UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Paroxysmal Nocturnal Haemoglobinuria Programme

Distribution - 252602 Sample - 195 Participant ID

Date Issued - 19 August 2025 Closing Date - 16 September 2025

Trial Comments

This exercise was issued to 175 participants of which 171 (97.7%) returned results at the time of report generation. Of the non-returning centres there was one pre notified non-return. One participant (0.58%) erroneously did not detect a neutrophil PNH clone for sample 195. Two participants (1.17%) erroneously did not detect a neutrophil PNH clone for sample 196. There were no methodological correlations between these laboratories

Sample Comments

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

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

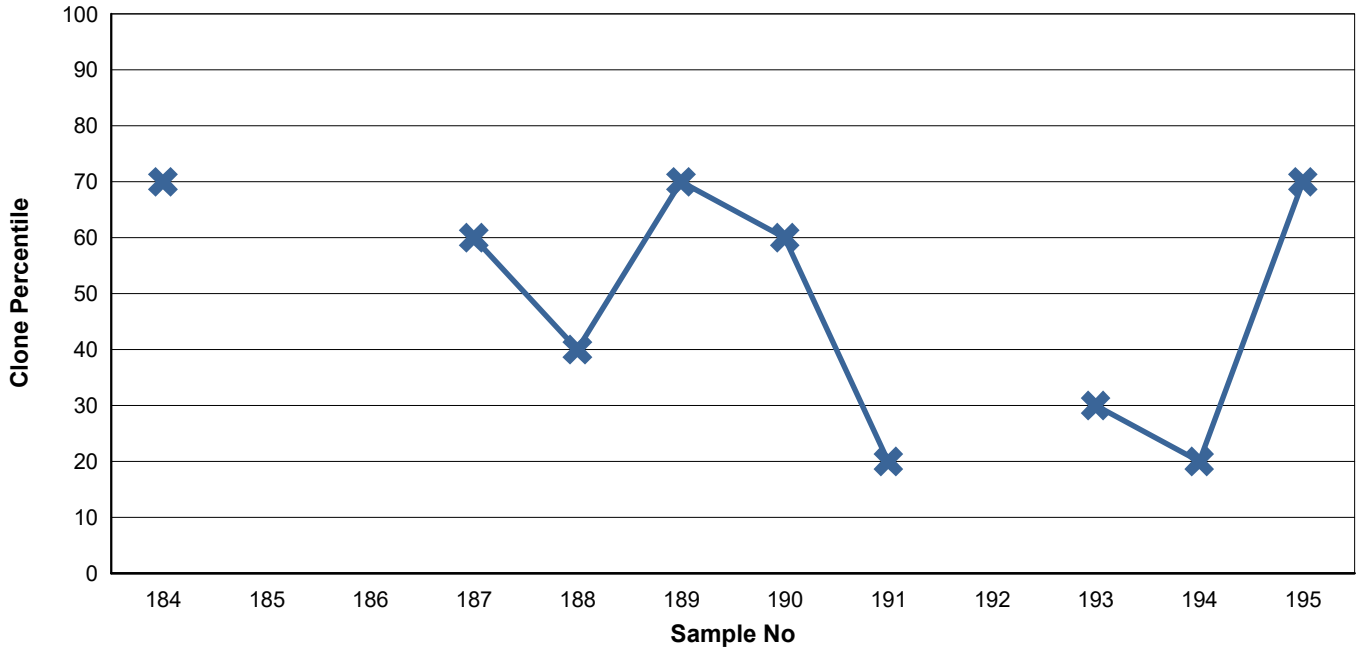
Percentage Value Results

Cell Population	Your Results (%)	Median Result (%)	Lower Quartile (%)	Upper Quartile (%)
Red Blood Cells PNH Clone	9.46	8.75	8.19	9.55
Monocytes PNH Clone	6.71	7.32	6.70	8.14
Neutrophils PNH Clone	14.67	10.70	10.20	11.53

