UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Measurable Residual Disease for ALL by Flow Cytometry Programme

All Participant Report

Distribution - 242501 | Sample - 134 | Participant ID -

Date Issued - 07 May 2024 | Closing Date - 28 May 2024 | Machine Used - FACSLyric

Trial Comments

This exercise was issued to 178 participants of which 152 (85.4%) returned results at the time of report generation. Of the non returning centres, 12 had requested an extension to the exercise deadline and there were 2 pre notified non returns.

As part of our transition to the ISO/IEC 17043:2023 standards, all quantitative reports now include a box showing the Uncertainty of the Assigned Value (Robust Mean) next to the Robust SD.

Sample Comments

The sample was manufactured by UK NEQAS using a B-ALL patient sample and a stabilised whole blood unit

Results and Performance

Percentage MRD Population	Your Results (%)	Robust Mean (%)	Robust SD (%)	Uncertainty of the Assigned Value (Robust Mean)
	0.0350	0.0366	0.0095	± 0.00

Percentage MRD Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
	-0.17	Satisfactory	11	0	1

***z Score Limits Definitions**

Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.

	Your Result	Consensus*
Interpretation	MRD Detected Below Quantifiable Limits	MRD Present
Total Denominator Events	181,316	490,383
Total Number of MRD Events	64	178
Percentage MRD	0.0350	0.0366
Total Number of Events Acquired	205,531	686,760

* consensus data shown are median values for events acquired and robust mean for percentage MRD

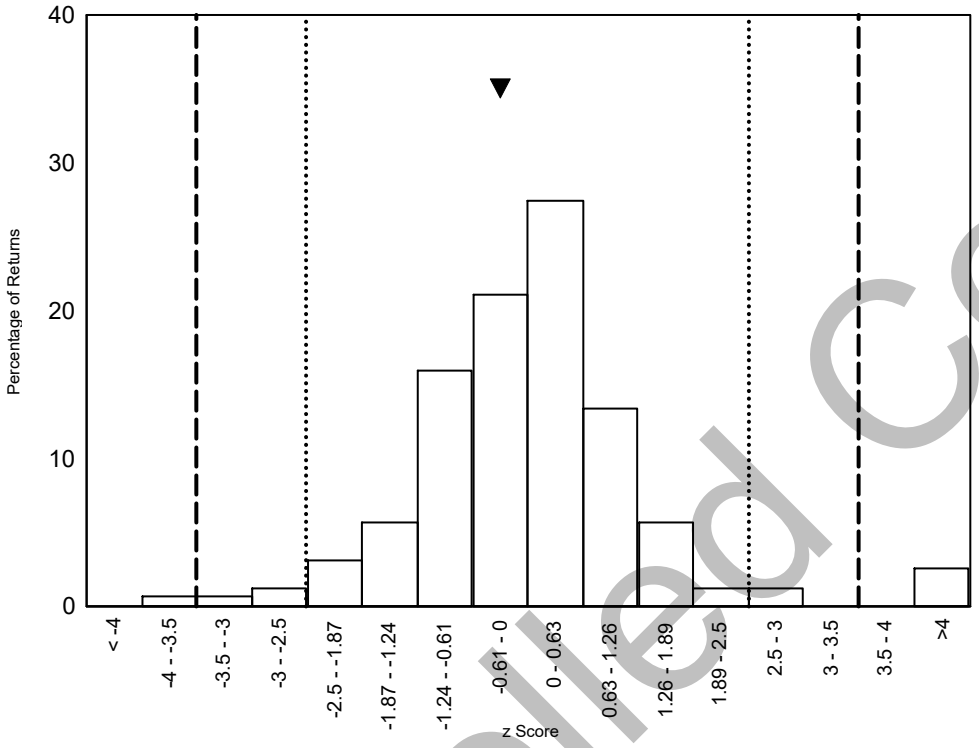
	Your Technique	Returns (n)
Lysis Method	Stain Lyse Wash	92
Doublets Excluded	Yes	126
Number of MRD Events to Define a Population	20	59
Denominator Used	All BM Nucleated Cells	80
Stated Limit of Detection of Assay	5.0000%	
Stated Limit of Quantification of Assay	%	
Calculated Limit of Detection Based on Events Collected	0.0110%	

