

Paroxysmal Nocturnal Haemoglobinuria Programme

Distribution - 222302

Sample - 159

Participant ID -

Date Issued - 08 June 2022

Closing Date - 29 June 2022

Trial Comments

This exercise was issued to 163 participants. Please note: this report was generated after the issue of the initial trial report due to the laboratory request for an extension to result submission.

Sample Comments

This sample was manufactured from consented stabilised PNH material in a background of stabilised whole blood only.

Results and Performance

Your Results

Cell Population	Your Results	Consensus Result
Red Blood Cells PNH Clone	Present	Clone Present
Monocytes PNH Clone	Present	Clone Present
Neutrophils PNH Clone	Present	Clone Present

Your Performance

Cell Population	Performance Status for this Trial	Performance Status Classification Over 12 Sample Period	
		Satisfactory	Critical
Red Blood Cells PNH Clone	Satisfactory	12	0
Monocytes PNH Clone	Satisfactory	12	0
Neutrophils PNH Clone	Satisfactory	12	0

N/A = Not Applicable

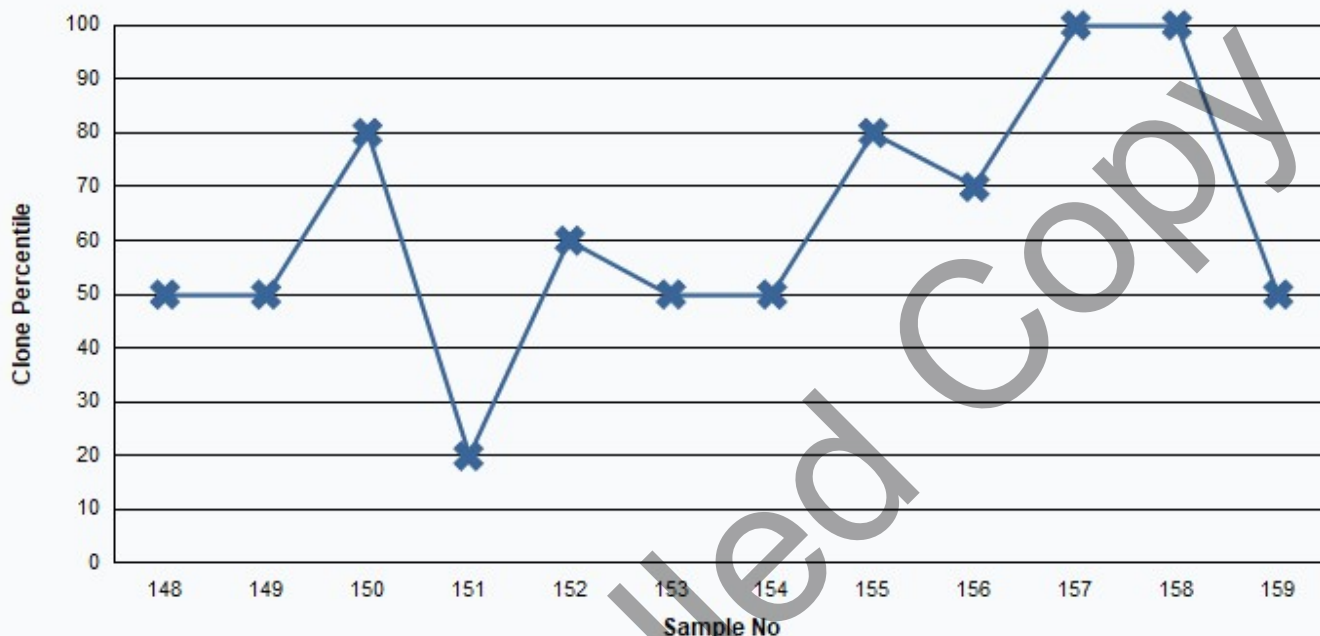
Percentage Value Results

Cell Population	Your Results (%)	Median Result (%)	Lower Quartile (%)	Upper Quartile (%)
Red Blood Cells PNH Clone	1.27	1.27	1.10	1.46
Monocytes PNH Clone	9.50	11.03	7.97	12.64
Neutrophils PNH Clone	4.00	4.50	4.10	4.86