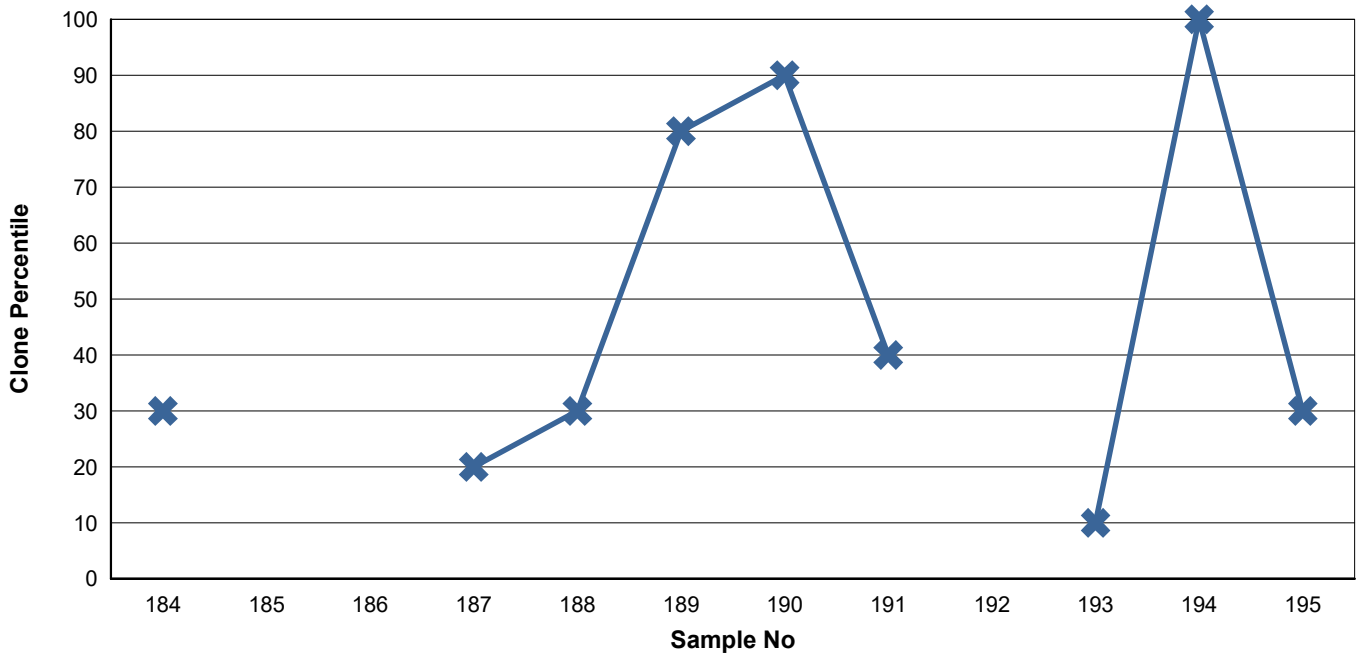
Paroxysmal Nocturnal Haemoglobinuria Programme

