UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 222301

Participant ID - 43347

Date Issued - 11 July 2022

Closing Date - 19 August 2022

Trial Comments

This trial was issued to 166 participants, of which 154 (92.8%) returned results. Of the non returns, five participants pre-notified us of their intended non return and three participants requested an extension to results submission. A further two laboratories were excluded from scoring because of ongoing difficulties when shipping samples to Belgium .

Sample Comments

Two cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 163 and NPM1 164). Sample NPM1 163 was formulated to be positive for a NPM1 Type A duplication, with sample NPM1 164 manufactured to be negative.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 163	Mutation Detected	Mutation Detected
Sample NPM1 164	No Mutation Detected	No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 163	146	8
Sample NPM1 164	0	154

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	115
cDNA	39

PCR Type

	Returns
Single PCR	85
Real-Time PCR	34
Multiplex PCR	24
Melting Curve Analysis	7
Sequencing	3

Protocol Type

	Returns
In-house Assay	122
Qiagen NPM1 Mutascreen Kit	14
Qiagen NPM1 mut A, B & D MutaQuant Kits	10
Illumina TruSight Myeloid Sequencing Panel	3
Ion Torrent Oncomine Myeloid Panel	2
Qiagen NPM1 mut A MutaQuant Kits	2
Ion AmpliSeq Cancer Hotspot Panel v2	1

Analysis Type

	Returns
Capillary Electrophoresis	82
Real-Time PCR Fluorescent Detection	37
Sanger Sequencing	11
Next Generation Sequencing (Miseq)	9
Agarose Gel Electrophoresis	5
High Resolution Melt	4
NGS (ThermoFisher Ion Torrent)	3
Illumina NextSeq 500	2
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	28
Falini B. et al (2005) N Engl J Med, 352(3):254-266	18
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	14
Falini B. et al (2007) Blood, 109(3):874-885	10
Gale R. et al (2008) Blood, 111(5):2776-2784	10
Thiede C. et al (2006) Blood, 107(10):4011-4020	10
Döhner K. et al (2005) Blood, 106(12):3740-3746	9
In-house method (no published reference available)	9
Schnittger S. et al (2005) Blood, 106(12):3733-3739	8
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	7
Belgian Molecular Diagnostic Group	6
Boissel N. et al (2005) Blood, 106(10):3618-3620	6
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	5
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	4
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	4
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	4
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	3
Chou WC. et al (2007) Leukemia, 21(5):998-1004	2
Falini B. et al (2006) Blood 108(6):1999-2005	2
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	2

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Trial Comments

Sample NPM1 163

- In line with sample formulation, 146 of 154 (94.8%) participants returning results identified an *NPM1* variant in sample NPM1 163.
- Of the eight participants reporting a false negative for NPM1 163, all utilised an in-house assay, four with capillary electrophoretic analysis, three utilised Sanger sequencing and one and used Real-Time Fluorescent detection.
- *NPM1* variants have been reported at allelic frequencies of ~5% in patients with newly diagnosed AML[1,2]. **As such, the detection of *NPM1* variants necessitates an assay with the appropriate limit of detection to identify low level variants at diagnosis.**
- Ninety-eight participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 74 (75.5%) identified a change consistent with the Type A[3] duplication of a TCTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature NM_002520.7(NPM1):c.860_863dup, systematic exon numbering of the *NPM1* transcript applied). Of these, two participants reported a c.863_864insTCTG. HGVS recommendations state that variants should be described as a duplication when a copy of one or more nucleotides are inserted directly 3' of the original nucleotides, when compared to the reference sequence[4]. Furthermore, listing the duplicated nucleotide sequence is not endorsed as this creates a longer description with redundant information.
- A further 18 laboratories (18.4%) reported a 4 bp duplication / insertion but did not specify further details. One participant (1.0%) reported an insertion but did not specify the size of the insertion, one laboratory (1.0%) incorrectly detected a *NPM1* Type D insertion, one (1.0%) reportedly detected both *NPM1* Type A and D duplication / insertion events.
- One participant reported a c.863_864insTTTG variant using reference sequence NM_002520 (no further variant transcript information provided). This variant is consistent with a *NPM1* Type G variant.
- One participant reported a c.964_965insTCTG variant using reference sequence NM_002520.7. The positional numbering for the NM_002520.7 reference sequence only extends to c.885.

Sample NPM1 164

- All participants (n=154) returning results did not detect an *NPM1* variant in sample NPM1 164.

The persistent presence of the *NPM1* variant(s) in patients with *NPM1* positive AML has shown that this is a stable marker to determine molecular assessment of measurable residual disease (MRD) at specific clinical time points[5]. **For participants interested in EQA for MRD assessment using *NPM1* (and other AML markers), UK NEQAS LI have recently developed a new pilot programme, 'Acute Myeloid Leukaemia Measurable Residual Disease by Molecular Methods'. If participants require further information about this programme, please contact admin@uknegasli.co.uk.**

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References

1. Patel, S.S. *et al*. High *NPM1*-mutant allele burden at diagnosis predicts unfavorable outcomes in de novo AML. *Blood*. **131**(25): 2816-2825 (2018).

2. Rothenberg-Thurley, M. *et al*. *NPM1* Variant Allele Frequency and Outcomes in AML. Blood. **132**(Supplement 1): 1486 (2018).

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

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

5. Schuurhuis, G.J. *et al.* Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*. **131**(12), 1275-1291 (2018).

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/