NPM1 Mutation Status Programme

Distribution - 212203

Participant ID - 43347

Date Issued - 23 March 2022

Closing Date - 29 April 2022

Trial Comments

Of the non returns, three laboratories notified us of their intended non return and one laboratory submitted a request for an extension to results submission. A further two laboratories were excluded from scoring because of ongoing difficulties when shipping samples to Belgium and Spain. We are working with Belgian and Spanish participants to resolve these issues.

Sample Comments

Two vials of cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 161 and NPM1 162). Both trial samples were formulated to be positive for a NPM1 Type A duplication. In addition, an educational sample (NPM1 Edu E) 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 D c.863_864insCCTG p.(Trp288Cysfs*12) insertion.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 161	Mutation Detected	Mutation Detected
Sample NPM1 162	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 161	154	2
Sample NPM1 162	152	4

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	3	0

N/A = Not Applicable

Sheffield Teaching Hospitals NHS Foundation Trust, a UKAS accredited proficiency testing provider No. 7804, operating UK NEQAS for Leucocyte Immunophenotyping.

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Template

	Returns
DNA	112
cDNA	43

PCR Type

	Returns
Single PCR	87
Real-Time PCR	33
Multiplex PCR	26
Melting Curve Analysis	6
Sequencing	1

Protocol Type

	Returns
In-house Assay	123
Qiagen NPM1 Mutascreen Kit	14
Qiagen NPM1 mut A, B & D MutaQuant Kits	9
Illumina TruSight Myeloid Sequencing Panel	2
Ion Torrent Oncomine Myeloid Panel	2
Qiagen NPM1 mut A MutaQuant Kits	2
Imegen NPM1 Kit	1
Ion AmpliSeq Cancer Hotspot Panel v2	1
Myeloid Solution by Sophia Genetics	1

Analysis Type

	Returns
Capillary Electrophoresis	81
Real-Time PCR Fluorescent Detection	38
Sanger Sequencing	10
Next Generation Sequencing (Miseq)	8
Agarose Gel Electrophoresis	5
High Resolution Melt	4
NGS (ThermoFisher Ion Torrent)	4
Illumina NextSeq 500	2
Illumina MiniSeq	1
Illumina NextSeq 2000	1
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	22
Falini B. et al (2005) N Engl J Med, 352(3):254-266	17
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	12
Gale R. et al (2008) Blood, 111(5):2776-2784	11
Thiede C. et al (2006) Blood, 107(10):4011-4020	11
Falini B. et al (2007) Blood, 109(3):874-885	10
In-house method (no published reference available)	10
Schnittger S. et al (2005) Blood, 106(12):3733-3739	10
Döhner K. et al (2005) Blood, 106(12):3740-3746	9
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	8
Boissel N. et al (2005) Blood, 106(10):3618-3620	6
Belgian Molecular Diagnostic Group	5
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	5
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	4
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	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
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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Sample NPM1 161

- In line with sample formulation, 154 of 156 (98.7%) participants returning results identified an *NPM1* variant in sample NPM1 161.
- Of the two participants reporting a false negative for NPM1 161, both utilised an in-house assay, one with capillary electrophoretic analysis and one utilised Sanger sequencing. Both participants also reported NPM1 162 to be negative for an *NPM1* variant
- One hundred and thirteen participants returned information relating to the type of *NPM1* variant detected. In line with sample formulation, 87 (77.0%) identified a change consistent with the Type A[1] 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, four participants reported an alternative description of c.863_864insTCTG, one reported c.860_863insTCTG and one reported c.862_863insTCTG. 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[2].
- A further 21 laboratories (18.6%) reported a 4 bp duplication / insertion but did not specify further details. One participant (0.9%) reported an insertion but did not specify the size of the insertion, one laboratory (0.9%) incorrectly detected a *NPM1* type D insertion, one (0.9%) reportedly detected both *NPM1* type A and D duplication / insertion events.
- One participant reported a c.863_864dupTCTG using reference sequence NM_002520.7. The predicted protein change in Alamut for this variant description is p.(Gln289Glyfs*12) and is not consistent with the expected Type A duplication.
- 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 162

- In line with sample formulation, 152 of 156 (97.4%) participants returning results identified an *NPM1* variant in sample NPM1 162.
- As previously stated, two participants reporting an out of consensus result for this sample also reported an out of consensus result for NPM1 161. For the remaining two participants one performed next generation sequencing using the Ion Torrent Oncomine Myeloid Panel and one participant utilised an in-house assay with capillary electrophoretic analysis.
- In line with expectation, 83 of the 107 laboratories returning information relating to variant type (77.6%) 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_002520.7:c.860_863dup, systematic exon numbering of the *NPM1* transcript applied). Of these, four participants reported an alternative variant description of c.863_864insTCTG, one reported c.860_863insTCTG and one reported

c.862_863insTCTG. 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[2].

- A further 20 laboratories (18.6%) reported a 4 bp duplication / insertion but did not specify further details. One participant (0.9%) reported an insertion but did not specify the size of the insertion, one laboratory (0.9%) incorrectly detected *NPM1* type A and D duplications and as with NPM1 161, one participant reported a c.964_965insTCTG variant and one a c.863_864dupTCTG variant using the NM_002520.7 reference sequence.

