

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

Distribution - 151602

Participant ID -

Date Issued - 22 December 2015

Closing Date - 29 January 2016

Trial Comments

Two vials of lyophilised cell line derived material (samples KIT 114 and KIT 115) were distributed to 48 participants for KIT p.Asp816Val (D816V) mutation analysis. In this trial, 45 (93.8%) participants returned results. Of the 3 participants who failed to return results, 2 laboratories pre-notified us of their non return. We would like to acknowledge the Mayo Clinic for the use of their material in this programme.

Sample Comments

To best reflect mutant levels typically seen in clinical mastocytosis cases, sample KIT 114 was formulated to feature a minor population of 0.1% KIT p.Asp816Val positive cells in a predominantly unmutated (wildtype) background. Sample KIT 114 represented the lowest mutation load KIT positive sample we have distributed to date. Sample KIT 115 was manufactured to include no KIT p.Asp816Val positive cells.

Results and Performance

Your Results

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

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample KIT 114	23	22
Sample KIT 115	1	44

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.

Pilot *KIT* p.Asp816Val (D816V) Mutation Status for Mast Cell Disease Programme**Template Type**

	Returns
DNA	41
cDNA	4

PCR Type

	Returns
Allele Specific PCR	16
Single PCR	11
Real-Time PCR	10
Droplet Digital PCR	4
Allele Specific Competitive Blocker PCR	3
Multiplex PCR	1

Protocol Type

	Returns
In-house Assay	40
BioRad PrimePCR ddPCR kit	4
LifeTechnologies TaqMan kit	1

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	22
Agarose Gel Electrophoresis	7
Sanger Sequencing	6
Droplet Digital PCR	4
Next Generation Sequencing	3
Capillary Electrophoresis	1
Enzyme Digest	1
Mass Spectrometry	1

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Journal Reference for Assay

	Returns
Kristensen T. et al (2011). JMD, 13:2, 180-188	16
In-house method	14
Schumacher J. et al (2008). JCP, 61, 109-114	4
Tan A. et al (2006). Clin Chem, 52:12, 2250-2257	3
Lawley W. et al (2005). Mutat Res, 572, 1-13	2
Longley BJ. et al (1999). Proc Natl Acad Sci, 96:4, 1609-1614	2
Orfao A. et al (2007). Br J Haematol, 138:1, 12-30	2
Sotlar K. et al (2003). Am J Pathol, 162:3, 737-746	2
Shimada A. et al (2006). Blood, 107, 1806-1809	1
Nagata H. et al (1995). PNAS, 92:23, 10560-4	1

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Final comments

- In line with sample formulation, 23/45 (51.1%) participants detected the *KIT* p.Asp816Val mutation in sample KIT 114. 22/45 (48.9%) participants failed to detect the *KIT* p.Asp816Val mutation in sample KIT 114.
- In line with sample formulation, 44/45 (97.8%) returning laboratories provided a 'no mutation detected' result for sample KIT 115. There was only one false positive for sample KIT 115 and this participant failed to detect the mutation in sample KIT 114, indicative of a sample mix up.
- Of the 22 participants who failed to detect the low level of *KIT* p.Asp816Val mutation in sample KIT 114:
 - All 4 participants basing their assay on a cDNA template method failed to detect the mutation in sample KIT 114.
 - Of the 11 participants using the Single PCR method, 10 (90.9%) failed to detect the mutation in sample KIT 114. 5 (50%) of the participants using Real Time PCR also failed to detect the mutation.
 - All 3 participants utilising Next Generation Sequencing as their analysis method failed to detect the mutation in sample KIT 114 (although one appeared to be due to a sample swap). It is important to ensure sufficient depth of read coverage to facilitate detection of clinically relevant low-level *KIT* mutation in mast cell disease.
 - All participants (5/5) using Sanger Sequencing as their analysis method, without some form of artificial mutation amplification such as PCR clamping, failed to detect the mutation in sample KIT 114.
 - 2/4 participants (50.0%) using ddPCR failed to detect the mutation in sample KIT 114.
- **In the absence of mast cell purification pre-processing, the detection of the *KIT* p.Asp816Val mutation in a mastocytosis setting necessitates an assay with the appropriate sensitivity to detect low level mutations.** A study by Kristensen *et al*¹ in patients with mastocytosis found a range of mutation positive cell fractions from 0.03% to 97%, with a median of 0.9% in bone marrow samples. In the same study, the mutation level in skin biopsies ranged from 3% to 23% (median 8%). To mimic mutant levels typically seen in clinical cases, sample KIT 114 was formulated to feature a minor population of *KIT* p.Asp816Val positive cells in a predominantly unmutated (wildtype) background.
- **The European Competence Network on Mastocytosis has recently published their recommendations for *KIT* mutation analysis in mast cell neoplasms².** The review includes consensus methodologies for diagnostic mutation testing and the follow-up monitoring of mastocytosis patients.
- The NCBI *KIT* gene webpage (<http://www.ncbi.nlm.nih.gov/gene/3815>) 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 variants³.

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References

- 1 Kristensen, T., Vestergaard, H., & 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 : JMD*, **13**(2), 180-188 (2011).
- 2 Arock M., Sotlar K., Akin C. *et al.* *KIT* mutation analysis in mast cell neoplasm recommendations of the European Competence Network on Mastocytosis. *Leukemia*. **29**(6):1223-1232 (2015).
- 3 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

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Sheffield, S10 2QD

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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) authorising 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 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.