UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 202102

Participant ID - 43347

Date Issued - 18 November 2020

Closing Date - 08 January 2021

Trial Comments

FINAL REPORT: This trial was issued to 145 participants; of which 141 (97.2%) returned results. Of the non returns, three laboratories notified us of their intended non return and one laboratory submitted a request 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 153 and NPM1 154). NPM1 153 was manufactured to carry a NPM1 variant (Type A, 4bp duplication) and NPM1 154 was formulated to be negative for an NPM1 duplication.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 153	No Mutation Detected	Mutation Detected
Sample NPM1 154	No Mutation Detected	No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 153	136	5
Sample NPM1 154	3	138

Your Performance

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

N/A = Not Applicable

NPM1 Mutation Status Programme

Template

	Returns
DNA	105
cDNA	36

PCR Type

	Returns
Single PCR	76
Real-Time PCR	33
Multiplex PCR	27
Melting Curve Analysis	4

Protocol Type

	Returns
In-house Assay	114
Qiagen NPM1 Mutascreen Kit	14
Qiagen NPM1 mut A, B & D MutaQuant Kits	5
Illumina TruSight Myeloid Sequencing Panel	4
Ion AmpliSeq Cancer Hotspot Panel v2	3
Qiagen NPM1 mut A MutaQuant Kits	1

Analysis Type

	Returns
Capillary Electrophoresis	73
Real-Time PCR Fluorescent Detection	34
Next Generation Sequencing (Miseq)	11
Sanger Sequencing	7
Agarose Gel Electrophoresis	6
NGS (ThermoFisher Ion Torrent)	4
High Resolution Melt	3
Illumina NextSeq 500	1
Pyrosequencing	1

NPM1 Mutation Status Programme

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	12
In-house method (no published reference available)	11
Thiede C. et al (2006) Blood, 107(10):4011-4020	11
Falini B. et al (2007) Blood, 109(3):874-885	9
Gale R. et al (2008) Blood, 111(5):2776-2784	9
Schnittger S. et al (2005) Blood, 106(12):3733-3739	9
Döhner K. et al (2005) Blood, 106(12):3740-3746	8
Boissel N. et al (2005) Blood, 106(10):3618-3620	7
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	7
Belgian Molecular Diagnostic Group	6
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	5
Falini B. et al (2006) Blood 108(6):1999-2005	4
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	4
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	3
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	3
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	3
Calvo KL et al (2008) Eur J Haematol 82: 69-72	2
Konoplev S. et al (2009) Cancer 115(20): 4737-4744	2
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	2
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	2

NPM1 Mutation Status Programme

Trial Comments

Sample NPM1 153

- In line with sample formulation, 136 out of 141 (96.5%) participants returning results detected a *NPM1* variant in sample NPM1 153.
- The five out-of-consensus false negative results for this sample were returned by laboratories using an in-house single PCR based assay. Two of the participants utilised capillary electrophoresis as an analysis method, one used next generation sequencing, one used Sanger sequencing and one utilised agarose gel electrophoresis.
- One hundred and one laboratories returned a description of the *NPM1* variant detected in this sample.
- In line with expectation, seventy five laboratories (74.3%) 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). Of these, three participants reported an alternative variant description of c.863_864insTCTG (two participants utilising reference sequence NM_002520.6, with one participant not providing a reference sequence), one reported c.860_863insTCTG (reference sequence NM_002520.6), one reported c.772_773insTCTG (reference sequence NM_199185) and one participant reported c.859_860insTCTG (reference sequence ENST00000296930). 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 (19.8%) reported a 4 bp duplication / insertion but did not specify further details, whilst three participants merely stated that an insertion had been detected and one indicated that an insertion had been detected in exon 12.
- One laboratory incorrectly detected a 4 bp deletion. Another laboratory incorrectly detected NPM1 type A, B and D duplications.

Sample NPM1 154

- In line with sample formulation, 138 out of 141 (97.9%) participants returning results did not detect a *NPM1* variant in sample NPM1 154.
- The three out-of-consensus false positive results were returned by participants for this sample. Of the three, two utilised an in-house assay, one with high resolution melt analysis and one with Real-Time PCR fluorescent detection. The third participant utilised the Qiagen NPM1 A, B and D MutaQuant kit with Real-Time PCR fluorescent detection.

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
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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/