Centile graph of Percentage Results

Red Blood Cells PNH Clone Percentage Population over last 12 months

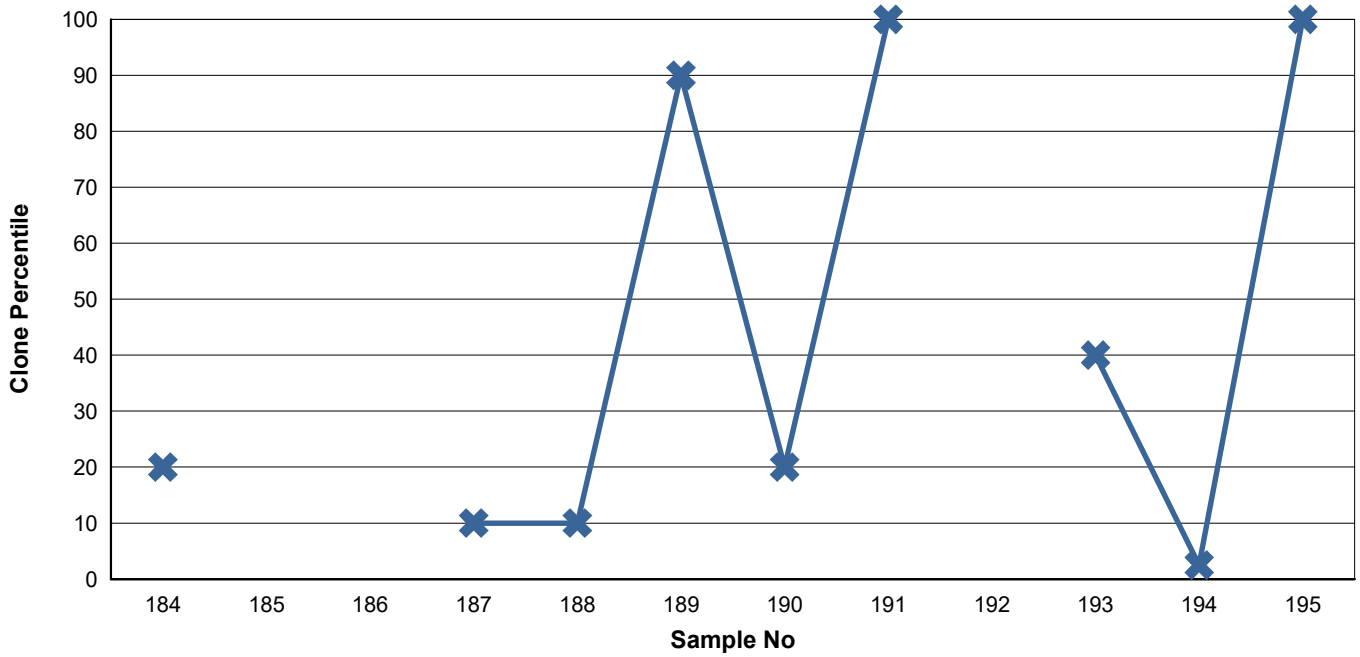


Monocytes PNH Clone Percentage Population over last 12 months



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 (%)
Navios	21	21	0	9.54	8.63	9.90
DxFLEX	22	22	0	9.30	8.67	12.36
FACSCanto II	38	38	0	8.72	8.00	9.22
FACSLyric	57	57	0	8.65	7.94	9.17

Monocytes PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
Navios	23	23	0	7.50	6.76	8.24
DxFLEX	26	26	0	7.30	6.26	8.15
FACSCanto II	40	40	0	7.49	6.81	8.50
FACSLyric	58	58	0	7.22	6.40	7.87

Neutrophils PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
Navios	23	23	0	10.73	10.11	12.25
DxFLEX	26	26	0	10.45	9.97	11.20
FACSCanto II	43	43	0	10.97	10.56	11.71
FACSLyric	62	62	0	10.70	10.26	11.24

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	131	131	0	8.79	8.24	9.56

Monocytes PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD33/CD45	29	29	0	8.00	7.20	8.70
CD45/CD64	94	94	0	7.25	6.53	7.85

Neutrophils PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD15	21	21	0	10.70	10.30	11.65
CD15/CD45	108	108	0	10.70	10.20	11.22

Paroxysmal Nocturnal Haemoglobinuria Programme

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	133	133	0	8.73	8.14	9.46

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	35	35	0	7.30	6.37	7.83
CD14/FLAER	81	81	0	7.25	6.70	8.10

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	33	33	0	10.80	10.15	11.54
CD24/FLAER	87	87	0	10.71	10.21	11.35

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	15	0	15
CD59	149	0	149

*** Please 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	112	0	112
CD157	57	0	57
CD24	7	0	7
^CD55	4	0	4
^CD59	3	0	3
CD66b	1	0	1
FLAER	152	0	152

*** Please 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**

Neutrophils PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
CD157	53	1	54
CD16	20	0	20
CD24	116	0	116
^CD55	6	0	6
^CD59	3	0	3
^CD66b	8	0	8
FLAER	157	1	158

*** Please 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**

^ Antigens marked above, in the GPI linked antibodies specific statistics table, are not considered best practice and laboratories should avoid using these.
Please see 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-SensitivityTesting. Cytometry Part B 2018; 94B: 23-48 for further explanation.

Paroxysmal Nocturnal Haemoglobinuria Programme

Methodology

Red Blood Cells

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD235a	KC16	Beckman Coulter	FITC	Yes
Testing	CD59	MEM-43	Life Technologies	PE	Yes

Monocytes

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD45	J33	Beckman Coulter	KO	Yes
Gating	CD15	HI98	BD Biosciences	APC	No
Gating	CD64	10.1	BD Biosciences	BV421	No
Testing	CD14	MoP9	BD Biosciences	APC-H7	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Neutrophils

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD45	J33	Beckman Coulter	KO	Yes
Testing	CD24	ALB9	Beckman Coulter	PE	Yes
Gating	CD15	HI98	BD Biosciences	APC	No
Gating	CD64	10.1	BD Biosciences	BV421	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Recommended Reading

Please see 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

Paroxysmal Nocturnal Haemoglobinuria Programme

Distribution - 252602 Sample - 196 Participant ID -

Date Issued - 19 August 2025 Closing Date - 16 September 2025

Trial Comments

This exercise was issued to 175 participants of which 171 (97.7%) returned results at the time of report generation. Of the non-returning centres there was one pre notified non-return. One participant (0.58%) erroneously did not detect a neutrophil PNH clone for sample 195. Two participants (1.17%) erroneously did not detect a neutrophil PNH clone for sample 196. There were no methodological correlations between these laboratories.

