UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Measurable Residual Disease for ALL by Flow Cytometry Programme

Distribution - 232402 Sample - 130 Participant ID - 4000?

Date Issued - 14 August 2023 Closing Date - 11 September 2023 Machine Used - FACSCanto II

Trial Comments

This exercise was issued to 175 participants of which 152 (87%) returned results at the time of report generation. Of the non returning centres, 10 had requested an extension to the exercise deadline, 12 were non returns and 1 was a pre notified non-return. 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

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

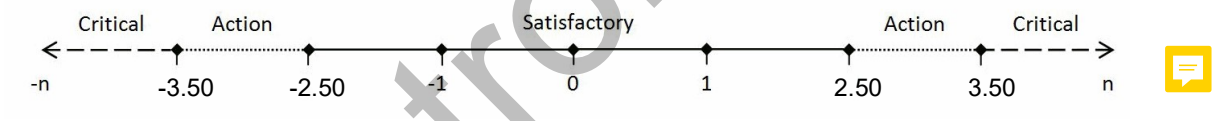
Results and Performance

Percentage MRD Population	Your Results (%)	Robust Mean (%)	Robust SD (%)
	0.0410	0.0382	0.0116

Percentage MRD Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
	0.24	Satisfactory	9	2	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 Present	MRD Present
Total Denominator Events	90,715	431,635
Total Number of MRD Events	37	154
Percentage MRD	0.0410	0.0382
Total Number of Events Acquired	150,290	644,987

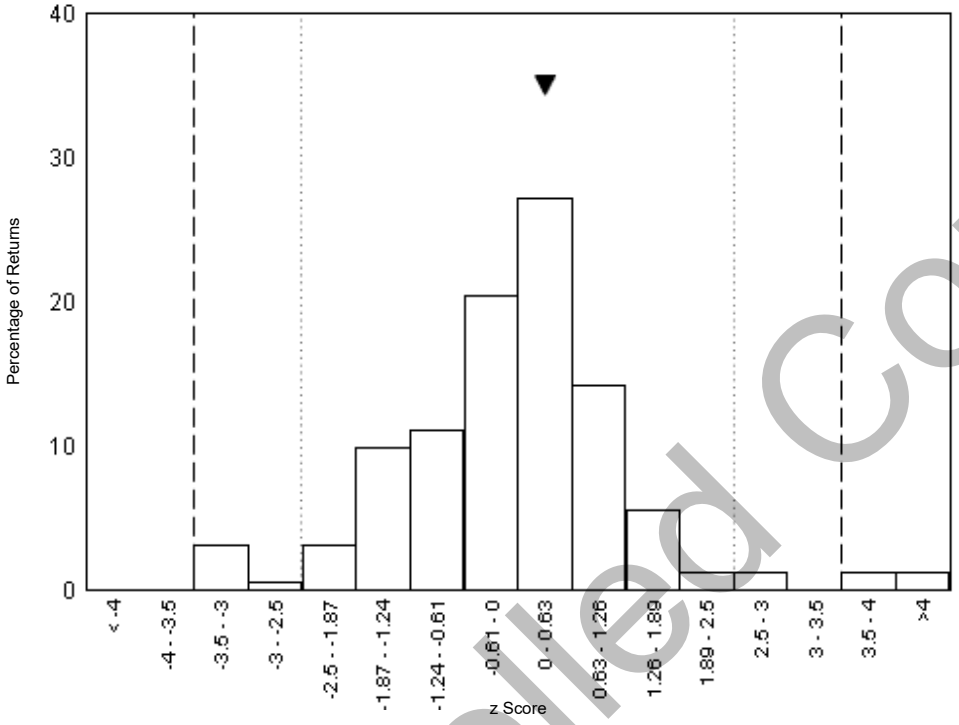
* consensus data shown are median values for events acquired and robust mean for percentage MRD

	Your Technique	Returns (n)
Lysis Method	Stain Lyse Wash	91
Doublets Excluded	Yes	31
Number of MRD Events to Define a Population	20	54
Denominator Used	Total Leucocytes	60
Stated Limit of Detection of Assay	1.0000%	
Calculated Limit of Detection Based on Events Collected	0.0220%	