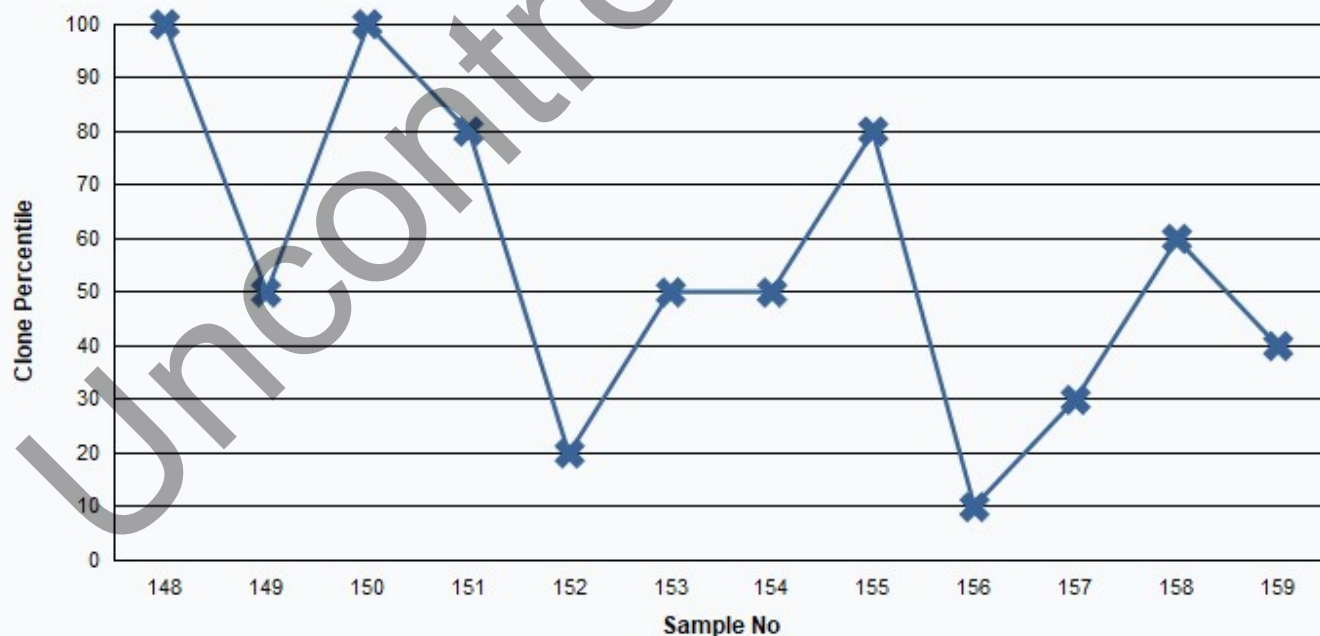
Paroxysmal Nocturnal Haemoglobinuria Programme