Measurable Residual Disease for ALL by Flow Cytometry Programme

Histograms of Participant z Scores

Percentage MRD Population -

Please note ▼ denotes your result

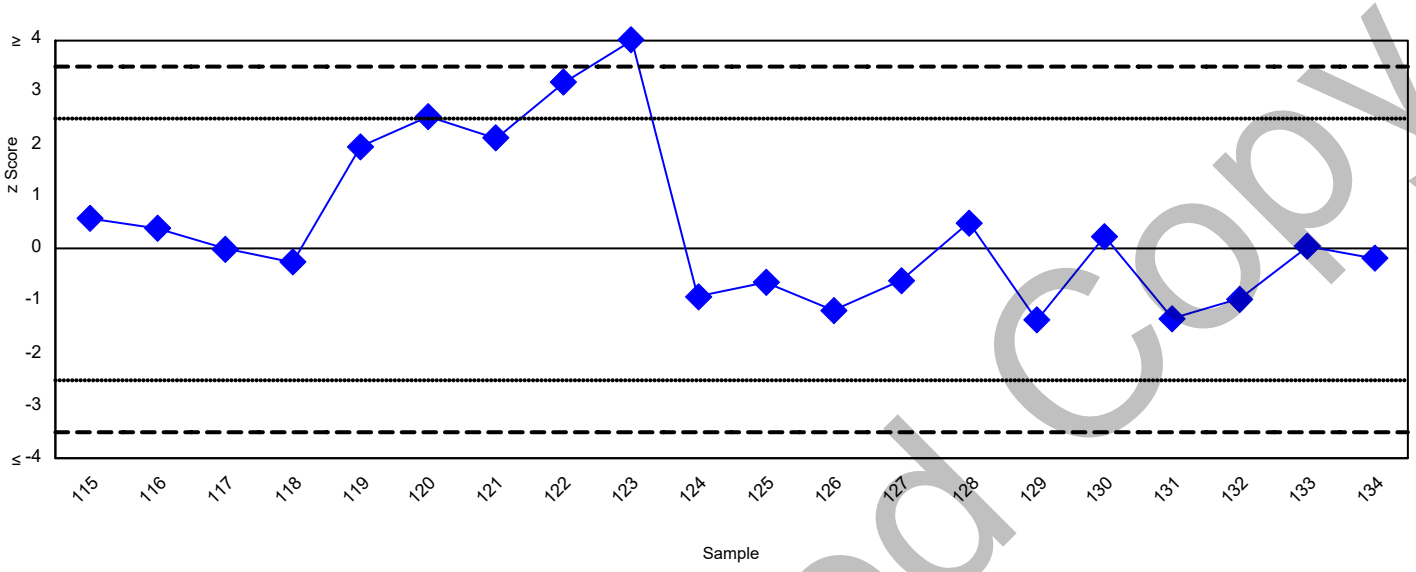


Measurable Residual Disease for ALL by Flow Cytometry Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

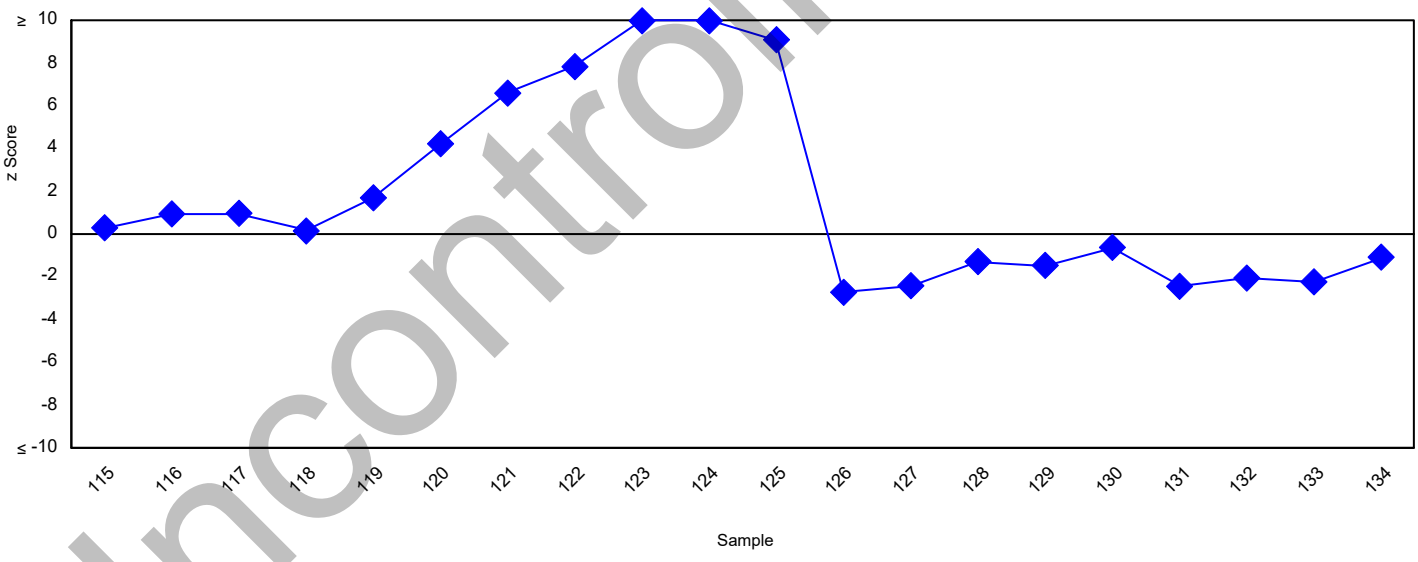
Values (Percentage MRD Population)



