UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 161702

Participant ID -

Date Issued - 09 November 2016

Closing Date - 09 December 2016

Trial Comments

The trial was issued to 119 participants for NPM1 analysis. 117 (98.3%) returned results.

Sample Comments

Two vials of lyophilised samples were manufactured and issued by UK NEQAS LI (sample refs NPM1 129, NPM1 130).
Samples NPM1 129 and NPM1 130 were cell line based and were formulated to be negative for an NPM1 mutation.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 129		No Mutation Detected
Sample NPM1 130		No Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 129	3	114
Sample NPM1 130	2	115

Your Performance

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

N/A = Not Applicable

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Template

	Returns
DNA	79
cDNA	38

PCR Type

	Returns
Single PCR	59
Real-Time PCR	25
Multiplex PCR	24
Melting Curve Analysis	7
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	98
Qiagen NPM1 Mutascreen Kit	8
Qiagen NPM1 mut A, B & D MutaQuant Kits	6
Ion AmpliSeq Cancer Hotspot Panel v2	2
Qiagen NPM1 mut A MutaQuant Kits	2
Illumina TruSight Myeloid Sequencing Panel	1

Analysis Type

	Returns
Capillary Electrophoresis	64
Real-Time PCR Fluorescent Detection	28
Sanger Sequencing	8
Next Generation Sequencing (Ion Torrent)	6
High Resolution Melt	5
Agarose Gel Electrophoresis	4
Pyrosequencing	2

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Journal Reference for Assay

	Returns
Gorello P. et al (2006) Leukemia, 20(6) 1103-1108	16
Falini B. et al (2005) N Engl J Med, 352(3):254-266	15
Schnittger S. et al (2005) Blood, 106(12):3733-3739	11
Thiede C. et al (2006) Blood, 107(10):4011-4020	11
Huang Q. et al (2008) Br J Haematol, 142:(3)489-492	8
In-house method (no published reference available)	8
Noguera N. et al (2005) Leukemia, 19(8):1479-1482	7
Belgian Molecular Diagnostic Group	6
Falini B. et al (2007) Blood, 109(3):874-885	6
Tan AY. et al (2008) J Haemtol Oncol, 1, 10	6
Gale R. et al (2008) Blood, 111(5):2776-2784	5
Boissel N. et al (2005) Blood, 106(10):3618-3620	4
Döhner K. et al (2005) Blood, 106(12):3740-3746	4
Lin LI. et al (2006) Leukemia, 20(10):1899-1903	4
Falini B. et al (2006) Blood 108(6):1999-2005	3
Scholl S. et al (2007) Leuk Res, 31(9):1205-1211	3
Szankasi P. et al (2008) J Mol Diagn, 10(3)236–241	3
Thiede C. et al (2006) Leukemia, 20(10):1897-1899	3
Bench AJ. et al (2012) Int J Lab Hematol. 34(1):21-34	2
Pitiot AS. at al (2007) Leukemia 21(7):1564-1566	2

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Final Comments

In line with sample formulation 114 (97.4%) participants returning results for this trial did not identify a *NPM1* mutation in samples NPM1 129. Three laboratories (2.6%) detected a false positive; two of which used Real-Time PCR Fluorescent Detection and one that used Sanger Sequencing as their analysis method.

In line with sample formulation 115 (98.3%) participants returning results for this trial did not identify a *NPM1* mutation in samples NPM1 130. Two laboratories (1.7%) detected a false positive; one of which used Real-Time PCR Fluorescent Detection and one that used Sanger Sequencing as their analysis method. The two participants that detected an *NPM1* mutation in sample NPM1 130 were the same participants that detected it in sample NPM1 129.

Although the two samples were not made in duplicate, they were both manufactured with the same *NPM1* negative cell line. One participant reported a "NM_002520.6:c.859_860insTCAT" mutation in NPM1 129 and a "NM_002520.6:c.860_863dupTCTG" mutation in NPM1 130. One participant reported a "het_del 28027 t" in both NPM1 129 and NPM1 130. One participant detected NPM1 type A in NPM1 129.

The template, PCR, protocol and analysis type utilised by our participants has not changed significantly over the course of the last four NPM1 trials issued.

NPM1 Mutation Status

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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United Kingdom
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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 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/