UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

NPM1 Mutation Status Programme

Distribution - 252601

Participant ID - 43347

Date Issued - 14 July 2025

Closing Date - 22 August 2025

Trial Comments

This trial was issued to 163 participants, of which 160 (98.2%) returned results. Of the non returns, one participant pre-notified us of their intended non return and one participant requested an extension to results submission.

Sample Comments

Two vials of cell line based lyophilised samples were manufactured and issued by UK NEQAS LI (sample references NPM1 181 and NPM1 182). Sample NPM1 181 was formulated to be negative for a NPM1 Type A duplication and NPM1 182 was manufactured to be positive for a NPM1 Type A duplication.

Results and Performance

Your Results

NPM1 Mutation Status	Your Results	Consensus Result
Sample NPM1 181	No Mutation Detected	No Mutation Detected
Sample NPM1 182	Mutation Detected	Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample NPM1 181	0	160
Sample NPM1 182	159	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	128
cDNA	32

PCR Type

	Returns
Single PCR	78
Real-Time PCR	35
Multiplex PCR	22
Sequencing	17
Melting Curve Analysis	7
Single PCR with Clamping	1

Protocol Type

	Returns
In-house Assay	125
Qiagen NPM1 Mutascreen Kit	11
Diatech EasyPGX ready NPM1 Screening	7
Oncomine Myeloid Research Assay	5
Qiagen NPM1 mut A, B & D MutaQuant Kits	5
Ion Torrent Oncomine Myeloid Panel	3
Cepheid Xpert NPM1 Mutation Assay	2
Ampliseq for Illumina Myeloid Panel	1
Imegen NPM1 Kit	1

Analysis Type

	Returns
Capillary Electrophoresis	88
Real-Time PCR Fluorescent Detection	33
NGS (ThermoFisher Ion Torrent)	8
Sanger Sequencing	8
High Resolution Melt	7
Agarose Gel Electrophoresis	4
NGS (Illumina)	4
Digital PCR (Biorad)	3
Next Generation Sequencing (Miseq)	2
Illumina NextSeq 2000	1
Illumina NextSeq 500	1
Illumina NextSeq 550	1

NPM1 Mutation Status Programme

Journal Reference for Assay

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

NPM1 Mutation Status Programme

Trial Comments

Sample NPM1 181

- In line with sample formulation, 160 of 160 (100%) participants returning results reported the absence of an *NPM1* variant in sample NPM1 181.

Sample NPM1 182

- One hundred and fifty-nine (99.4%) participants returning results identified an *NPM1* variant in sample NPM1 182.
- The participant reporting an out of consensus false negative result, utilised an in-house assay with Sanger sequencing. Despite reporting the absence of a variant in NPM1 182, the participant reported HGVS nomenclature of c.860_863dupTCTG, suggestive of a possible data entry error.
- One hundred and nine participants provided information relating to the type of *NPM1* variant detected in NPM1 182. In line with sample formulation, 83 (76.1%) 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, five participants described the duplicated nucleotide material (c.860_863dupTCTG). HGVS recommendations do not endorse listing the duplicated nucleotide sequence as this creates a longer description with redundant information[2].
- A further 22 laboratories (20.2%) reported a 4 bp insertion but did not specify further details and one participant reported an insertion but did not specify the insertion size.
- One participant reported detection of a Type B *NPM1* variant. A further participant reported a c.847_1010insN[4] variant using the NM_002520.7 reference sequence.
- A final participant reported that the variant detected affected the amino acid residue tryptophan at position 288 (W288) of the NPM1 protein, however the specific variant could not be identified with the methodology utilised.

The persistent presence of the *NPM1* variant(s) in patients with *NPM1*+ AML has shown that this is a stable marker to determine molecular assessment of measurable residual disease (MRD) at specific clinical time points[3]. **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'[4]. 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) Nomenclature. Available at: https://hgvs-nomenclature.org/stable/ (v21.1.3). (Accessed: 14 October 2025).

3. 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).

4. Scott, S. *et al*. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. *Blood Adv*. **7**(14), 3686-3694 (2023).

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
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600
e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (acting Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.

4.8.2 d) Pre issue and post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were externally provided.

4.8.2 d) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

4.8.2 g) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel is informed 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/