Centile graph of Percentage Results

Red Blood Cells PNH Clone Percentage Population over last 12 months

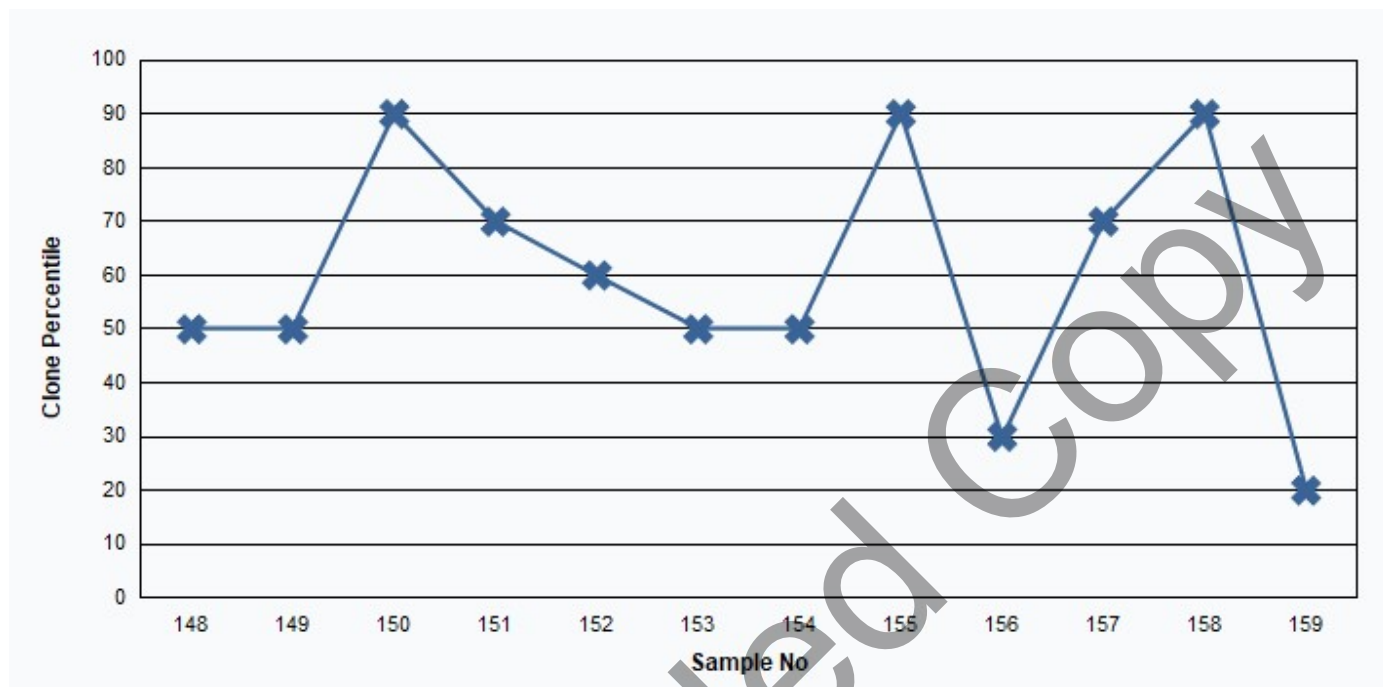


Monocytes PNH Clone Percentage Population over last 12 months



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Neutrophils PNH Clone Percentage Population over last 12 months



Paroxysmal Nocturnal Haemoglobinuria Programme

Flow Cytometer Specific Statistics

(Please note only groups of >20 returns are displayed)



Red Blood Cell PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	28	28	0	1.28	1.08	1.42
Navios	34	34	0	1.28	1.08	1.62
FACSCanto II	49	49	0	1.30	1.10	1.45

Monocytes PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	27	27	0	11.10	9.40	12.42
Navios	38	38	0	9.65	7.79	11.60
FACSCanto II	51	50	1	11.40	9.20	13.66

Neutrophils PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	33	33	0	4.50	4.10	4.76
Navios	38	38	0	4.46	4.11	5.02
FACSCanto II	57	56	1	4.46	4.05	4.83

Paroxysmal Nocturnal Haemoglobinuria Programme

Gating Antibodies Used Statistics

(Please note only groups of >20 returns are displayed)



Red Blood Cell PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD235a	111	111	0	1.28	1.11	1.44

Monocytes PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD33/CD45	30	30	0	9.35	7.44	11.88
CD45/CD64	68	68	0	11.36	9.38	13.10

Neutrophils PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD15	23	23	0	4.30	4.13	4.65
CD15/CD45	83	83	0	4.50	4.11	4.80

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GPI Linked Antibodies Used Statistics

(Please note only groups of >20 returns are displayed)