Measurable Residual Disease for ALL by Flow Cytometry Programme

Histograms of Participant z Scores

Percentage MRD Population -
Please note ▼ denotes your result

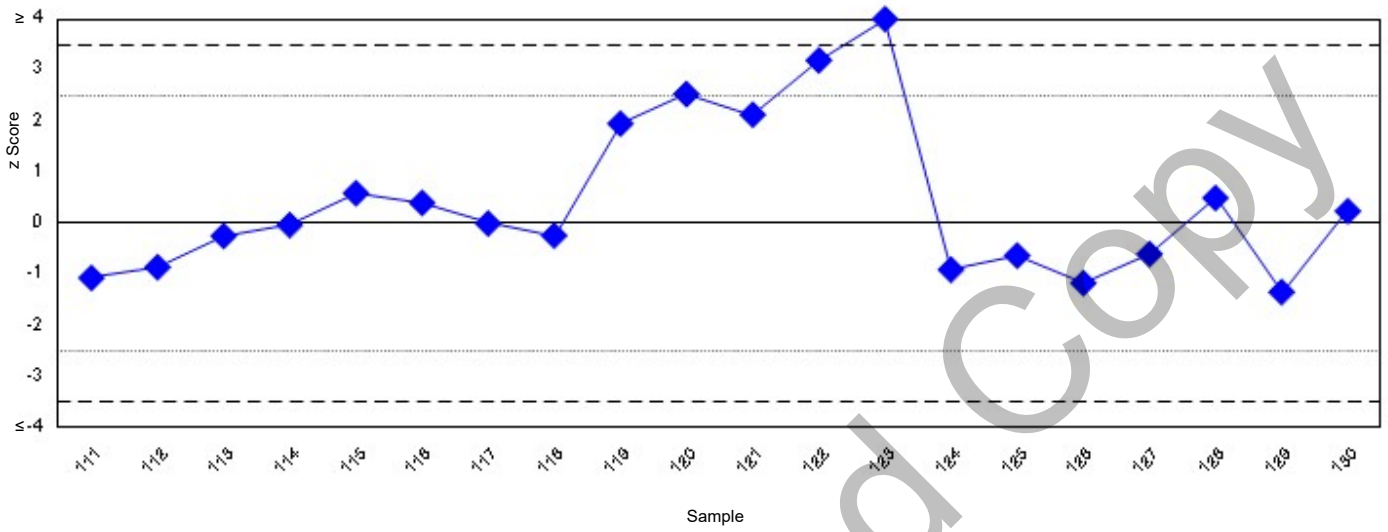


Measurable Residual Disease for ALL by Flow Cytometry Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

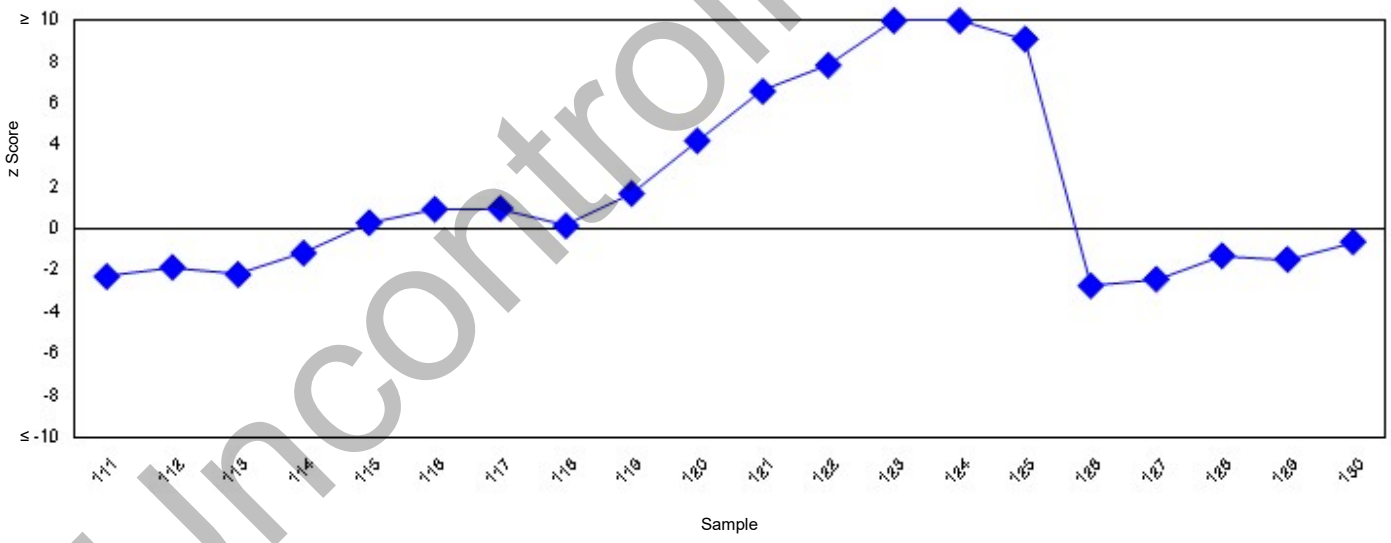
Values (Percentage MRD Population)



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	0.0411	0.0178	0.0380	0.0300	0.0516
FACSCanto II	45	0.0389	0.0053	0.0400	0.0340	0.0420
FACSLyric	45	0.0387	0.0108	0.0400	0.0300	0.0460
Navios	35	0.0346	0.0137	0.0357	0.0248	0.0415

