UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

KIT p.Asp816Val (D816V) Mutation Status for Mast Cell Disease Programme

Distribution - 222301 Participant ID -

Date Issued - 05 May 2022 Closing Date - 03 June 2022

Trial Comments

Two vials of lyophilised cell line material (samples refs KIT 150 and KIT 151) were distributed to 86 participants for KIT NM_000222.3:c.2447A>T p.Asp816Val (D816V) variant analysis. For this trial, 84 (97.7%) participants returned results. Two laboratories pre-notified us of their non return.

Sample Comments

Sample KIT 150 was manufactured to feature a population of 0.6% KIT NM_000222.3:c.2447A>T p.Asp816Val heterozygous positive cells in a non-mutated (wildtype) background. Sample KIT 151 featured 'wildtype' cells only.

Results and Performance

Your Results

KIT Mutation Status	Your Results	Consensus Result
Sample KIT 150		Mutation Detected
Sample KIT 151		No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample KIT 150	81	3
Sample KIT 151	1	83

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	79
cDNA	5

PCR Type

	Returns
Allele Specific PCR	26
Real-Time PCR	23
Droplet Digital PCR	21
Single PCR	7
Multiplex PCR	3
Allele Specific Competitive Blocker PCR	2
Chip Digital PCR	2

Protocol Type

	Returns
In-house Assay	60
BioRad PrimePCR ddPCR kit	16
LifeTechnologies TaqMan kit	5
Plentiplex Mastocytosis kit	3

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	43
Digital PCR	23
Agarose Gel Electrophoresis	6
NGS (Other)	5
Sanger Sequencing	4
Capillary Electrophoresis	3

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Journal Reference for Assay

	Returns
Kristensen T. et al (2011). JMD, 13:2, 180-188	34
In-house method	16
Schumacher J. et al (2008). JCP, 61, 109-114	7
Lawley W. et al (2005). Mutat Res, 572, 1-13	1
Longley BJ. et al (1999). Proc Natl Acad Sci, 96:4, 1609-1614	1
Orfao A. et al (2007). Br J Haematol, 138:1, 12-30	1
Tan A. et al (2006). Clin Chem, 52:12, 2250-2257	1
Sotlar K. et al (2003). Am J Pathol, 162:3, 737-746	1
Nagata H. et al (1995). PNAS, 92:23, 10560-4	1

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Final Comments

In line with sample formulation, 81/84 (96.4%) participants returning results reported detection of the *KIT* c.2447A>T p.(Asp816Val) variant (mutation) in sample KIT 150. The three laboratories returning a false negative result utilised various methodologies (DNA input material): allele specific PCR with agarose gel electrophoresis, multiplex PCR with NGS and single PCR with Sanger sequencing. The lyophilised samples provided for this trial are not suitable for mast cell enrichment pre-processing. One of the laboratories failing to detect the variant informed us that they do not perform routine mast cell enrichment pre-processing on clinical samples. The remaining two laboratories did not complete the related question (hosted on JotForm) as part of their results return.

In the absence of mast cell enrichment, Sanger sequencing or the visualisation of allele specific PCR products on an agarose gel may not afford the required assay sensitivity[1-2]. **Detection of the *KIT* c.2447A>T p.(Asp816Val) variant (NM_000222.3) present at a low level (including <1%) is clinically relevant in the context of mast cell disease.** A study by Kristensen *et al*.[3] in patients with mastocytosis found a range of mutation (variant) positive cell fractions from 0.03% to 97%, with a median of 0.9% in bone marrow samples. In the same study, the variant level in skin biopsies ranged from 3% to 23% (median 8%).

Of the 3/4 Sanger sequencing users able to identify the variant in sample KIT 150, all extracted RNA and utilised cDNA as assay input material (to analyse expressed variant load) rather than gDNA. Please refer to the caveat below regarding the focus of sample formulations for this programme.

In line with sample formulation 83/84 (98.8%) participants returning results did not detect a *KIT* c.2447A>T p.(Asp816Val) variant (mutation) in sample KIT 151. The single laboratory submitting a false positive result (DNA input material) utilised real time PCR with fluorescent detection (PlentiPlex Mastocytosis commercial kit).

Approximately 35% (30/84) of participants returned accompanying quantitative information. Results from those laboratories analysing gDNA are summarised in the table below. **Please note sample formulations for this programme focus on clinically relevant genomic *KIT* c.2447A>T p.(Asp816Val) variant levels.** Care should be taken by those participants utilising cDNA as assay input material (n=5) not to use the quantification statistics to benchmark the performance of an assay which targets mRNA to determine expressed variant load.

Percentage (%) *KIT* NM_000222.3:c.2447A>T p.(Asp816Val) variant^		
	Sample KIT 150	**Sample KIT 151**
n*	29	N/A
Mean	0.33	N/A
Median	0.21	N/A
IQR	0.13	N/A

^ % variant = (variant/(wildtype+variant))x100.
* Includes only those laboratories using extracted gDNA as assay input material. Note the lyophilised samples provided for this trial are not suitable for mast cell enrichment pre-processing.
IQR = Interquartile range
N/A = Not applicable

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Four returning laboratories reported the routine mast cell enrichment of clinical samples prior to extraction; techniques include mononuclear cell fraction density gradient centrifugation (n=2), flow cytometric sorting (n=1) and magnetic bead separation (n=1).

The NCBI *KIT* gene webpage (http://www.ncbi.nlm.nih.gov/gene/3815) is a valuable resource for obtaining relevant reference sequences at the DNA and protein level. The Matched Annotation from NCBI and EMBL-EBI (MANE) project release v1.0 is now available[4-5]. It states RefSeq NM_000222.3/Ensembl ENST00000288135.6 (isoform 1) as the MANE Select *KIT* transcript of choice for clinical reporting. Please note, Locus Reference Genomic (LRG)[6] reference sequences are no longer actively maintained and use of Ensembl/RefSeq transcripts specified by the MANE collaboration are preferred for all genes where available.

The Human Genome Variation Society (HGVS) provides a series of recommendations with the aim of standardising nomenclature for the description of sequence variants[7-8]. Parentheses are used in this report to denote predicted protein variant descriptions. However, we acknowledge that this approach to protein nomenclature would not be appropriate for the minority of participants extracting RNA and utilising cDNA as assay input material.

References

1 Arock, M., Sotlar, K., Akin, C., Broesby-Olsen, S., Hoermann, G., Escribano, L., *et al.* KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 29(6):1223-32 (2015).

2 Martelli, M., Monaldi, C., De Santis, S., Bruno, S., Mancini, M., Cavo, M., and Soverini S. Recent Advances in the Molecular Biology of systemic Mastocytosis: Implications for Diagnosis, Prognosis, and Therapy. *Int J Mol Sci* 21:(11):3987 (2020).

3 Kristensen, T., Vestergaard, H., and Møller, M. B. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. *The Journal of Molecular Diagnostics* 13(2), 180-188 (2011).

4 https://www.ncbi.nlm.nih.gov/refseq/MANE/

5 Morales, J., Pujar, S., Loveland, J. E., *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* 604(7905):310-315 (2022).

6 http://ftp.ebi.ac.uk/pub/databases/lrgex/LRG_307.xml

7 http://varnomen.hgvs.org/

8 Den Dunnen, J.T., Dalgleish, R., Maglott, D.R. *et al*. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human Mutation* 37(6):564-569 (2016).

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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:
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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) 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. Where subcontracting occurs, it is placed with a competent subcontractor and the EQA provider is responsible for this work.

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/

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