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Values (Percentage MRD Population)



Measurable Residual Disease for ALL by Flow Cytometry Programme

Flow Cytometer Specific Statistics

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
DxFLEX	12	0.0399	0.0095	0.0392	0.0348	0.0463
FACSCanto II	38	0.0379	0.0084	0.0389	0.0309	0.0433
FACSLyric	56	0.0369	0.0079	0.0365	0.0318	0.0413
Navios	33	0.0360	0.0109	0.0360	0.0290	0.0445

MRD Group Specific Statistics

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
IBFM	27	0.0344	0.0084	0.0348	0.0290	0.0409
Non-Affiliated	83	0.0377	0.0110	0.0383	0.0300	0.0450
NOPHO	17	0.0399	0.0022	0.0400	0.0388	0.0405
Other	23	0.0352	0.0063	0.0350	0.0310	0.0400

Technique Specific Statistics

Lysis Technique

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
Lyse Stain Wash	52	0.0353	0.0096	0.0350	0.0300	0.0413
Stain Lyse Wash	94	0.0377	0.0090	0.0380	0.0320	0.0430

Doublet Exclusion

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
No	26	0.0332	0.0045	0.0326	0.0300	0.0373
Yes	126	0.0374	0.0095	0.0384	0.0300	0.0440

Denominator

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
All BM Nucleated Cells	82	0.0359	0.0085	0.0358	0.0300	0.0410
Total Leucocytes	62	0.0379	0.0096	0.0390	0.0300	0.0438

Measurable Residual Disease for ALL by Flow Cytometry Programme

Reported Staining Intensity (numbers differ from antibody usage table as not all centres submitted full results)

Antigen	Absent		Weak		Strong		Total
	n	%	n	%	n	%	
CD10	16	11.4	33	23.4	70	49.7	141
CD19	1	0.7	17	12.5	96	70.6	136
CD34	83	62.4	25	18.8	5	3.8	133
CD45	2	1.6	90	70.3	16	12.5	128
CD38	2	1.6	19	15.1	86	68.3	126
CD20	86	68.8	14	11.2	5	4.0	125
CD58	4	6.2	10	15.4	41	63.1	65
CD81	2	3.1	19	29.7	33	51.6	64
CD123	6	12.0	7	14.0	28	56.0	50
CD66c	17	37.0	5	10.9	14	30.4	46
CD22	3	8.1	23	62.2	7	18.9	37
CD73	9	32.1	8	28.6	4	14.3	28
CD304	7	31.8	5	22.7	3	13.6	22
CD24	8	38.1	10	47.6	2	9.5	21
CD33	7	36.8	6	31.6	4	21.1	19
CD66c/CD123	2	11.8	3	17.7	12	70.6	17
CD73/CD304	3	27.3	4	36.4	4	36.4	11
CD13	5	45.5	2	18.2	2	18.2	11
CD3	8	80.0	0	0.0	0	0.0	10

Measurable Residual Disease for ALL by Flow Cytometry Programme

Table showing the breakdown of participant returns according to antibody manufacturer

N.B. To allow for concise reports only antigens tested by >=25% of participants and with manufacturer group >20 users across all reagents are shown. Please note, numbers in all tables may not equal the number of participants due to not all participants providing all information or to some participants performing multiple combinations of techniques. The 'Total Results' column reflects all results returned, as small user groups are not shown on the table individual row totals may differ from that shown in 'Total Results'.