Sample Comments

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

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

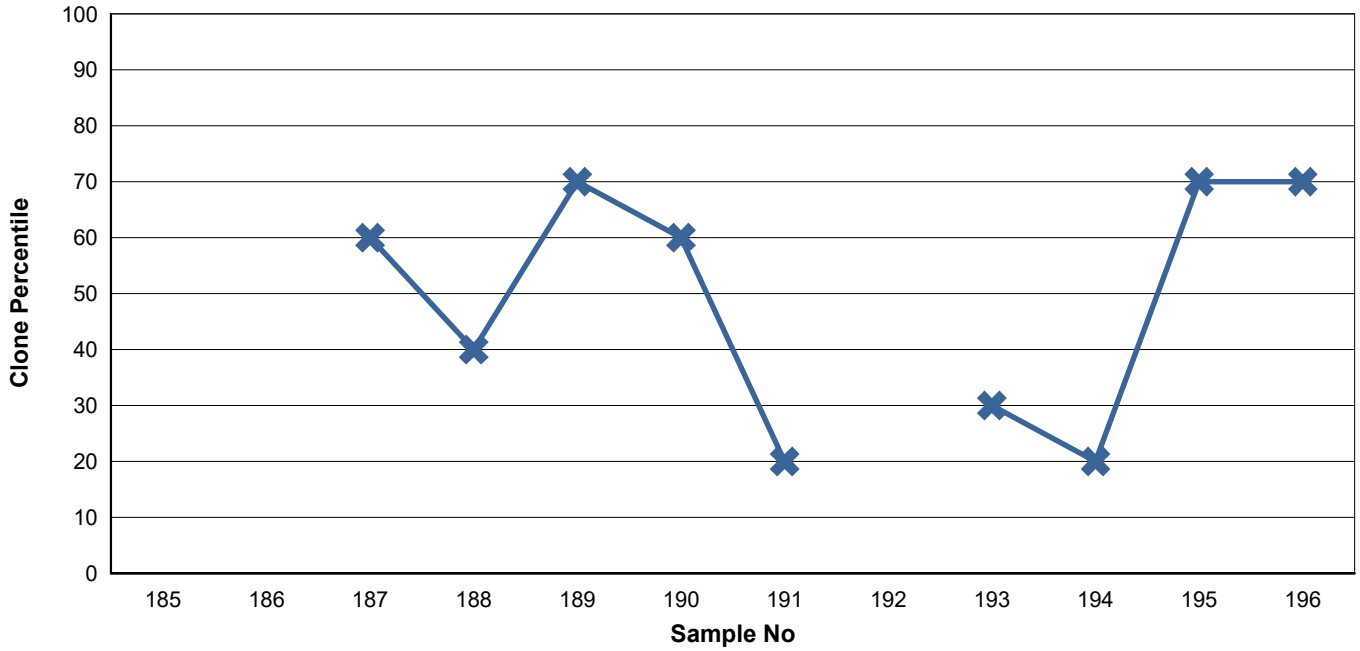
Percentage Value Results

Cell Population	Your Results (%)	Median Result (%)	Lower Quartile (%)	Upper Quartile (%)
Red Blood Cells PNH Clone	1.25	1.16	1.02	1.38
Monocytes PNH Clone	7.00	4.50	3.14	5.50
Neutrophils PNH Clone	2.58	3.01	2.70	3.30

