

NPM1 Mutation Status Programme

Distribution - 151601 

Participant ID - 

Date Issued - 05 August 2015 

Closing Date - 04 September 2015 

Trial Comments

The trial was issued to 98 participants for NPM1 analysis. 95 (97.0%) participants returned results; 3 did not, 1 of whom prenotified us of their non return. Please note, data tables do not necessarily add up to the total participation as late results are excluded from analysis.

Sample Comments

Two vials of lyophilised samples were manufactured and issued by UKNEQAS LI (sample refs NPM1 120 and NPM1 121). Sample NPM1 120 was cell line based and formulated to be negative for a NPM1 mutation. NPM1 121 was a lyophilised patient sample positive for a NPM1 mutation.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 120 	No Mutation Detected 	No Mutation Detected 
Sample NPM1 121	No Mutation Detected 	Mutation Detected 

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 120	2	92
Sample NPM1 121	85	7

Your Performance

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

N/A = Not Applicable

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Template

	Returns
DNA	69
cDNA	25

PCR Type

	Returns
Single PCR	47
Multiplex PCR	23
Real-Time PCR	16
Melting Curve Analysis	7
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	80
Qiagen NPM1 Mutascreen Kit	8
Qiagen NPM1 Mutaquant Kit	5
Ion AmpliSeq Cancer Hotspot Panel v2	1

Analysis Type

	Returns
Capillary Electrophoresis	49
Real-Time PCR Fluorescent Detection	20
Sanger Sequencing	12
High Resolution Melt	6
Next Generation Sequencing (Ion Torrent)	4
Agarose Gel Electrophoresis	2
Pyrosequencing	1

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6):1103-1108	11
Falini B. et al (2005) N Engl J Med, 352(3):254-266	9
Schnittger S. et al (2005) Blood, 106(12):3733-3739	9
Thiede C. et al (2006) Blood, 107(10):4011-4020	9
Huang Q. et al (2008) Br J Haematol, 142(3):489-492	8
Falini B. et al (2007) Blood, 109(3):874-885	7
Belgian Molecular Diagnostic Group	6
In-house method (no published reference available)	6
Tan AY. et al (2008) J Haematol Oncol, 1, 10	6
Bench AJ. et al (2012) Int J Lab Hematol. 34(1):21-34	4
Boissel N. et al (2005) Blood, 106(10):3618-3620	4
Gale R. et al (2008) Blood, 111(5):2776-2784	4
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	4
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	4
Döhner K. et al (2005) Blood, 106(12):3740-3747	3
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	3
Chou WC. et al (2007) Leukemia, 21(9):993-1004	2
Falini B. et al (2006) Blood 108(6):1999-2005	2
Lazenby M. et al (2014) Leukemia 28(10):1953-1959	2
Lin LI. et al (2006) Leukemia 20(10):1899-1903	2
Pitiot AS. et al (2007) Leukemia 21(7):1564-1566	2
Szankasi P. et al (2008) J Mol Diagn, 10(3):236-241	2
Verhaak RG. et al (2005) Blood, 106(12):3747-3754	2

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Final Comments

For sample NPM1 120, 92 (97.9%) participants returning results for this trial did not identify a *NPM1* mutation, in line with sample formulation. Of the two false positive results, one participant used a real-time PCR approach and the other used single PCR alongside agarose gel detection.

For sample NPM1 121, 85 (92.4%) participants returning results for this trial detected a *NPM1* mutation, in line with sample formulation. Of the 7 participants that failed to detect a *NPM1* mutation, 6 used real-time PCR (4 Qiagen Mutaquant kit and 2 In-house) and 1 used Sanger sequencing.

Sample NPM1 121 was a lyophilised peripheral blood sample taken from a patient with Acute Myeloid Leukaemia (AML). Pre issue testing revealed the sample to carry a c.867_868insAGGA p.(Trp290Argfs*10) or p.(Trp290ArgfsTer10) mutation (*NPM1* NM_002520.6), a 4bp insert (AGGA) located 4 bases 3' downstream of the usual insert location for type A, B, and D mutations. Functionally this coding change works in the same way as the more prevalent A, B, and D mutations; a net insertion of 4bp, leading to a frameshift and an elongated protein containing a novel nuclear export signal which induces an increased transport of the NPM1 protein to the cytoplasm (Thiede, Koch, et al., 2006).