Antigen	Result	Intensity	Total Results (All Data)	BD Biosciences	Beckman Coulter	Cytognos	BioLegend	exbio	Miltenyi Biotec	Pharmingen	Cytek Bioscience	Other	Immunotech	invitrogen	eBioscience	Life Technologies	Dako	Immunostep
CD10	Negative (-)	Absent	16	10	3	3												
	Positive (+)	Weak	33	14	11	1	3	1			1		1	1				
		Strong	70	41	14	8	1	3				1					2	
CD19	Negative (-)	Absent	1		1													
	Positive (+)	Weak	17	7	5	1	3						1					
		Strong	96	31	44	11	3	3	2		1	1						
CD34	Negative (-)	Absent	83	45	21	7	1	4			1	1	1	1				
		Weak	1	1														
	Positive (+)	Weak	24	18	1	3	1	1										
		Strong	5	2	1	2												
CD45	Negative (-)	Absent	2	1												1		
	Positive (+)	Weak	90	44	22	11	4	3			1		1	2		2		
		Strong	16	6	3	1	2							1		1		2
CD38	Negative (-)	Absent	2		1	1												
	Positive (+)	Weak	19	11	2	3				2			1					
		Strong	86	46	23	9	4	2			1	1						
CD20	Negative (-)	Absent	85	40	20	9	9	3			1	1			1		1	
		Weak	1	1														
		Strong	1						1									
	Positive (+)	Absent	1				1											
		Weak	13	6		2	5											
		Strong	4	2	1	1												
CD58	Negative (-)	Absent	4	2	1										1			
	Positive (+)	Weak	10	6	3					1								
		Strong	41	13	18		1	1		5	1	1			1			
CD123	Negative (-)	Absent	6	2	2	1			1									
	Positive (+)	Weak	7	5			1		1									
		Strong	28	15		1			10		1	1						
CD22	Negative (-)	Absent	3		1			1			1							
	Positive (+)	Weak	23	10	6		1	1	1			1		1	2			
		Strong	7	4	2		1											
CD66c	Negative (-)	Absent	17	5	8	1				1	1		1					
	Positive (+)	Weak	5	2	1					2								
		Strong	14	2	10	1						1						
CD81	Negative (-)	Absent	2	1									1					
	Positive (+)	Weak	19	11		4	1	2		1								
		Strong	33	15	5	8		1		3	1							

Measurable Residual Disease for ALL by Flow Cytometry Programme

Table showing the breakdown of participant returns according to antibody fluorochrome

N.B. To allow for concise reports only antigens tested by >=25% of participants and with groups >20 users across all reagents are shown. Please note, numbers in all tables may not equal the number of participants due to not all participants providing all information or to some participants performing multiple combinations of techniques. The 'Total Results' column reflects all results returned, as small user groups are not shown on the table individual row totals may differ from that shown in 'Total Results'.

Antigen	Result	Intensity	Total Results (All Data)	PE	APC	FITC	PE-CY7	PerCP-CY5.5	APC-H7	Pacific Blue	V450	PC7	V500	ECD	PerCP-CY5	APC-Alexa 750	BV421	V500-C
CD10	Negative (-)	Absent	16	1	10	1	2											
	Positive (+)	Weak	33	3	14		1		1			1		1		1	2	
		Strong	70	13	31	1	6					2		2			3	
CD19	Negative (-)	Absent	1									1						
	Positive (+)	Weak	17	1	1		7	1				2			2		1	
		Strong	96		13		55	2				12		5		2	2	
CD34	Negative (-)	Absent	83	5	7	1	9	29			1	3		5	8	1		
		Weak	1	1														
	Positive (+)	Weak	24	1	2			15						1	4			
		Strong	5				2	2										
CD45	Negative (-)	Absent	2															
	Positive (+)	Weak	90		1		1	2	8		1	1	15	2	1			13
		Strong	16						2				3	1				1
CD38	Negative (-)	Absent	2													1		
	Positive (+)	Weak	19					3	6	1						2		
		Strong	86	3	2	8	1	3	28	1	3				1	8	1	
CD20	Negative (-)	Absent	85		1	10	2	3	2	25	21		1	1	1	2	2	2
		Weak	1															
		Strong	1															
	Positive (+)	Absent	1							1								
		Weak	13						1	7	4							
		Strong	4						1	2								
CD58	Negative (-)	Absent	4		1	3												
	Positive (+)	Weak	10	1	2	7												
		Strong	41	7	4	25	1										1	
CD123	Negative (-)	Absent	6	2			1					1						
	Positive (+)	Weak	7	5	1												1	
		Strong	28	19	1										1		1	
CD22	Negative (-)	Absent	3	1														
	Positive (+)	Weak	23	9	6									1			1	
		Strong	7	2	2	1											1	
CD66c	Negative (-)	Absent	17	12		3												
	Positive (+)	Weak	5	5														
		Strong	14	12		1												
CD81	Negative (-)	Absent	2		1	1												
	Positive (+)	Weak	19		1	12			3									
		Strong	33	1	3	25			1	1								