Red Blood Cell PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD59	118	118	0	1.28	1.10	1.47

Monocytes PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD157/FLAER	25	25	0	11.82	10.23	12.79
CD14/FLAER	79	79	0	11.00	7.86	12.75

Neutrophils PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD157/FLAER	26	26	0	4.52	3.81	4.96
CD24/FLAER	87	86	1	4.46	4.11	4.86

Paroxysmal Nocturnal Haemoglobinuria Programme

GPI Linked Antibodies Specific Statistics

Red Blood Cell PNH Clone



GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
^CD55	12	0	12
CD59	131	0	131

se note the numbers in the above tables will not match the number of laboratories enrolled in the programme as each centre uses a panel with multiple antibodies

Monocytes PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
CD14	99	0	99
CD157	40	0	40
CD24	6	1	7
^CD55	7	0	7
^CD59	4	0	4
FLAER	130	1	131

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Neutrophils PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
CD157	38	0	38
CD16	19	0	19
CD24	108	1	109
^CD55	6	0	6
^CD59	5	0	5
^CD66b	7	0	7
FLAER	138	1	139

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^ Antigens marked above, in the GPI linked antibodies specific statistics table, are not considered best practice and laboratories should avoid using these.

Paroxysmal Nocturnal Haemoglobinuria Programme

Methodology



Red Blood Cells

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Testing	CD59	MEM-43	Life Technologies	PE	Yes
Gating	CD235a	GAR-2	BD Biosciences	FITC	No

Monocytes

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD64	N/A	Beckman Coulter	PC7	No
Testing	CD14	MoP9	BD Biosciences	APC-H7	No
Gating	CD45	2D1	BD Biosciences	PerCP	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Neutrophils

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD15	N/A	BD Biosciences	AlexaFluor 647	No
Gating	CD45	2D1	BD Biosciences	PerCP	No
Testing	CD24	ML5	BD Biosciences	PE	Yes
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Recommended Reading

Sutherland DR, Illingworth A, Marinov I, Ortiz F, Andreasen J, Payne D, Wallace PK and Keeney M. ICCS/ESCCA Consensus Guidelines to detect GPI-deficient cells in Paroxysmal Nocturnal Hemoglobinuria (PNH) and related Disorders Part 2 - Reagent Selection and Assay Optimization for High-Sensitivity Testing. Cytometry, Part B 2018; 94B: 23-48.

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Neutrophils PNH Clone	Present	Clone Present

Your Performance

Cell Population	Performance Status for this Trial	Performance Status Classification Over 12 Sample Period	
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Monocytes PNH Clone	Satisfactory	12	0
Neutrophils PNH Clone	Satisfactory	12	0

