UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Lymphoplasmacytic Lymphoma / Waldenström Macroglobulinaemia Programme

Distribution – LPLWM 252601

Participant –

Date Issued – 02 June 2025

Closing Date – 04 July 2025

This programme has now achieved ISO 17043:2010 accreditation and is now performance monitored.

Trial Comments

This trial was issued to 80 participants; 78 (97.5%) returned results.

Sample Comments

Two lyophilised samples (LPLWM 124 and 125) were prepared and distributed by UK NEQAS LI. Samples LPLWM 124 and LPLWM 125 were manufactured to be positive for the NM_002468.5(*MYD88*):c.755T>C p.(Leu252Pro) (MANE select sequence) (historical variant nomenclature NM_002468.4(*MYD88*):c.794T>C p.(Leu265Pro)) variant. We would like to acknowledge Professor Steven Treon (Dana-Farber Cancer Institute), who kindly donated the cell line material used in this programme.

Results and performance

Your Results

***MYD88* Variant (Mutation) Status**	**Your Results**	**Consensus Result**
Sample LPLWM 124	Variant detected	Variant detected
Sample LPLWM 125	Variant detected	Variant detected

All Participant Results

***MYD88* Variant (Mutation) Status**	**Variant Detected (Returns)**	**No Variant Detected (Returns)**
Sample LPLWM 124	77	1
Sample LPLWM 125	78	0

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	2	0

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Methods

Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Template Type

Template	Count
gDNA	76
cDNA	2

PCR Type

PCR method	Count
Digital PCR	27
Allele Specific PCR	23
Real-Time PCR	11
Single PCR	7
PCR for Next Generation Sequencing	7
LNA PCR	2
MLPA	1
Extension PCR for MALDI-TOF	1
Multiplex PCR	1

Protocol Type

Assay Protocol	Count
In-house designed	40
Biorad PrimePCR ddPCR Mutation Assay: MYD88 p.L265P	26
PlentiPlex™ MYD88 L265P assay	4
Qiagen qBiomarker MYD88	2
MLPA	1
Ion Torrent Liverpool Lymphoma Network Community Lymphoid NGS Panel	1
Marsden Haem v2	1
Qiagen Targeted DNA Custom Panel	1
Agilent SureSelect custom panel	1
Twist Bioscience target enrichment panel	1
TRUPCR® MYD88 Mutation Detection Kit	1

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Analysis Type

Analysis method	Count
Digital PCR	27
Real-Time PCR fluorescent detection	24
Agarose gel electrophoresis	10
Next Generation Sequencing - Illumina	7
Sanger sequencing	3
Capillary electrophoresis	2
Next Generation Sequencing - Thermofisher Ion Torrent	2
Mass spectrometry	1
Acrylamide gel electrophoresis (PAGE)	1
High resolution melting analysis	1
Tapestation	1

Journal Reference for Assay

Assay Journal Reference	Count
Treon, S. P. *et al*. N Engl J Med. 2012; 367(9):826-833	16
Varettoni, M., *et al*. Blood. 2013; 121(13):2522-2528	10
Jimenez, C., *et al*. Appl Immunohistochem Mol Morphol. 2014; 22(10):768-773	9
Xu, L., *et al*. Blood. 2013;121(11):2051-2058	9
Pratt, G., *et al*. Br. J. Haematol. 2022; 197(2):171-187	3
Mori, N., *et al*. PLOS ONE. 2013; 8(11):1-9	2
Kaiser, L., *et al*. Leukaemia. 2021; 35(2):333-345	2
Drandi, D., *et al*. Haematologica. 2018;103(6):1029-1037	2
Staiger, A. M., *et al*. Br J Haematol. 2015; 171(1):145-148	2
Drandi, D., *et al*. Methods Mol. Biol. 2023; 2621:57-72	1
Poulaine, S., *et al*. Blood. 2013; 121(22):4504-11	1
Chen, Q., *et al*. J Mol Diagn. 2007; 9(2):272-276	1
Shin, S.Y., *et al*. Blood Res. 2016;51(3):181-186	1
Véronèse, L., *et al*. Cancer Genet. 2013; 206:19-25	1

Reported Limit of Detection

Limit of Detection (%)	Count
0-0.01	15
0.011-0.1	28
0.11-1.0	21
1.1-10.0	6
>10	1

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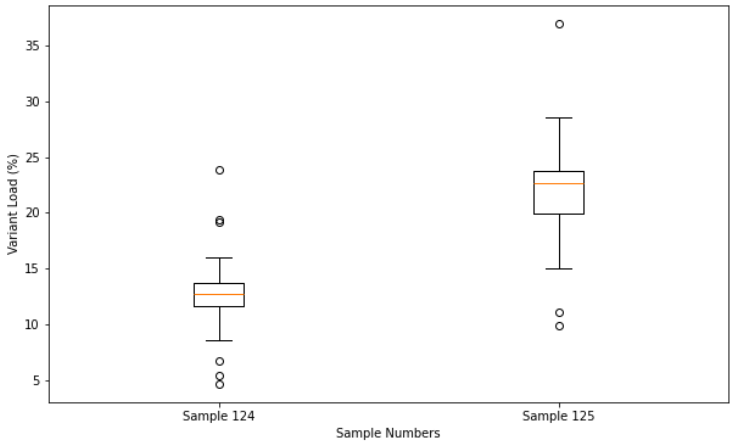

Box and whisker plot to show variant allele frequency reported by participants for samples LPLWM 124 and 125. The graph represents all variant allele frequency data submitted by participants. The middle (orange) line inside the boxes represents the median of the data, with the bottom and top edges of the box representing the 25th and 75th centiles. The 'whiskers' extending from the box represent the most extreme data points that are no more than 1.5x the interquartile range (IQR). The circles beyond the whiskers represent data outliers.

Trial Comments

- 77 out of 78 (98.7%) participants correctly reported 'Variant detected' for the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in LPLWM 124.
- The participant(s) reporting a false negative result in sample LPLWM 124 utilised Illumina Next Generation Sequencing with an Agilent Sureselect custom panel.
- 78 out of 78 participants (100.0%) correctly reported 'Variant detected' for the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in LPLWM 125.
- Thirty-nine participants returned quantification data for sample LPLWM 124 and forty returned quantification data for sample LPLWM 125.
- Thirty-two participants utilised gDNA template and the Variant/(Variant+Wildtype) x 100 calculation method. One participant used cDNA template with the Variant/(Variant+Wildtype) x 100 calculation method. One participant utilised gDNA template and the Variant/Wildtype x 100 calculation method, with one participant using cDNA template and the Variant/Wildtype x 100 calculation method. Two participants utilised gDNA template with fractional abundance as the calculation method. One participant used gDNA template and 1/2^(deltaCT between variant and wild type) x 100 calculation method. One participant utilised gDNA template and stated use of 'Other' in the calculation method but provided no further information. A further participant provided quantification information for LPLWM 124 and 125 but provided no method for calculating the quantitative data.
- The median variant load reported for LPLWM 124 (gDNA input material, Variant/(Variant+WT) x 100 quantification calculation) was 12.8 %, with an interquartile range (IQR) of 2.0%. Variant loads ranged from 5.4-16.0%.
- The median variant load reported for LPLWM 125 (gDNA input material, Variant/(Variant+WT) x 100 quantification calculation) was 22.9%, with an interquartile range (IQR) of 3.7%. Variant loads ranged from 9.9-28.6%.

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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
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/