UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Lymphoplasmacytic Lymphoma / Waldenström Macroglobulinaemia Programme (Not Accredited)

Distribution – LPLWM 222302

Participant –

Date Issued – 14 November 2022

Closing Date – 23 December 2022

Trial Comments

This study was issued to 60 participants; 58 (96.7%) returned results.

Sample Comments

Two lyophilised samples (LPLWM 112 and 113) were prepared and distributed by UK NEQAS LI. Sample LPLWM 112 was 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)). LPLWM 113 was formulated to be negative for the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) (MANE select sequence) 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 112	Variant detected	Variant detected
Sample LPLWM 113	No variant detected	No variant detected

All Participant Results

***MYD88* Variant (Mutation) Status**	**Variant Detected (Returns)**	**No Variant Detected (Returns)**
Sample LPLWM 112	56	2
Sample LPLWM 113	2	56

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

	Returns
gDNA	55
cDNA	2

PCR Type

	Returns
Allele Specific PCR	22
Real-Time PCR	13
Single PCR	12
Multiplex PCR	6
Digital PCR	5
LNA PCR	3
Next Generation Sequencing	3
Multiplex Ligation Dependent Probe Amplification (MLPA)	1

Protocol Type

	Returns
In-house Assay	33
Biorad PrimePCR ddPCR Mutation Assay	15
PlentiPlex MYD88 L265P Assay	3
Qiagen qBiomarker Somatic Mutation PCR Assay	2
Illumina TruSight Oncology 500	1
Custom Qiaseq Lymphoid Panel	1
ThermoFisher Taqman MYD88 p.L265P mutation assay	1
MRC Holland MLPA	1

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Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	20
Digital PCR	18
Agarose Gel Electrophoresis	5
Next Generation Sequencing (Illumina)	5
Capillary Electrophoresis	3
Pyrosequencing	2
Sanger Sequencing	1
Next Generation Sequencing (Other)	1
Next Generation Sequencing (ThermoFisher Scientific Ion Torrent)	1
Acrylamide Gel Electrophoresis	1

Journal Reference for Assay

	Returns
Xu, L., *et al. Blood* 2013;**121**(11): 2051-2058.	8
Jimenez, C., *et al*. *Appl Immunohistochem Mol Morphol* 2014;**22**(10):768-773.	7
Drandi, D., *et al*. *Haematologica* 2018;**103**(6):1029-1037.	3
Treon, S.P., *et al*. *N Engl J Med* 2012;**367**:826-833.	2
Varettoni, M., *et al. Blood* 2013;**121**(13):2522-2528.	2
Véronèse, L., *et al*. *Cancer Genet* 2013;**206**(1-2):19-25.	1
Shin, S.Y., *et al*. *Blood Res* 2016;**51**(3): 181-186.	1
Mori, N., *et al. PLoS One* 2013;**8**(11):1-9.	1
Staiger, A.M., *et al. Br J Haematol* 2015;**171**(1):145-158.	1
Hiemcke-Jiwa, L.S., *et al. Hematol Oncol* 2018;**36**(2):429-435.	1
Hamedeh, F., *et al*. *Mod Pathol* 2015;**28**:564-574.	1
Huggett, J. F., *et al*. *Clin Chem* 2013;**59**(6): 892-902.	1
Treon, S.P., *et al*. *Blood* 2014;**123**(18): 2791-2796.	1

Lymphoplasmacytic Lymphoma / Waldenström Macroglobulinaemia Programme (Not Accredited)

Trial Comments

- Fifty-six out of 58 participants (96.6%) correctly reported the presence of the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in LPLWM 112.
- Of the two participants reporting a false negative in sample LPLWM 112, one also reported an out of consensus false positive result for LPLWM 113, suggestive of a sample transposition event. The remaining participant utilised an in-house single PCR assay with agarose gel electrophoresis.
- Fifty-six participants (96.6%) reported the absence of the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in sample LPLWM 113.
- Two participants reported a false positive result, one reported an out of consensus result for LPLWM 112, suggestive of a sample transposition. The remaining participant utilised an in-house designed assay by digital PCR.
- Thirty participants returned quantification data for sample LPLWM 112.
- Twenty-four (80.0%) participants quantified the variant using the Variant/(Variant+WT) x 100 calculation, 23 of which utilised gDNA as input material. Three (10.0%) participants quantified the variant using the Variant/WT x 100 method, with two using gDNA and one using cDNA as the input material. One (3.3%) participant reported the use of Delta-Delta CT method of quantification with gDNA as input material. Two (6.7%) participants did not report the method by which they quantified the variant.
- The median variant load reported for LPLWM 112 (gDNA input material, Variant/(Variant+WT) x 100 quantification calculation) was 5.1%, with an interquartile range (IQR) of 2.5%. Variant loads ranged from 1-18.185%.
- The data returns for limit of detection (LOD) for participant *MYD88* assays ranged from 0.004-20%; with a median value of 0.6%. The participant reporting a LOD of 20% utilised Sanger sequencing and stated an LOD range of between 10-20%. Despite the stated LOD, the participant was able to detect the NM_002468.5(MYD88):c.755T>C p.(Leu252Pro) variant in sample LPLWM 112 (median VAF = 5.1%).

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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
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Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
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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/