Measurable Residual Disease for ALL by Flow Cytometry Programme

Distribution - 242501 | Sample - 135 | Participant ID -

Date Issued - 07 May 2024 | Closing Date - 28 May 2024 | Machine Used - FACSLyric

Trial Comments

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As part of our transition to the ISO/IEC 17043:2023 standards, all quantitative reports now include a box showing the Uncertainty of the Assigned Value (Robust Mean) next to the Robust SD.

Sample Comments

The sample was manufactured by UK NEQAS using a B-ALL patient sample and a stabilised whole blood unit

Results and Performance

Percentage MRD Population	Your Results (%)	Robust Mean (%)	Robust SD (%)	Uncertainty of the Assigned Value (Robust Mean)
	0.1340	0.1402	0.0299	± 0.00

Percentage MRD Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
	-0.21	Satisfactory	12	0	0

***z Score Limits Definitions**

Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.

	Your Result	Consensus*
Interpretation	MRD Detected Below Quantifiable Limits	MRD Present
Total Denominator Events	178,041	437,707
Total Number of MRD Events	239	642
Percentage MRD	0.1340	0.1402
Total Number of Events Acquired	204,433	627,946

* consensus data shown are median values for events acquired and robust mean for percentage MRD

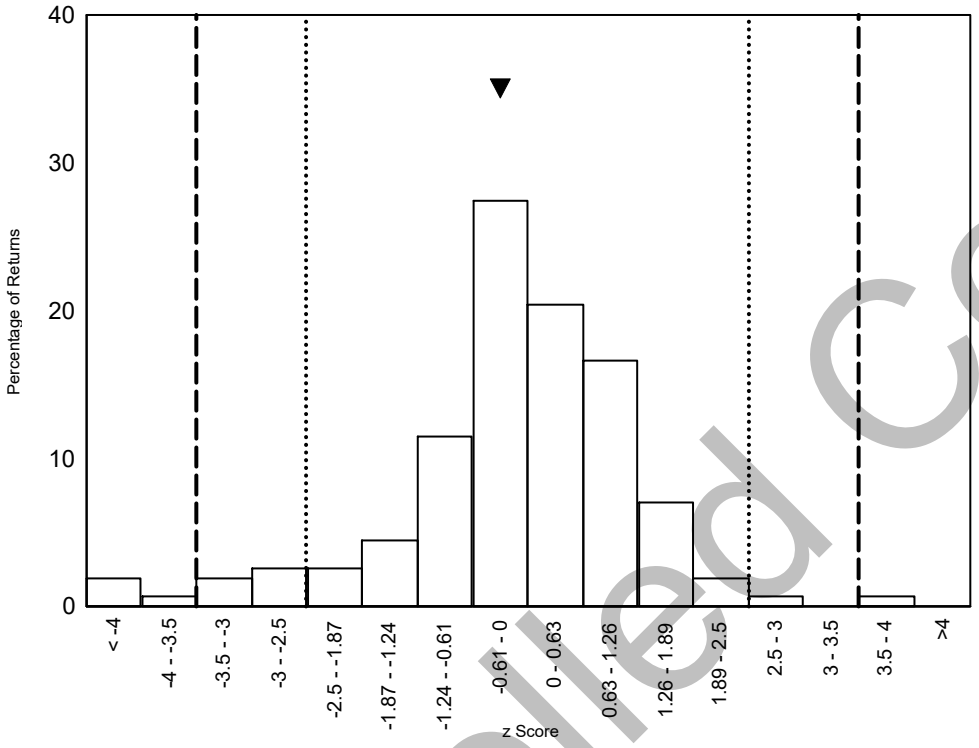
	Your Technique	Returns (n)
Lysis Method	Stain Lyse Wash	90
Doublets Excluded	Yes	126
Number of MRD Events to Define a Population	20	59
Denominator Used	All BM Nucleated Cells	80
Stated Limit of Detection of Assay	5.0000%	
Stated Limit of Quantification of Assay	%	
Calculated Limit of Detection Based on Events Collected	0.0112%	