N/A = Not Applicable

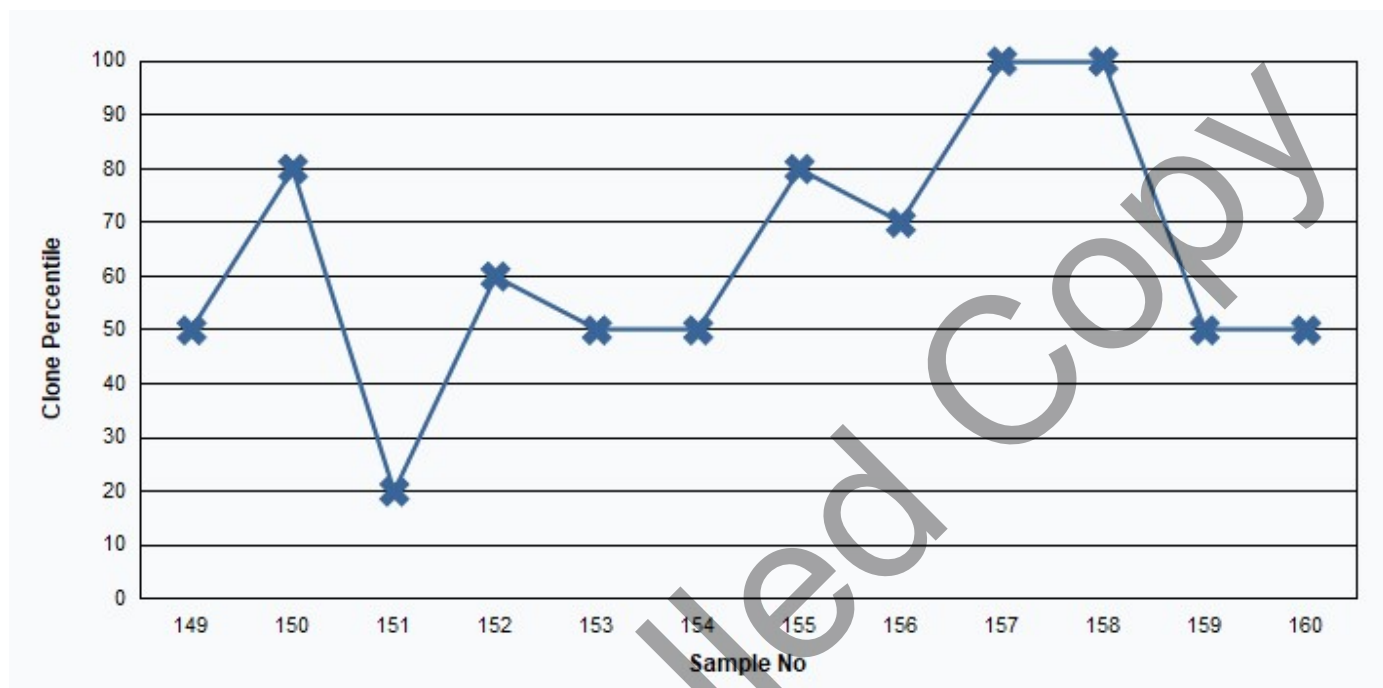
Percentage Value Results

Cell Population	Your Results (%)	Median Result (%)	Lower Quartile (%)	Upper Quartile (%)
