UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 202103

Participant ID - 43347

Date Issued - 04 March 2021

Closing Date - 09 April 2021

Trial Comments

FINAL REPORT: This trial was issued to 154 participants; of which 141 (91.6%) returned results. Of the non returns, three laboratories notified us of their intended non return and three laboratory submitted a request for an extension to 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 155 and NPM1 156). Both trial samples were formulated to be negative for an NPM1 duplication. In addition, an educational sample (NPM1 Edu C) was issued for exon 12 variant analysis. This sample was whole genome amplified DNA, derived from patient material and was positive for a NPM1 Type B (c.863_864insCATG) variant.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 155	No Mutation Detected	No Mutation Detected
Sample NPM1 156	No Mutation Detected	No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 155	1	140
Sample NPM1 156	0	141

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	2	1

N/A = Not Applicable

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Template

	Returns
DNA	112
cDNA	28

PCR Type

	Returns
Single PCR	80
Multiplex PCR	26
Real-Time PCR	25
Melting Curve Analysis	5

Protocol Type

	Returns
In-house Assay	116
Qiagen NPM1 Mutascreen Kit	14
Qiagen NPM1 mut A, B & D MutaQuant Kits	5
Illumina TruSight Myeloid Sequencing Panel	3
Ion AmpliSeq Cancer Hotspot Panel v2	2

Analysis Type

	Returns
Capillary Electrophoresis	78
Real-Time PCR Fluorescent Detection	29
Sanger Sequencing	8
Next Generation Sequencing (Miseq)	7
NGS (ThermoFisher Ion Torrent)	5
Agarose Gel Electrophoresis	4
High Resolution Melt	4
Illumina NextSeq 500	2
Illumina MiniSeq	1
Illumina NextSeq 550	1
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	21
Falini B. et al (2005) N Engl J Med, 352(3):254-266	13
In-house method (no published reference available)	11
Thiede C. et al (2006) Blood, 107(10):4011-4020	11
Schnittger S. et al (2005) Blood, 106(12):3733-3739	9
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	8
Döhner K. et al (2005) Blood, 106(12):3740-3746	7
Boissel N. et al (2005) Blood, 106(10):3618-3620	6
Falini B. et al (2007) Blood, 109(3):874-885	6
Gale R. et al (2008) Blood, 111(5):2776-2784	6
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	6
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	6
Belgian Molecular Diagnostic Group	5
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	5
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	4
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	4
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	4
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	3
Chou WC. et al (2007) Leukemia, 21(5):998-1004	2
Konoplev S. et al (2009) Cancer 115(20): 4737-4744	2
Lazenby M. et al (2014) Leukemia 28(10):1953-1959	2

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Trial Comments

- In line with sample formulation, 140 of 141 (99.3%) participants returning results did not detect an *NPM1* variant in sample NPM1 155.
- The single out-of-consensus participant indicated that a type A mutation had been detected using Qiagen NPM1 mut A, B & D MutaQuant kits with Real-Time PCR fluorescent detection.
- In line with sample formulation, all 141 returning participants did not detect an *NPM1* variant in sample NPM1 156.

Educational Sample NPM1 Edu C

- Eighty-seven participants returned a result for the educational DNA sample NPM1 Edu C. Results for this sample have not been scored.
- Eighty-five participants (97.7%) indicated that they detected a variant in this sample.
- The two laboratories (2.3%) returning false negative results both employed in-house single PCR based assays: one utilised agarose gel electrophoresis as an analysis method, the other used capillary electrophoresis.
- Of the 58 participants providing information on the variant detected, 44 (75.9%) stated that they had detected a Type B mutation, and an additional nine participants (15.5%) indicated that they had detected a 4bp insertion without specifying further details. A further participant stated that they had detected an insertion of TGCA but did not categorise the *NPM1* mutation type.
- Three laboratories (5.2%) reported that they detected a Type A mutation. These participants all employed in-house multiplex PCR based assays: two utilised capillary electrophoresis as an analysis method, the other used next generation sequencing. Interestingly this laboratory then correctly described a Type B variant using HGVS nomenclature.
- Finally, one participant (1.8%) indicated that they had detected a variant that was not Types A-D. This laboratory employed the Illumina TruSight Myeloid Sequencing Panel, and again interestingly went on to correctly describe a Type B variant according to HGVS nomenclature descriptions.

- The Type B[1] mutation is a 4 base pair insertion of a CATG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature[2] NM_002520.7:c.863_864insCATG p.(Trp288Cysfs*12), systematic exon numbering of the *NPM1* transcript applied).
- Of the 44 laboratories detecting a Type B mutation, 37 provided a DNA description using HGVS nomenclature, with 34 using the approved description c.863_864insCATG. The remaining three laboratories incorrectly described the Type B mutation as c.862_863insCATG, c.860_863insCATG or c.1745_1831dup (using either reference sequence NM_002520.6 or NM_002520.7).
- Of the 44 laboratories detecting a Type B mutation, 36 provided a protein description using HGVS nomenclature. For the predicted amino acid change associated with the *NPM1* variant; there was variable use of the HGVS nomenclature, as outlined in the table below.

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Protein nomenclature *NPM1* variant	n	Comments
p.(Trp288Cysfs*12)	10	Parenthesis reflecting the analysis of DNA and the predicted status of the protein level description. * or Ter are equally acceptable to indicate a termination/STOP codon. Shorthand descriptions of the frameshift are acceptable.
p.(Trp288CysfsTer12)	1	
p.(Trp288fs)	1	
p.(Trp288fs*12)	1	
p.Trp288fs	9	Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Shorthand descriptions of the frameshift are acceptable. * or Ter are equally acceptable to indicate a termination/STOP codon.
p.Trp288CysfsTer12	4	
p.Trp288Cysfs*12	2	
p.W288Cfs*12	2	Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Three letter amino acid code is preferred when describing protein changes.
p.(Trp288Cysfs*?)	1	This suggests that the predicted consequence of the frame shift variant changes Trp288 to Cys but the new reading frame does not encounter a new termination codon.
p.(Trp288CysfsTer*12)	1	Use of either * or Ter are acceptable to indicate a termination/STOP codon, not both.
p.W288fs	1	Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Three letter amino acid code is preferred when describing protein changes.
(p.Trp288fs)	1	Incorrect positioning of parentheses.
p.Trp288SerfsTer12	1	Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. Incorrect amino acid substitution at Trp288.
p.(Leu610_Glu611ins29)	1	Incompatible variant nomenclature and reference sequence (NM_002520). The variant nomenclature was also incompatible with the reference sequence at the c.DNA level.

Green = compliant with HGVS nomenclature. **Amber** = mostly compliant with HGVS nomenclature, some minor issues. **Red** = not compliant with HGVS nomenclature.

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Satisfaction Survey

- The satisfaction survey was answered by 92 participants. All participants stated that they were happy with the service provided by UKNEQAS LI.

We would like to take this opportunity to thank participants who returned data for NPM1 Educational Sample C. We are aware that the Covid-19 pandemic has made it difficult for laboratories across the world to deliver core services and would like to thank participants for their engagement with the *NPM1* programme during the 2020-2021 trial period.

References

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

2. Human Genome Variation Society (HGVS), https://varnomen.hgvs.org/ (v20.05).

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
Pegasus House, 4th Floor Suite
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Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: amanda.newbould@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/