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NPM1 Educational Sample Edu E

Sample Information

Sample NPM1 Edu E was issued as whole genome amplified material (WGA) derived from a patient with a NM_002520.7(*NPM1*):c.863_864insCCTG p.(Trp288Cysfs*12). Results for this sample have not been scored.

In total, 81 participants returned results for the educational DNA sample NPM1 Edu E.

Your Result

Sample	Participant	Your Result
NPM1 Edu E variant detected?	43347	Not Tested

All Participant Results

Sample	Variant Detected	No Variant Detected
NPM1 Edu E	77	4

Your Variant Results

	Your DNA sequence variant description	Your protein variant description
NPM1 Edu E	Not Tested	Not Tested

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- Seventy-seven out of 81 participants (95.1%) indicated that they detected a variant in sample NPM1 Edu E.
- Of the four laboratories reporting a false negative result, two employed in-house assays, one with agarose gel electrophoresis and one with Real-Time fluorescent detection. An additional participant utilised next generation sequencing (NGS) on the Illumina Trusight Myeloid Sequencing Panel. The remaining participant used the Qiagen NPM1 mut A MutaQuant kit designed to detect *NPM1* type A variants only, which likely explains the false negative result.
- Of the 54 participants providing information on the *NPM1* variant type detected, 42 (77.8%) stated that they had detected a Type D mutation, and an additional eight participants (14.8%) indicated that they had detected a 4bp insertion without specifying further details.
- Two participants (3.7%) reported the variant type to be Type A and two (3.7%) a 'possible' Type A. These participants all employed an in-house assay with capillary electrophoresis. One of the participants who indicated a 'possible' Type A stated that their methodology amplifies Type A and D *NPM1* variants.

The Type D[3] variant is a 4 base pair insertion of a CCTG tetranucleotide in exon 11 of the *NPM1* gene (approved HGVS nomenclature[2] NM_002520.7(*NPM1*):c.863_864insCCTG p.(Trp288Cysfs*12), systematic exon numbering of the *NPM1* transcript applied).

- Of the 42 laboratories detecting a Type D variant, 33 provided a DNA description using HGVS nomenclature, with 30 (90.9%) using the approved description c.863_864insCCTG. Of the remaining three laboratories, two (6%) described the variant as c.860_863dup. Despite this participant reporting detection of a *NPM1* Type D variant, this description is that of a *NPM1* Type A variant. A further participant (3%) described the variant as c.860_863dupCCTG (using reference sequence NM_002520.7).
- Of the 42 laboratories detecting a Type D mutation, 33 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 tables below. Table 1 reviews the use of protein nomenclature based on participants utilising DNA as assay starting material (n=27).

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Protein nomenclature *NPM1* variant[a]	n	Comments	
p.(Trp288Cysfs*12)	7	Parentheses 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.	Green
p.(Trp288CysfsTer12)	7		Green
p.Trp288Cysfs*12	3	Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected. * or Ter are equally acceptable to indicate a termination/STOP codon.	Amber
p.Trp288CysfsTer12	1		Amber
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.	Amber
p.Trp288fs	2	Shorthand descriptions of the frameshift are acceptable. Parentheses are required in this context as DNA has been analysed, thus any protein change is only predicted based on the DNA variant detected.	Amber
p.W288fs*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. Short format descriptions of frameshifts should include the first amino acid changed, its position and "fs" without any further details.	Amber
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.	Amber
(p.(Trp288Cysfs*12	1	Incorrect positioning of parentheses.	Amber
Trp288CysfsTer12	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. A single letter prefix is mandatory to indicate the type of reference sequence utilised, in this instance "p.".	Red

Table 1. Protein nomenclature review based on participants utilising DNA as the assay starting material. **Green** = compliant with HGVS nomenclature. **Amber** = mostly compliant with HGVS nomenclature, some minor issues. **Red** = not compliant with HGVS nomenclature.

- Six participants reporting protein nomenclature used cDNA as the assay starting material, of which four (66.7%) reported the protein description p.(Trp288Cysfs*12). Please note parentheses are not required if RNA or cDNA is the assay input material.
- One participant reported p.(W288Cfs*12). When describing protein changes, the three-letter amino acid code is preferred. Parentheses are not required if RNA or cDNA is the assay input material.
- One participant reported p.Trp288Cysfs*12 which is fully compliant with HGVS protein nomenclature.

- Of the 42 laboratories detecting a Type D mutation, 20 provided quantification information. The median variant allele frequency (VAF) was 39.7%, with an interquartile range of 6.3%. Reported VAFs ranged from 29.8-52.0%.

We would like to take this opportunity to thank participants who returned data for NPM1 Educational Sample E.

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[4]. **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@ukneqasli.co.uk.**

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).
3. Alpermann, T. *et al*. Molecular subtypes of NPM1 mutations have different clinical profiles, specific patterns of accompanying molecular mutations and varying outcomes in intermediate risk acute myeloid leukemia. *Haematologica*. **101**(2): e55-e58 (2016).
4. 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).

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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
Pegasus House, 4th Floor Suite
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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/