UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 202101

Participant ID - 43347

Date Issued - 21 July 2020

Closing Date - 28 August 2020

Trial Comments

FINAL REPORT: This trial was issued to 146 participants; of which 136 (93.1%) returned results. Of the non returns, five laboratories notified us of their intended non return and two laboratories submitted requests for an extension in results submission in light of the ongoing Covid-19 pandemic.

Sample Comments

Two lyophilised samples were manufactured and distributed by UK NEQAS LI. NPM1 151 and NPM1 152 were duplicate samples, formulated to carry a NPM1 variant (Type A, 4bp duplication), with the samples comprised of cell line material spiked into a pooled buffy coat.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 151	Mutation Detected	Mutation Detected
Sample NPM1 152	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 151	134	2
Sample NPM1 152	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

NPM1 Mutation Status Programme

Template

	Returns
DNA	101
cDNA	35

PCR Type

	Returns
Single PCR	74
Real-Time PCR	33
Multiplex PCR	21
Melting Curve Analysis	5

Protocol Type

	Returns
In-house Assay	107
Qiagen NPM1 Mutascreen Kit	12
Qiagen NPM1 mut A, B & D MutaQuant Kits	11
Illumina TruSight Myeloid Sequencing Panel	3
Ion AmpliSeq Cancer Hotspot Panel v2	2
Qiagen NPM1 mut A MutaQuant Kits	1

Analysis Type

	Returns
Capillary Electrophoresis	70
Real-Time PCR Fluorescent Detection	37
Next Generation Sequencing (Miseq)	9
Sanger Sequencing	7
Agarose Gel Electrophoresis	6
High Resolution Melt	3
NGS (ThermoFisher Ion Torrent)	3
Pyrosequencing	1

NPM1 Mutation Status Programme

Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	19
Falini B. et al (2007) Blood, 109(3):874-885	14
Schnittger S. et al (2005) Blood, 106(12):3733-3739	11
In-house method (no published reference available)	10
Falini B. et al (2005) N Engl J Med, 352(3):254-266	9
Gale R. et al (2008) Blood, 111(5):2776-2784	9
Thiede C. et al (2006) Blood, 107(10):4011-4020	9
Döhner K. et al (2005) Blood, 106(12):3740-3746	7
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	7
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	7
Belgian Molecular Diagnostic Group	6
Boissel N. et al (2005) Blood, 106(10):3618-3620	6
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	6
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	5
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	3
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	3
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	3
Chou WC. et al (2007) Leukemia, 21(5):998-1004	2
Falini B. et al (2006) Blood 108(6):1999-2005	2
Konoplev S. et al (2009) Cancer 115(20): 4737-4744	2

NPM1 Mutation Status Programme

Trial Comments

Sample NPM1 151

- In line with sample formulation, 134 out of 136 (98.5%) participants returning results detected a *NPM1* variant in sample NPM1 151.
- The two out-of-consensus false negative results for this sample were returned by laboratories using an in-house multiplex PCR based assay with capillary electrophoresis and an in-house real time PCR assay with fluorescent detection.

Sample NPM1 152

- In line with sample formulation, 135 out of 136 (99.3%) participants returning results detected a *NPM1* variant in sample NPM1 152.
- The laboratory returning an out-of-consensus false negative result also returned a false negative result for sample NPM1 151 (in-house real-time PCR with fluorescent detection).

Summary of variant descriptions

- NPM1 151 and 152 were duplicate samples, manufactured to have the same *NPM1* variant. Across both samples, 101 participants returned information relating to the *NPM1* variant detected.
- 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, four participants reported an alternative variant description:
    - c.863_864insTCTG (NM_002520.6; NG_016018.1; LRG-458)
    - c.863_864insTCTG (no reference sequence provided)
    - c.772_773insTCTG:p.L258fs (NM_199185, exon 10)
    - c.860_863dupCAGA (NM_002520.6).
- A further 21 laboratories (20.8%) reported a 4 bp duplication / insertion but did not specify further details, whilst two participants merely stated that an insertion or duplication had been detected and one indicated that an insertion had been detected in exon 12 (no reference sequence provided).
- One participant detected both a Type A and Type D duplication, and a further participant detected Types A, B and D.

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/