

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

Distribution - 151602

Participant ID -

Date Issued - 24 November 2015

Closing Date - 24 December 2015

Trial Comments

The trial was issued to 43 participants for BRAF p.Val600Glu (V600E) analysis. 41 (95.3%) participants returned results, 2 participants did not return, 1 of whom pre notified us.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI. Sample BRAF 114 was manufactured to be strongly positive for the BRAF p.Val600Glu mutation and sample BRAF 115 was manufactured to be negative for the BRAF p.Val600Glu mutation.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 114		Mutation Detected
Sample BRAF 115		No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 114	40	1
Sample BRAF 115	2	39

Your Performance*

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	N/A	N/A	N/A

* PLEASE NOTE: This is not currently a scored trial; however we are working towards accreditation for this programme.

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Template Type

	Returns
DNA	39
cDNA	1

PCR Type

	Returns
Allele Specific PCR	13
Single PCR	12
Real-Time PCR	11
Multiplex PCR	3
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	37
COBAS Kit	1
Diatech Kit	1
Entrogen Kit	1

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	17
Agarose Gel Electrophoresis	7
Sanger Sequencing	4
Capillary Electrophoresis	3
High Resolution Melt	2
Next Generation Sequencing	2
SNaPshot (Mini Sequencing)	2
Acrylamide Gel Electrophoresis (PAGE)	1
Mass Spectrometry	1
Pyrosequencing	1

Journal Reference for Assay

	Returns
Arcaini L. et al (2012). Blood, 119:1, 188-191	7
Tiacci E. et al (2012). Blood, 119:1 - 192-195	7
Boyd E. et al (2011). British Journal of Haematology, 155:5, 609-612	4
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	2

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Trial Summary

- In line with sample formulation, 40 (97.6%) participants returning results for this trial found sample *BRAF* 114 to be positive for the *BRAF* p.Val600Glu (V600E) mutation.
- In line with sample formulation, 39 (95.1%) participants found sample *BRAF* 115 to be negative for the *BRAF* p.Val600Glu (V600E) mutation.
- One participant failed to detect the mutation in *BRAF* 114 and detected one in *BRAF* 115 indicative of a sample mix up. Another participant detected a mutation in both samples, i.e. detected a false positive in *BRAF* 115 using DNA, and an in-house allele specific real-time PCR methodology.
- The use of commercial kits has fallen, with only 3 of the 40 (7.5%) participants who informed us of their protocol type using commercial kits; Lightmix (R) TibMolBiol Kit and PrimerDesign quasa *BRAF*V600E mutation kit are no longer used by participants returning results.
- Real-Time PCR Fluorescent Detection (17/41, 41.5%) remains the most popular analysis method. The use of next generation sequencing and high resolution melt curve analysis has fallen in recent trials.
- 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 variants¹.

Please contact Stuart Scott (stuart.scott@ukneqasli.co.uk) with any further suggestions or comments.

References:

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

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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) 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.