Measurable Residual Disease for ALL by Flow Cytometry Programme

Histograms of Participant z Scores

Percentage MRD Population -

Please note ▼ denotes your result

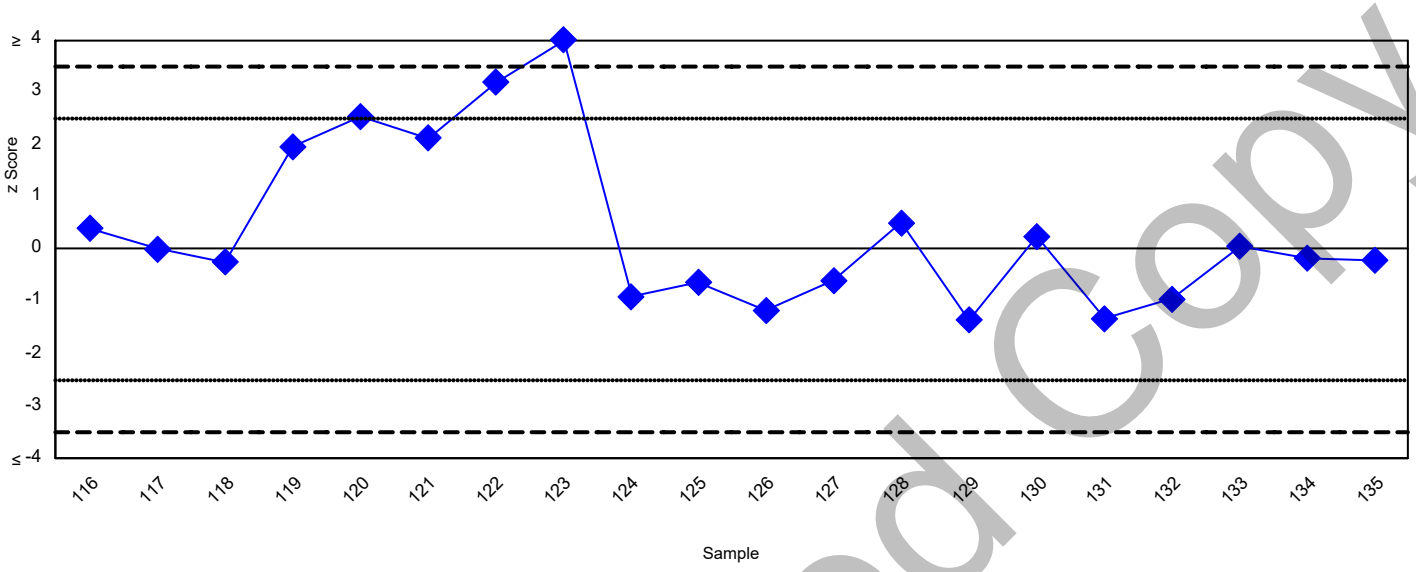


Measurable Residual Disease for ALL by Flow Cytometry Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

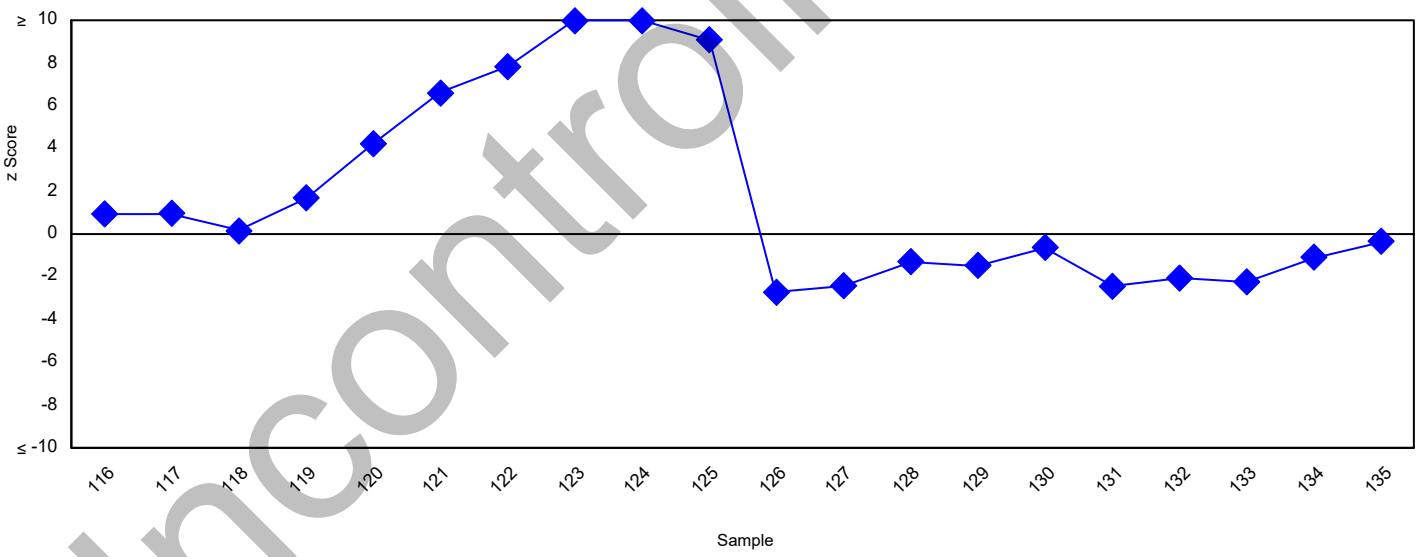
Values (Percentage MRD Population)