(1) NPM1 Wildtype gctattcaagatctctggcagtgaggaggtctctctttaagaaaatag
(2) Type A gctattcaagatctctg TCTG gaggaggaggaagtctctctttaagaaaatag
(3) NPM1 121 gctattcaagatctctggcag AGGaggaggaggaagtctctctttaagaaaatag

(1) Reference sequence *NPM1* NM_002520.6 (nucleophosmin isoform 1) with c.DNA sequence commencing at position 847 (start of exon 12). The stop/termination codon is highlighted. (2) Mutation c.860_863dupTCTG (Type A mutation), duplicated nucleotides are underscored with resulting additional nucleotides represented in uppercase. The stop/termination codon in the new reading frame is highlighted. (3) Mutation c.867_868insAGGA, inserted nucleotides are represented in uppercase. The stop/termination codon in the new reading frame is highlighted.

The c.867_868insAGGA mutation has been variously classified in the literature as Type Q (Rau & Brown, 2009), DD-9 (Thiede, Creutzig, et al., 2006) and MM (Schnittger et al., 2005). 58 participants provided a description of the mutation, ranging from the size of the insertion to the use of full Human Genome Variation Society (HGVS) nomenclature (<http://www.hgvs.org/mutnomen/>). 52 participants described the mutation in-line with the pre issue testing results. Of the 5 out-of-consensus results, 4 were mutation misclassifications (type I, B and 2x A) and 1 was a protein nomenclature issue (p.(Trp290Serfs*10)).

The Qiagen/Ipsogen Mutaquant kit is designed to detect type A, B and D mutations **ONLY**, thus will not pick up rarer mutations. In line with expectation, **all participants** who used the Mutaquant kit in isolation in this trial issue failed to detect a *NPM1* mutation in sample NPM1 121. Non type A, B or D mutations represent approximately 10% of reported mutations in *NPM1* in AML (Thiede, Creutzig, et al., 2006) and thus if laboratories use the Mutaquant kit in isolation these mutations will not be detected.

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Three participants commented that the sample was inadequate or failed their internal IQC, only one of whom contacted UKNEQAS LI for a repeat sample. All used cDNA as a template. It is worth considering that this was a lyophilised patient sample and not an over expressing cell line, so RNA may not be as prevalent. Please contact UKNEQAS LI if the samples do not pass your IQC and **do not submit results based on a suboptimal assay.**

We would like to thank Prof. David Grimwade and Dr Adam Ivey for their help sourcing sample NPM 121 and providing details of the sequence changes.

References

- Rau, R., & Brown, P. (2009). Nucleophosmin (NPM1) mutations in adult and childhood acute myeloid leukaemia: towards definition of a new leukaemia entity. *Hematological Oncology*, 27(4), 171–81. doi:10.1002/hon.904
- Schnittger, S., Schoch, C., Kern, W., Mecucci, C., Tschulik, C., Martelli, M. F., ... Falini, B. (2005). Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood*, 106(12), 3733–3739. doi:10.1182/blood-2005-06-2248
- Thiede, C., Creutzig, E., Illmer, T., Schaich, M., Haase, V., Ehninger, G., & Landt, O. (2006). Rapid and sensitive typing of NPM1 mutations using LNA-mediated PCR clamping. *Leukemia*, 20(10), 1897–9. doi:10.1038/sj.leu.2404367
- Thiede, C., Koch, S., Creutzig, E., Steudel, C., Illmer, T., Schaich, M., ... Leukami, D. S. (2006). Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML) RID A-1417-2008. *Blood*, 107(10), 4011–4020. doi:10.1182/blood-2005-08-3167

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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

463A Glossop Road

Sheffield, S10 2QD

United Kingdom

Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601

e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:

Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

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 n) 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.