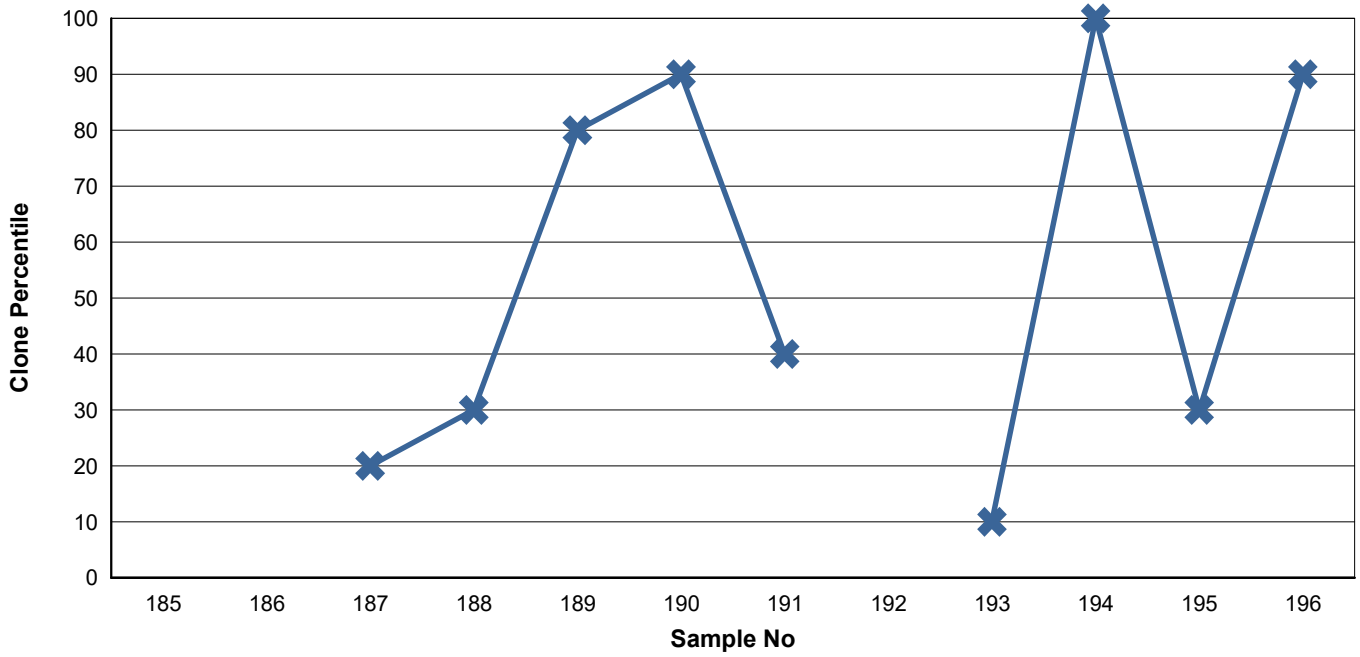
Paroxysmal Nocturnal Haemoglobinuria Programme