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Values (Percentage MRD Population)



Measurable Residual Disease for ALL by Flow Cytometry Programme

Flow Cytometer Specific Statistics	Percentage MRD					
Method	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
DxFLEX	12	0.1452	0.0180	0.1465	0.1355	0.1576
FACSCanto II	38	0.1403	0.0255	0.1400	0.1240	0.1544
FACSLyric	55	0.1431	0.0274	0.1490	0.1227	0.1600
Navios	32	0.1407	0.0373	0.1386	0.1193	0.1628

MRD Group Specific Statistics	Percentage MRD					
Method	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
IBFM	27	0.1427	0.0253	0.1400	0.1277	0.1585
Non-Affiliated	83	0.1386	0.0300	0.1392	0.1200	0.1595
NOPHO	17	0.1515	0.0341	0.1500	0.1400	0.1700
Other	22	0.1423	0.0257	0.1440	0.1323	0.1600

Technique Specific Statistics

Lysis Technique	Percentage MRD					
Method	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
Lyse Stain Wash	54	0.1362	0.0376	0.1400	0.1100	0.1608
Stain Lyse Wash	92	0.1423	0.0247	0.1400	0.1295	0.1593

Doublet Exclusion	Percentage MRD					
Method	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
No	26	0.1304	0.0250	0.1320	0.1118	0.1438
Yes	126	0.1422	0.0294	0.1420	0.1240	0.1610

Denominator	Percentage MRD					
Method	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
All BM Nucleated Cells	82	0.1375	0.0263	0.1381	0.1200	0.1550
Total Leucocytes	62	0.1448	0.0289	0.1470	0.1285	0.1640

Measurable Residual Disease for ALL by Flow Cytometry Programme

Reported Staining Intensity (numbers differ from antibody usage table as not all centres submitted full results)

Antigen	Absent		Weak		Strong		Total
	n	%	n	%	n	%	
CD10	16	11.3	33	23.2	71	50.0	142
CD19	1	0.7	18	13.1	96	70.1	137
CD34	84	62.7	24	17.9	6	4.5	134
CD45	2	1.6	88	68.2	19	14.7	129
CD38	1	0.8	18	14.2	89	70.1	127
CD20	87	69.1	14	11.1	5	4.0	126
CD58	4	6.0	10	14.9	42	62.7	67
CD81	2	3.1	20	31.3	33	51.6	64
CD123	6	12.0	7	14.0	28	56.0	50
CD66c	17	37.0	5	10.9	14	30.4	46
CD22	3	7.9	24	63.2	6	15.8	38
CD73	9	31.0	8	27.6	5	17.2	29
CD304	7	30.4	5	21.7	4	17.4	23
CD24	8	38.1	10	47.6	2	9.5	21
CD33	7	36.8	6	31.6	4	21.1	19
CD66c/CD123	2	11.8	3	17.7	12	70.6	17
CD73/CD304	3	27.3	4	36.4	4	36.4	11
CD13	5	45.5	2	18.2	2	18.2	11
CD3	8	80.0	0	0.0	0	0.0	10

Measurable Residual Disease for ALL by Flow Cytometry Programme

Table showing the breakdown of participant returns according to antibody manufacturer

N.B. To allow for concise reports only antigens tested by >=25% of participants and with manufacturer group >20 users across all reagents are shown. Please note, numbers in all tables may not equal the number of participants due to not all participants providing all information or to some participants performing multiple combinations of techniques. The 'Total Results' column reflects all results returned, as small user groups are not shown on the table individual row totals may differ from that shown in 'Total Results'.

