrogen Kit	2
COBAS Kit	1
Ion AmpliSeq Cancer Hotspot Panel v2	1
Oncomine Myeloid Research Assay	1
Qiagen therascreen BRAF	1
SensiScreen BRAF V600 Multiplex (FFPE) Assay	1

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	21
Digital PCR	19
NGS (Illumina)	7
NGS (ThermoFisher Ion Torrent)	5
Agarose Gel Electrophoresis	4
Capillary Electrophoresis	4
Acrylamide Gel Electrophoresis (PAGE)	2
Biocartis Idylla	2
SNaPshot (Mini Sequencing)	2
NGS (Other)	1
Pyrosequencing	1
Reverse Hybridisation	1
Sanger Sequencing	1

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Journal Reference for Assay

	Returns
Tiacci E. et al (2012). Blood, 119:1 - 192-195	17
Arcaini L. et al (2012). Blood, 119:1, 188-191	11
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	3
Guerrini, F. et al., (2016) Front Pharmacol, 7:363.	2
Huang T. et al (2013) Biomark Res, 16:1, 3	2
Rustad EH. et al. (2015) Blood Cancer J 5: e299	2
Wong C. et al (2005). J Clin Pathol, 58, 640-644.	2

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Trial Summary

- In line with sample formulation, all 70 participants (100%) returning results for this trial did not detect the *BRAF* p.Val600Glu (V600E) variant (mutation) in sample BRAF 152.
- Similarly, in line with formulation of sample BRAF 153, all 70 participants (100%) correctly detected the *BRAF* p.Val600Glu (V600E) variant.
- **UK NEQAS LI have previously outlined how Sanger sequencing, in the absence of an enrichment method, is considered an inadequate technique for the detection of *BRAF* p.Val600Glu in hairy cell leukaemia (HCL) due to reduced assay sensitivity; hairy cells have previously been reported at levels as low as 2% in peripheral blood from HCL patients[1].** Whilst there remains a single participant stating the use of Sanger sequencing as an analysis method in this trial, we are pleased to report that the Sanger sequencing user who returned out-of-consensus results in the previous trial (BRAF 222301) is now declaring the use of digital PCR and their results are in consensus for the current BRAF 222302 trial distribution.

General Comments

- The Human Genome Variation Society (HGVS) nomenclature guidelines now include a recommendation to use transcript reference sequences specified by the Matched Annotation from the NCBI and the EMBL-EBI (MANE) collaboration project[2]. In the preliminary versions of the MANE project, the MANE Select transcript for *BRAF* was designated as NM_001374258.1 / ENST00000644969.2 (producing the *BRAF* c.1919T>A p.Val640Glu description). However, in response to feedback and in recognition of the legacy nomenclature for this gene, the MANE Select transcript has now been revised to NM_004333.6 / ENST00000646891.2, as specified in the recently published MANE v1.0 transcript set[3]. The NM_001374258.1 reference sequence (longest transcript), has been retained as the MANE Plus Clinical transcript, and its use is also acceptable. The use of Locus Reference Genomic (LRG) reference sequences is no longer advocated by the HGVS when a MANE transcript option is available[2].
- The nomenclature used in this programme, *BRAF* c.1799T>A p.Val600Glu, complies with the current MANE Select transcript NM_004333.6. Please note that at the time of this report, some annotation resources have yet to be updated to specify the current MANE Select transcript, and therefore caution should be exercised when using software tools that may not be aligned with MANE v1.0.
- We thank participants for their continued engagement in the *BRAF* p.Val600Glu (V600E) Mutation Status for Hairy Cell Leukaemia programme.

References

1. Arcaini, L. *et al.* The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. *Blood* **119**: 188–191 (2012).
2. den Dunnen, J.T. *et al*. HGVS recommendations for the description of sequence variants: 2016 update. *Hum.Mutat.* **37**: 564-569 (2016). https://varnomen.hgvs.org/recommendations/general/ (Accessed October 2022).
3. Morales, J. *et al*. A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* **604**: 310-315 (2022).

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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) No activities in relation to this EQA exercise were subcontracted.

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/