UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 192003

Participant ID - 43347

Date Issued - 12 March 2020

Closing Date - 17 April 2020

Trial Comments

FINAL REPORT: This trial was issued to 147 participants; of which 136 (92.5%) returned results. Of the non returns, one laboratory notified us of their intended non return and six laboratories submitted requests for an extension in results submission in light of the ongoing Covid-19 pandemic.

Sample Comments

Two vials of cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 149 and NPM1 150). NPM1 149 was manufactured to be negative for an NPM1 duplication and NPM1 150 was formulated to carry a NPM1 variant (Type A, 4bp duplication).

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 149	No Mutation Detected	No Mutation Detected
Sample NPM1 150	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 149	2	134
Sample NPM1 150	135	1

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

	Returns
DNA	95
cDNA	39

PCR Type

	Returns
Single PCR	75
Real-Time PCR	33
Multiplex PCR	21
Melting Curve Analysis	3

Protocol Type

	Returns
In-house Assay	104
Qiagen NPM1 Mutascreen Kit	12
Qiagen NPM1 mut A, B & D MutaQuant Kits	10
Illumina TruSight Myeloid Sequencing Panel	4
Ion AmpliSeq Cancer Hotspot Panel v2	2
Qiagen NPM1 mut A MutaQuant Kits	2

Analysis Type

	Returns
Capillary Electrophoresis	65
Real-Time PCR Fluorescent Detection	33
Next Generation Sequencing (Miseq)	11
Agarose Gel Electrophoresis	8
Sanger Sequencing	8
High Resolution Melt	4
NGS (ThermoFisher Ion Torrent)	4
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	20
Thiede C. et al (2006) Blood, 107(10):4011-4020	13
Falini B. et al (2005) N Engl J Med, 352(3):254-266	12
Falini B. et al (2007) Blood, 109(3):874-885	12
Schnittger S. et al (2005) Blood, 106(12):3733-3739	11
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	10
Gale R. et al (2008) Blood, 111(5):2776-2784	8
In-house method (no published reference available)	8
Döhner K. et al (2005) Blood, 106(12):3740-3746	7
Belgian Molecular Diagnostic Group	6
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	5
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	5
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	4
Boissel N. et al (2005) Blood, 106(10):3618-3620	3
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	3
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	3
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	3
Bench AJ. et al (2012) Int J Lab Hematol. 34(1):21-34	2
Lazenby M. et al (2014) Leukemia 28(10):1953-1959	2
Ottone T. et al (2008) J Mol Diagn 10: 212-216	2
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	2

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Trial Comments

Sample NPM1 149

- In line with sample formulation, 134 out of 136 (98.5%) participants returning results found sample NPM1 149 to be negative for a *NPM1* variant.
- One of the two of consensus false positives appeared to be due to a sample transposition event. For the remaining result, the participant potentially analysed NPM1 150 twice.

Sample NPM1 150

- In line with sample formulation, 135 out of 136 (99.3%) participants returning results detected a *NPM1* variant in sample NPM1 150.
- The out of consensus false positive appeared to be due to a sample transposition event between NPM1 149 and NPM1 150.
- For sample NPM1 150, the majority of participants identified a single change consistent with the Type A[1] duplication of a TCTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature NM_002520.6:c.860_863dup, systematic exon numbering of the *NPM1* transcript applied). Four participants reported an alternative variant description of c.863_864insTCTG. One participant detected both a Type A and Type D duplication, with one participant reporting the detection of a Type D duplication.

2019-2020 Overview

- This represents the final trial issue as part of the 2019-2020 trial schedule period. As such, analysis has been performed for the course of the last 12 months to provide an overall summary of the year.
- Over the course of the last 12 months, there have been a total of 5 out of consensus results out of a total of 830 results submitted in this programme, representing 0.6% of results. Of the out of consensus results, 3 (60%) represented false positives, one of which was the result of a sample transposition and 2 (40%) represented false negatives.

References

1. Falini, B. *et al.* Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype. *N. Engl. J. Med.* **352,** 254–266 (2005).

NPM1 Mutation Status Programme
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: nicola.rose@ukneqasli.co.uk

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/

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/