Antigen	Result	Intensity	Total Results (All Data)	BD Biosciences	Beckman Coulter	Cytognos	BioLegend	exbio	Miltenyi Biotec	Pharmingen	Cytek Bioscience	Other	Immunotech	invitrogen	eBioscience	Life Technologies	Dako	Immunostep
CD10	Negative (-)	Absent	16	10	3	3												
	Positive (+)	Weak	33	14	11	1	3	1			1		1	1				
		Strong	71	42	14	8	1	3				1					2	
CD19	Negative (-)	Absent	1		1													
	Positive (+)	Weak	18	7	5	2	3						1					
		Strong	96	31	45	10	3	3	2		1	1						
CD34	Negative (-)	Absent	84	46	21	7	1	4			1	1	1	1				
		Weak	1	1														
		Strong	1	1														
	Positive (+)	Weak	23	17	1	3	1	1										
		Strong	5	2	1	2												
CD45	Negative (-)	Absent	2	1												1		
	Positive (+)	Weak	88	42	22	11	4	3			1		1	2		2		
		Strong	19	8	3	1	2							1		2		2
CD38	Negative (-)	Absent	1		1													
	Positive (+)	Weak	18	11	1	3				2			1					
		Strong	89	46	25	10	4	2			1	1						
CD20	Negative (-)	Absent	87	40	20	9	11	3			1	1			1		1	
		Weak	1	1														
		Strong	1						1									
	Positive (+)	Weak	13	6		2	5											
		Strong	4	2	1	1												
CD58	Negative (-)	Absent	4	2	1										1			
	Positive (+)	Weak	10	6	3					1								
		Strong	42	13	19		1	1		5	1	1			1			
CD123	Negative (-)	Absent	6	2	2	1			1									
	Positive (+)	Weak	7	5			1		1									
		Strong	28	15					11		1	1						
CD22	Negative (-)	Absent	3		1			1			1							
	Positive (+)	Weak	24	10	6		2	1	1			1		1	2			
		Strong	6	4	2													
CD66c	Negative (-)	Absent	17	5	8	1				1	1		1					
	Positive (+)	Weak	5	2	1					2								
		Strong	14	2	11							1						
CD81	Negative (-)	Absent	2	1									1					
	Positive (+)	Weak	20	12		4	1	2		1								
		Strong	33	15	5	8		1		3	1							

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Table showing the breakdown of participant returns according to antibody fluorochrome

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Antigen	Result	Intensity	Total Results (All Data)	PE	APC	FITC	PE-CY7	PerCP-CY5.5	APC-H7	Pacific Blue	V450	PC7	V500	ECD	PerCP-CY5	APC-Alexa 750	BV421	V500-C
CD10	Negative (-)	Absent	16	1	10	1	2											
	Positive (+)	Weak	33	3	14		1		1			1		1		1	2	
		Strong	71	13	32	1	6					2		2			3	
CD19	Negative (-)	Absent	1									1						
	Positive (+)	Weak	18	1	1		8	1				2			2		1	
		Strong	96		13		54	2				13		5		2	2	
CD34	Negative (-)	Absent	84	5	7	1	9	32			1	3		5	7	1		
		Weak	1	1														
		Strong	1		1													
	Positive (+)	Weak	23	1	1			14						1	5			
		Strong	5				2	2										
CD45	Negative (-)	Absent	2															
	Positive (+)	Weak	88		1	1	1	2	7			1	15	2	1			13
		Strong	19						3		1		3	1				1
CD38	Negative (-)	Absent	1													1		
	Positive (+)	Weak	18					3	6	1						1		
		Strong	89	3	2	7	1	3	28	1	3				1	10	1	
CD20	Negative (-)	Absent	87		1	10	2	2	2	27	21		1	1	2	2	2	2
		Weak	1															
		Strong	1															
	Positive (+)	Weak	13						1	7	4							
		Strong	4						1	2								
CD58	Negative (-)	Absent	4		1	3												
	Positive (+)	Weak	10	1	2	7												
		Strong	42	7	4	26	1										1	
CD123	Negative (-)	Absent	6	2			1					1						
	Positive (+)	Weak	7	5	1												1	
		Strong	28	19	1										1		1	
CD22	Negative (-)	Absent	3	1														
	Positive (+)	Weak	24	9	6									1			2	
		Strong	6	2	2	1												
CD66c	Negative (-)	Absent	17	12		3												
	Positive (+)	Weak	5	5														
		Strong	14	12		1												
CD81	Negative (-)	Absent	2		1	1												
	Positive (+)	Weak	20		1	13			3									
		Strong	33	1	3	25			1	1								

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 (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) No 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/

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/