Centile graph of Percentage Results

Red Blood Cells PNH Clone Percentage Population over last 12 months

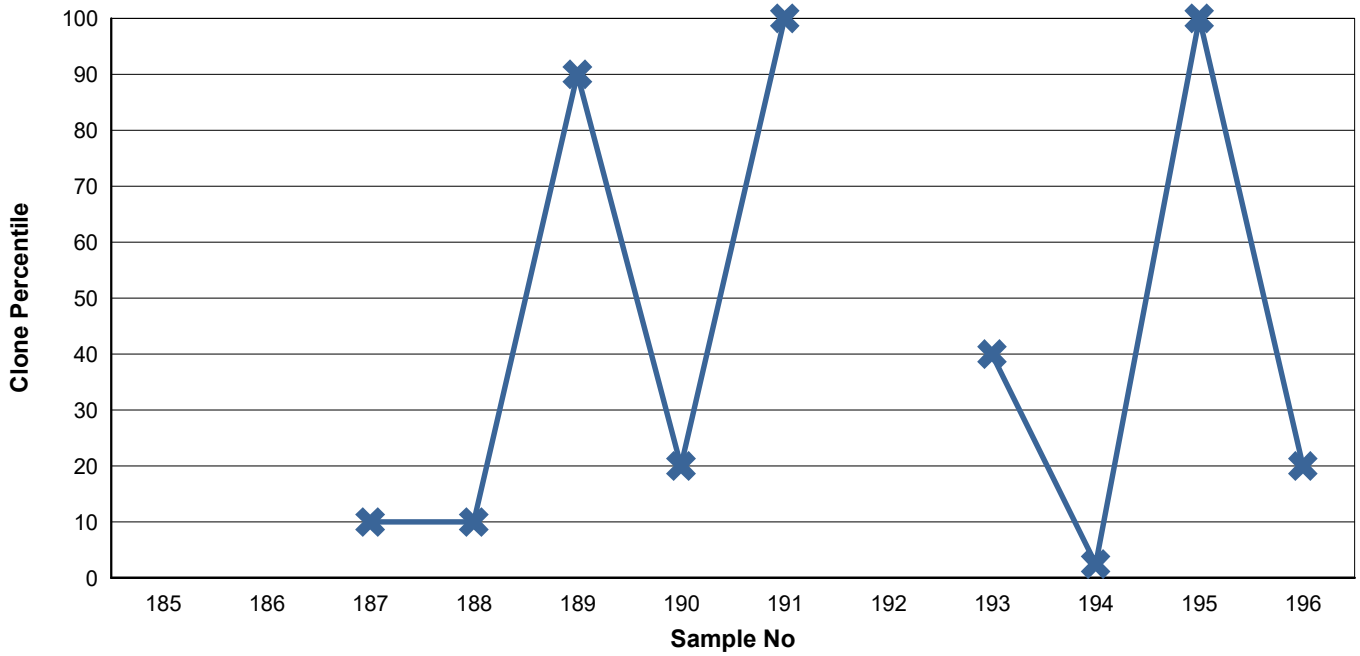


Monocytes PNH Clone Percentage Population over last 12 months



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 (%)
Navios	21	21	0	1.20	1.10	1.45
DxFLEX	22	22	0	1.20	1.03	1.93
FACSCanto II	38	37	1	1.21	1.15	1.39
FACSLyric	57	56	1	1.09	1.00	1.20

Monocytes PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
Navios	23	23	0	4.34	3.50	5.22
DxFLEX	26	26	0	4.45	2.32	5.67
FACSCanto II	40	40	0	4.20	3.00	5.40
FACSLyric	58	58	0	4.67	3.16	5.43

Neutrophils PNH Clone

Flow Cytometer	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
Navios	23	23	0	3.00	2.59	3.33
DxFLEX	26	26	0	3.14	2.81	3.32
FACSCanto II	43	43	0	2.99	2.70	3.32
FACSLyric	62	62	0	3.01	2.75	3.22

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	131	130	1	1.16	1.03	1.36

Monocytes PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD33/CD45	29	29	0	3.24	2.31	4.00
CD45/CD64	94	94	0	4.88	4.00	5.65

Neutrophils PNH Clone

Gating Strategy Used	Returns	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Median Clone Size (%)	Lower Quartile (%)	Upper Quartile (%)
CD15	21	21	0	3.00	2.90	3.17
CD15/CD45	108	108	0	3.07	2.73	3.30

Paroxysmal Nocturnal Haemoglobinuria Programme

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	133	132	1	1.17	1.02	1.37

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	35	35	0	4.80	3.80	5.69
CD14/FLAER	81	81	0	4.39	3.00	5.06

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	33	33	0	3.00	2.74	3.36
CD24/FLAER	87	87	0	3.01	2.70	3.27

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	14	1	15
CD59	147	2	149

*** Please 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	112	0	112
CD157	57	0	57
CD24	7	0	7
^CD55	4	0	4
^CD59	3	0	3
CD66b	1	0	1
FLAER	152	0	152

*** Please 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**

Neutrophils PNH Clone

GPI Linked Antibody	Laboratories Reporting Clone Present	Laboratories Reporting Clone Absent	Laboratories using as part of their panel*
CD157	53	1	54
CD16	20	0	20
CD24	116	0	116
^CD55	5	1	6
^CD59	3	0	3
^CD66b	7	1	8
FLAER	156	2	158

*** Please 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**

^ Antigens marked above, in the GPI linked antibodies specific statistics table, are not considered best practice and laboratories should avoid using these.
Please see 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-SensitivityTesting. Cytometry Part B 2018; 94B: 23-48 for further explanation.

Paroxysmal Nocturnal Haemoglobinuria Programme

Methodology

Red Blood Cells

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD235a	KC16	Beckman Coulter	FITC	Yes
Testing	CD59	MEM-43	Life Technologies	PE	Yes

Monocytes

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD45	J33	Beckman Coulter	KO	Yes
Gating	CD15	HI98	BD Biosciences	APC	No
Gating	CD64	10.1	BD Biosciences	BV421	No
Testing	CD14	MoP9	BD Biosciences	APC-H7	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Neutrophils

Reagents	Antibody	Clone	Manufacturer	Fluorochrome	Best Practice
Gating	CD45	J33	Beckman Coulter	KO	Yes
Testing	CD24	ALB9	Beckman Coulter	PE	Yes
Gating	CD15	HI98	BD Biosciences	APC	No
Gating	CD64	10.1	BD Biosciences	BV421	No
Testing	FLAER	N/A	Cedarlane/Pinewood Scientific	AlexaFluor 488	Yes

Recommended Reading

Please see 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

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 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 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 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: here
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 operating 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 authorised 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