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

Distribution - 202101

Participant ID - 4XXXX

Date Issued - 29 April 2020

Closing Date - 05 June 2020

Trial Comments

FINAL REPORT: This trial was issued to 74 participants, of which 69 (93.2%) returned results. Of the non returns, one participant notified us of their intended non return and three laboratories submitted requests for an extension in results submission in light of the ongoing Covid-19 pandemic.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI . Samples BRAF 138 was manufatured to be positive for the BRAF p.Val600Glu (V600E) variant and BRAF 139 was formulated to be negative.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 138		Mutation Detected
Sample BRAF 139		No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 138	69	0
Sample BRAF 139	1	68

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	68
cDNA	1

PCR Type

	Returns
Real-Time PCR	17
Allele Specific PCR	14
Droplet Digital PCR	13
Single PCR	11
PCR for Next generation Sequencing	10
Multiplex PCR	3
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	48
BioRad PrimePCR ddPCR kit	10
Biocartis Idylla	2
Diatech Kit	2
Entrogen Kit	2
Ion AmpliSeq Cancer Hotspot Panel v2	2
Genmark BRAF V600E Assay	1
PNAClamp BRAF Mutation Detection	1
Qiagen therascreen BRAF	1

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	21
Digital PCR	14
Agarose Gel Electrophoresis	8
Capillary Electrophoresis	6
NGS (Illumina)	6
NGS (ThermoFisher Ion Torrent)	6
Acrylamide Gel Electrophoresis (PAGE)	2
Biocartis Idylla	2
High Resolution Melt	1
Pyrosequencing	1
Sanger Sequencing	1
SNaPshot (Mini Sequencing)	1

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Journal Reference for Assay

	Returns
Tiacci E. et al (2012). Blood, 119:1 - 192-195	15
Arcaini L. et al (2012). Blood, 119:1, 188-191	10
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	2
Wong C. et al (2005). J Clin Pathol, 58, 640-644.	2

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Trial Summary

- In line with sample formulation, 69 out of 69 (100%) participants returning results for this trial detected the *BRAF* p.Val600Glu (V600E) mutation in sample BRAF 138.
- For BRAF 139, 68/69 (98.6%) participants returning results reported the sample to be negative the *BRAF* p.Val600Glu (V600E) mutation.
- The one participant who reported a false positive result for BRAF 139 utilised an in-house method with Real-Time fluorescent PCR analysis.
- **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 the lack of sensitivity of the assay. With hairy cells having previously been reported at levels of 2% in peripheral blood in patients HCL[1].** For two of the three trial distributions in the previous trial year (BRAF 192002 and 192003), Sanger Sequencing was not used as an analysis method by participants returning results. However, for this trial (BRAF 202101) one participant reported the use of Sanger Sequencing as an analysis method.

References

1. Arcaini, L. *et al.* Brief report The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. *Blood* **119,** 188–192 (2012).

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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
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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) 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/