UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Lymphoplasmacytic Lymphoma / Waldenström Macroglobulinaemia Programme (Not Accredited)

Distribution – LPLWM 232402
Date Issued – 08 November 2023

Participant –
Closing Date – 15 December 2023

Trial Comments

This trial was issued to 67 participants; 64 (95.5%) returned results.

Sample Comments

Two lyophilised samples (LPLWM 116 and 117) were prepared and distributed by UK NEQAS LI. Samples LPLWM 116 was manufactured to be to be negative and 117 was designed 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)). 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 116	No variant detected	No variant detected
Sample LPLWM 117	Variant detected	Variant detected

All Participant Results

***MYD88* Variant (Mutation) Status**	**Variant Detected (Returns)**	**No Variant Detected (Returns)**
Sample LPLWM 116	0	64
Sample LPLWM 117	60	4

Your Performance

Performance	Performance Status for this sample	Performance Status Classification Over 12 Month Period	
		Satisfactory	Critical
n/a	n/a	n/a	n/a

Please note: this programme is not currently performance monitored. We will work towards a performance monitoring system as the programme develops.

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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	63
cDNA	1

PCR Type

PCR method	Count
Allele Specific PCR	32
Single PCR	13
Real-Time PCR	10
Multiplex PCR	7
PCR for Next Generation Sequencing (NGS)	3
Digital PCR	3
LNA PCR	2

Protocol Type

Assay Protocol	Count
In-house designed	40
BioRad PrimePCR ddPCR Mutation Assay: MYD88 p.L265P	16
PlentiPlex MYD88 L265P assay	4
MLPA P038 probe mix	1
Qiagen QBioMarker MYD88	1
Roche RMHhaemv2	1
Qiaseq Targeted DNA Custom Panel	1

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Analysis Type

Analysis method	Count
Digital PCR	19
Real-Time PCR fluorescent detection	19
Agarose gel electrophoresis	7
Next Generation Sequencing - Illumina	7
Capillary electrophoresis	5
Pyrosequencing	2
Sanger sequencing	2
Next Generation Sequencing - ThermoFisher Ion Torrent	1
Next Generation Sequencing - Other	1
Acrylamide gel electrophoresis (PAGE)	1
High resolution melting analysis	1

Journal Reference for Assay

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

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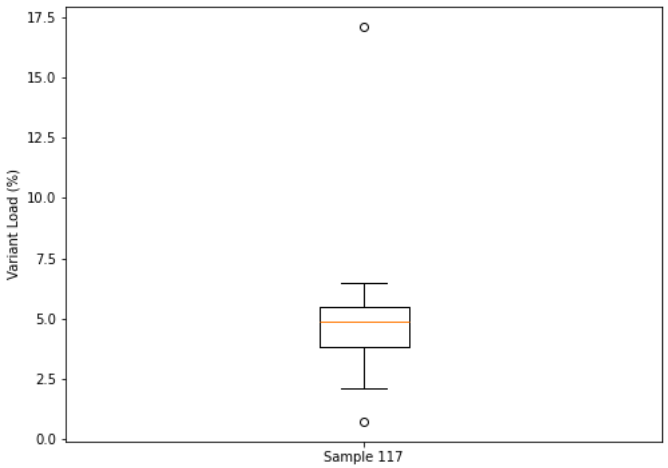
Reported Limit of Detection

Limit of Detection	Count
0-0.01	20
0.011-0.1	12
0.11-1.0	13
1.1-10.0	8
>10	1



Box and whisker plot to show variant allele frequency reported by participants for sample LPLWM 117. The middle (orange) line inside the box 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.

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Trial Comments

- Sixty-four out of 64 (100.0%) participants correctly reported 'No variant detected' for the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in LPLWM 116.
- Sixty out of 64 participants (93.8%) correctly reported 'Variant detected' for the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in LPLWM 117.
- Of the four participant(s) reporting a false negative result in sample LPLWM 117 three utilised an in-house designed assay (two with Real-Time fluorescent detection and one with capillary electrophoresis). A further participant reported an out of consensus false negative result utilising the droplet digital BioRad Primer PCR MYD88 p.L265P assay, despite submitting a quantitative variant allelic frequency of 3.76%.
- Thirty-two participants returned quantification data for samples LPLWM 117.
- Twenty-four (75%) participants quantified the variant using the Variant/(Variant+Wildtype) x 100 calculation method, with gDNA used as input material. Four (12.5%) participants used the Variant/Wildtype x 100 calculation method, three utilising gDNA and one using cDNA as the input material. One (3.1%) participant reported the use of the Delta CT method of quantification using gDNA input material. A further participant reported use of fractional abundance, using gDNA as input material. Two participants did not provide information relating to the method of quantification.
- The median variant load reported for LPLWM 117 (gDNA input material, Variant/(Variant+WT) x 100 quantification calculation) was 4.8%, with an IQR of 1.7%. Variant loads ranged from 0.7-6.5%.

Please note, this programme is currently being assessed for UKAS ISO 17043:2010 accreditation and once achieved, we will seek to implement performance monitoring.

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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: amanda.newbould@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/