

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

Distribution - 252601

Participant ID -

Date Issued - 10 April 2025

Closing Date - 09 May 2025

Trial Comments

This trial was issued to 78 participants; at the time of reporting, 76 (97.4%) have submitted results. Of the remaining two participants, one requested an extension to the submission deadline, and one pre-notified UK NEQAS LI of their intended non-return for this trial.

Sample Comments

Two vials of lyophilised cell line derived material were manufactured and issued by UK NEQAS LI. Both BRAF 168 and BRAF 169 were manufactured to be positive for the NM_004333.6(BRAF):c.1799T>A p.(Val600Glu) variant.

Results and Performance

Your Results

BRAF Mutation Status	Your Results	Consensus Result
Sample BRAF 168	Mutation Detected	Mutation Detected
Sample BRAF 169	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample BRAF 168	76	0
Sample BRAF 169	76	0

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	3	0

N/A = Not Applicable

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Template Type

	Returns
DNA	75
cDNA	1

Please note that only PCR, Protocol and Analysis types used by ≥ 2 participants are included in the tables below.

PCR Type

	Returns
Droplet Digital PCR	21
Allele Specific PCR	13
Real-Time PCR	13
PCR for Next generation Sequencing	11
Single PCR	11
Multiplex PCR	5

Protocol Type

	Returns
In-house Assay	45
BioRad PrimePCR ddPCR kit	18
Diatech Pharmacogenetics Easy Kit	4
Biocartis Idylla	2
Lightmix (R) TibMolBiol Kit	2
Oncomine Myeloid Research Assay	2

Analysis Type

	Returns
Digital PCR (Biorad)	20
Real-Time PCR Fluorescent Detection	17
NGS (Illumina)	9
Capillary Electrophoresis	7
NGS (ThermoFisher Ion Torrent)	6
Agarose Gel Electrophoresis	5
Sanger Sequencing	3
Acrylamide Gel Electrophoresis (PAGE)	2
Biocartis Idylla	2
Digital PCR (Other)	2
High Resolution Melt	2

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Journal Reference for Assay

	Returns
Arcaini L. et al (2012). Blood, 119:1, 188-191	11
Tiacci E. et al (2012). Blood, 119:1 - 192-195	11
Liu X. et al. (2020) Front Genet 11: 147	4
Rustad EH. et al. (2015) Blood Cancer J 5: e299	4
Wong C. et al (2005). J Clin Pathol, 58, 640-644.	4
Ellison G. et al (2010). J Exp Clin Cancer Res; 115, 21-28	3
Boyd E. et al (2011). British Journal of Haematology, 155:5, 609-612	2
Guerrini, F. et al., (2016) Front Pharmacol, 7:363.	2
Huang T. et al (2013) Biomark Res, 16:1, 3	2

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Trial Summary

Sample BRAF 168

- In line with sample formulation, all 76 participants returning results for this trial (100%) detected the *BRAF* c.1799T>A p.(Val600Glu) variant in sample BRAF 168.

Sample BRAF 169

- In line with sample formulation, all 76 participants (100%) also detected the *BRAF* c.1799T>A p.(Val600Glu) variant in sample BRAF 169.

It is well recognised that the level of disease in peripheral blood in hairy cell leukaemia (HCL) is often very low and that bone marrow aspirate may not be possible or be dilute due to marrow fibrosis¹⁻³. Hairy cells have been reported at levels as low as 1-2% in peripheral blood^{1,2} and UK NEQAS LI has previously outlined how, in the absence of an enrichment method, assays with reduced sensitivity are considered inadequate for the detection of *BRAF* c.1799T>A p.(Val600Glu) in HCL. Consensus guidelines for the diagnosis and management of patients with HCL recommend that all patients are evaluated for this variant using a sensitive molecular assay, and that **highly sensitive techniques (e.g. allele-specific PCR or next-generation sequencing) should be used in preference to less sensitive techniques (e.g. Sanger sequencing, pyrosequencing, or melting curve analysis) in order to avoid false-negative results³.**

Whilst not presenting an issue for performance in the current trial, three participants stated the use of Sanger sequencing as an analysis method, and only one has previously made us aware that they use an enrichment technique for clinical samples.

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 project⁴. 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 was revised to NM_004333.6 / ENST00000646891.2, as specified in the published MANE v1.0 transcript set⁵. 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 available⁴.
- 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 some annotation resources may not yet 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.

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- We thank participants for their continued engagement in the BRAF p.Val600Glu (V600E) Mutation Status for Hairy Cell Leukaemia programme and welcome suggestions or ideas for programme development (admin@ukneqasli.co.uk).

References

1. Arcaini, L. *et al.* The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. *Blood* **119**(1): 188–191 (2012).
2. Cornfield, D. B. *et al.* The diagnosis of hairy cell leukemia can be established by flow cytometric analysis of peripheral blood, even in patients with low levels of circulating malignant cells. *Am J Hematol* **67**: 223–226 (2001).
3. Grever, M. R. *et al.* Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. *Blood* **129**: 553–560 (2017).
4. den Dunnen, J.T. *et al.* HGVS recommendations for the description of sequence variants: 2016 update. *Hum.Mutat.* **37**(6): 564-569 (2016).
<https://hgvs-nomenclature.org/stable/recommendations/general/> (v21.1.2, accessed June 2025).
5. Morales, J. *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* **604**(7905): 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

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e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited. Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider. No other activities in relation to this EQA exercise were externally provided. Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

4.8.2 g) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRINTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFIZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel is informed when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict standard operating 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 authorised 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/>