UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

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

Distribution - 171802

Participant ID -

Date Issued - 27 July 2017

Closing Date - 25 August 2017

Trial Comments

The trial was issued to 51 participants for BRAF p.Val600Glu (V600E) analysis. 51 (100%) participants returned results. One participant did not return the result for BRAF 123 only and pre notified us of this.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI . Samples BRAF 122 and BRAF 123 were manufactured to be positive for the BRAF p.Val600Glu (V600E) variant with BRAF 122 showing a particularly high mutation load.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 122		Mutation Detected
Sample BRAF 123		Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 122	51	0
Sample BRAF 123	50	0

Your Performance

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

N/A = Not Applicable

Sheffield Teaching Hospitals NHS Foundation Trust, a UKAS proficiency testing provider No. 7804, operating UK NEQAS for Leucocyte Immunophenotyping.

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Template Type

	Returns
DNA	50
cDNA	1

PCR Type

	Returns
Allele Specific PCR	15
Single PCR	15
Real-Time PCR	13
Multiplex PCR	5
Single PCR with Clamping	2
Droplet Digital PCR	1

Protocol Type

	Returns
In-house Assay	43
PNAClamp BRAF Mutation Detection	3
Entrogen Kit	2
BioRad PrimePCR ddPCR kit	1
COBAS Kit	1
Diatech Kit	1

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	21
Next Generation Sequencing	8
Agarose Gel Electrophoresis	6
Capillary Electrophoresis	4
Sanger Sequencing	4
Acrylamide Gel Electrophoresis (PAGE)	3
Droplet Digital PCR	2
High Resolution Melt	1
Mass Spectometry	1
SNaPshot (Mini Sequencing)	1

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Journal Reference for Assay

	Returns
Tiacci E. et al (2012). Blood, 119:1 - 192-195	10
Arcaini L. et al (2012). Blood, 119:1, 188-191	9
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	2
Huang T. et al (2013) Biomark Res, 16:1, 3	2

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Trial Summary

- In line with sample formulation, 51/51 (100%) participants returning results for this trial found sample BRAF 122 to be positive for the *BRAF* p.Val600Glu (V600E) mutation.
- In line with sample formulation, 50/50 (100%) participants returning results found sample BRAF 123 to be positive for the *BRAF* p.Val600Glu (V600E) mutation (one result being excluded due to technical issues).
- Real-Time PCR Fluorescent Detection (21/51, 41.2%) remains the most popular analysis method. Next Generation Sequencing has replaced Agarose Gel Electrophoresis as the second most popular analysis method at 15.7% (8/51) and 11.8% (6/51) respectively. Analysis methods with an inadequate limit of detection i.e. Sanger Sequencing are still being used by some participants.
- Hairy cells have been reported at levels of 2% in peripheral blood in patients with hairy cell leukaemia (HCL) (Arcaini et al., 2012) differentiating the technical requirements of *BRAF* analysis in HCL from the solid tumour setting where less sensitive technologies, such as Sanger sequencing, are adequate.
- The NCBI GenBank *BRAF* gene webpage (http://www.ncbi.nlm.nih.gov/gene/673) is a valuable resource for obtaining relevant reference sequences at both a protein and DNA level. It features numerous links to related information including PubMed, dbSNP and the Ensembl or UCSC genome browsers.
- The Human Genome Variation Society (HGVS) provides a series of recommendations with the aim of standardising the nomenclature for the description of sequence variants (den Dunnen & Antonarakis, 2000).

References:

Arcaini, L., Zibellini, S., Boveri, E., Riboni, R., Rattotti, S., Varettoni, M., … Cazzola, M. (2012). The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. Blood, 119(1), 188–91.

den Dunnen, J. T., & Antonarakis, S. E. (2000). Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Human Mutation, 15(1), 7– 12.

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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, 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 Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations 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.

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/