Red Blood Cells PNH Clone	0.94	0.96	0.80	1.12
Monocytes PNH Clone	2.10	1.66	1.29	2.10
Neutrophils PNH Clone	1.10	1.09	1.00	1.15

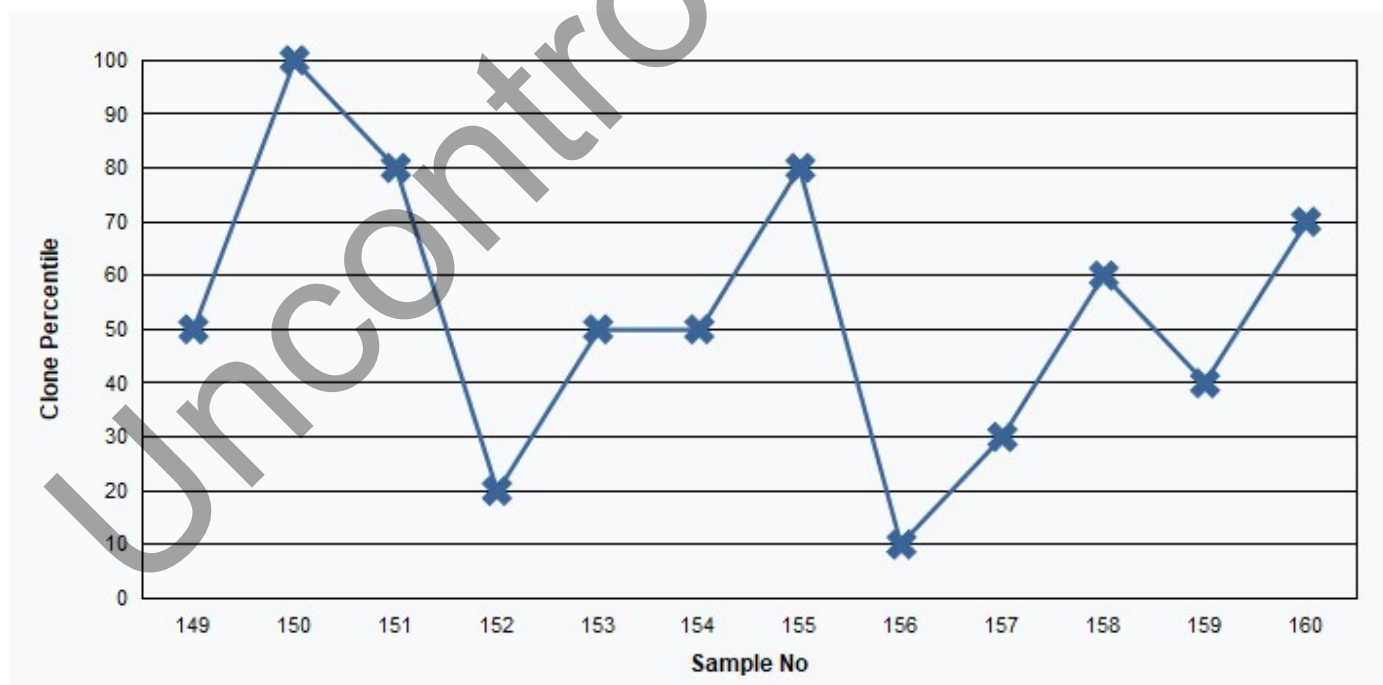
Paroxysmal Nocturnal Haemoglobinuria Programme

Centile graph of Percentage Results

Red Blood Cells PNH Clone Percentage Population over last 12 months

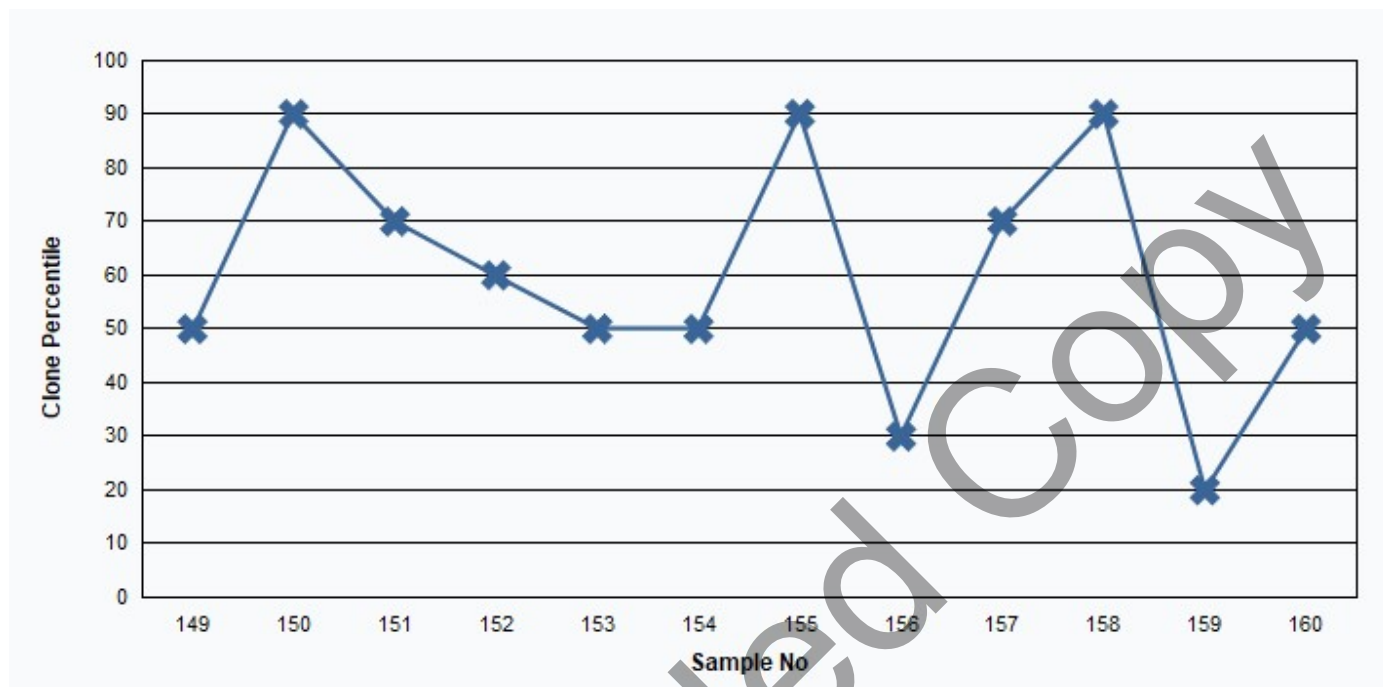


Monocytes PNH Clone Percentage Population over last 12 months



Paroxysmal Nocturnal Haemoglobinuria Programme

Neutrophils PNH Clone Percentage Population over last 12 months



Paroxysmal Nocturnal Haemoglobinuria Programme

Flow Cytometer Specific Statistics

(Please note only groups of >20 returns are displayed)

Red Blood Cell PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	28	28	0	0.94	0.77	1.03
Navios	34	34	0	1.00	0.80	1.27
FACSCanto II	49	48	1	1.00	0.84	1.10

Monocytes PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	27	27	0	1.40	1.24	1.76
Navios	37	36	1	1.59	1.30	2.49
FACSCanto II	51	49	2	1.80	1.40	2.25

Neutrophils PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
FACSLytic	33	33	0	1.07	1.00	1.10
Navios	38	38	0	1.09	1.00	1.19
FACSCanto II	57	56	1	1.10	1.00	1.19

Paroxysmal Nocturnal Haemoglobinuria Programme

Gating Antibodies Used Statistics

(Please note only groups of >20 returns are displayed)

Red Blood Cell PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD235a	111	110	1	1.00	0.83	1.14

Monocytes PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD33/CD45	30	30	0	1.88	1.33	2.89
CD45/CD64	68	66	2	1.62	1.34	1.86

Neutrophils PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD15	23	23	0	1.10	1.00	1.16
CD15/CD45	83	83	0	1.10	1.01	1.17

Paroxysmal Nocturnal Haemoglobinuria Programme

GPI Linked Antibodies Used Statistics

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Red Blood Cell PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD59	118	117	1	0.98	0.80	1.12

Monocytes PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD157/FLAER	25	25	0	1.75	1.58	1.88
CD14/FLAER	78	76	2	1.61	1.19	2.18

Neutrophils PNH Clone

GPI Linked Antibodies Combination Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD157/FLAER	26	26	0	1.09	1.02	1.11
CD24/FLAER	87	86	1	1.08	1.00	1.16

Paroxysmal Nocturnal Haemoglobinuria Programme

GPI Linked Antibodies Specific Statistics

Red Blood Cell PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
^CD55	12	0	12
CD59	130	1	131

se note the numbers in the above tables will not match the number of laboratories enrolled in the programme as each centre uses a panel with multiple antibodies

Monocytes PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
CD14	96	2	98
CD157	40	0	40
CD24	6	1	7
^CD55	7	0	7
^CD59	4	0	4
FLAER	127	3	130

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Neutrophils PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
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CD16	19	0	19
CD24	108	1	109
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^CD59	5	0	5
^CD66b	7	0	7
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Gating	CD45	2D1	BD Biosciences	PerCP	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Neutrophils

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD15	N/A	BD Biosciences	AlexaFluor 647	No
Gating	CD45	2D1	BD Biosciences	PerCP	No
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Recommended Reading

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Paroxysmal Nocturnal Haemoglobinuria 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 coordinators of UK NEQAS LI programmes are Mr Liam Whitby and Mr Stuart Scott.

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.

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/