MRD Group Specific Statistics

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
IBFM	29	0.0387	0.0122	0.0390	0.0320	0.0480
Non-Affiliated	85	0.0371	0.0110	0.0383	0.0280	0.0427
NOPHO	14	0.0421	0.0102	0.0425	0.0343	0.0493
Other	18	0.0392	0.0126	0.0415	0.0303	0.0468

Technique Specific Statistics

Lysis Technique

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
Lyse Stain Wash	52	0.0353	0.0120	0.0379	0.0255	0.0423
Stain Lyse Wash	91	0.0408	0.0100	0.0400	0.0354	0.0480

Doublet Exclusion

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
No	120	0.0383	0.0114	0.0400	0.0310	0.0460
Yes	31	0.0376	0.0126	0.0380	0.0284	0.0439

Denominator

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
All BM Nucleated Cells	80	0.0399	0.0105	0.0400	0.0338	0.0473
Total Leucocytes	60	0.0356	0.0115	0.0380	0.0278	0.0420

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	2	1.6	60	46.9	64	50.0	128
CD19	3	2.5	26	21.3	91	74.6	122
CD34	95	81.2	15	12.8	5	4.3	117
CD38	3	2.7	8	7.1	101	89.4	113
CD45	2	1.9	84	77.8	21	19.4	108
CD20	84	79.3	15	14.2	6	5.7	106
CD58	18	30.0	24	40.0	17	28.3	60
CD81	7	12.7	27	49.1	21	38.2	55
CD66c	1	2.0	9	18.4	39	79.6	49
CD123	9	22.5	9	22.5	22	55.0	40
CD22	6	16.7	21	58.3	9	25.0	36
CD24	0	0.0	1	5.3	18	94.7	19
CD73	12	70.6	3	17.7	2	11.8	17
CD33	13	86.7	1	6.7	1	6.7	15
CD304	8	57.1	3	21.4	3	21.4	14
CD9	0	0.0	4	33.3	8	66.7	12
CD13	9	81.8	1	9.1	1	9.1	11
CD66c/CD123	0	0.0	2	20.0	8	80.0	10
CD3	8	100.0	0	0.0	0	0.0	8
HLADR	0	0.0	1	16.7	5	83.3	6

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	Other	eBioscience	Dako	invitrogen	Life Technologies	Immunostep	Sysmex	Immunotech
CD10	Negative (-)	Absent	2	2														
	Positive (+)	Weak	60	38	17	1	3	1										
		Strong	64	27	19	7	2	4		1	1		3					
CD19	Negative (-)	Absent	3	2	1													
		Weak	1		1													
	Positive (+)	Weak	25	8	15	1		1										
		Strong	91	31	43	7	6	1	2		1							
CD34	Negative (-)	Absent	95	56	26	5	1	6			1							
		Weak	4	4														
		Strong	1	1														
	Positive (+)	Weak	11	8	1	1	1											
		Strong	4	1	1	2												
CD45	Negative (-)	Absent	2	1											1			
		Weak	1	1														
	Positive (+)	Weak	83	35	27	7	4	2			1			2	2	2	1	
		Strong	21	12	5	1	1							1	1			
CD38	Negative (-)	Absent	3	2	1													
	Positive (+)	Weak	8	5	3													
		Strong	101	49	33	9	4	2		2	1	1						
CD20	Negative (-)	Absent	84	41	22	6	11	2					2					
		Weak	2	1			1											
		Strong	3	2					1									
	Positive (+)	Weak	13	6	2	2	2				1							
		Strong	3	3														
CD58	Negative (-)	Absent	18	8	8					1		1						
		Weak	1	1														
		Strong	1	1														
	Positive (+)	Weak	23	7	10					4	1	1						
		Strong	16	5	7		1	1		2								
CD123	Negative (-)	Absent	9	3	3		2		1									
	Positive (+)	Weak	9	6	2		1											
		Strong	22	11		1			9		1							
CD22	Negative (-)	Absent	6	1	4			1										
	Positive (+)	Weak	21	8	5			2	1		1	2		2				
		Strong	9	4	4		1											
CD66c	Negative (-)	Absent	1		1													
	Positive (+)	Weak	9	2	6													1
		Strong	39	12	24	1				1	1							
CD81	Negative (-)	Absent	7	7														
	Positive (+)	Weak	27	13	3	4	2	2		3								
		Strong	21	13	3	4		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	PC7	V450	APC-Alexa 750	ECD	APC-Alexa 700	BV421	V500	V500-C
CD10	Negative (-)	Absent	2	1	1													
	Positive (+)	Weak	60	6	27		5		1		1		3	1	2	6		
		Strong	64	11	31	1	5				3			1	2			
CD19	Negative (-)	Absent	3		1		1				1							
		Weak	1								1							
	Positive (+)	Weak	25		2		11	2			2		1	2	1			
		Strong	91	1	14		49	2			15		2	4	2	1		
CD34	Negative (-)	Absent	95	6	8	1	8	39			5		1	7	4			
		Weak	4				1	3										
		Strong	1					1										
	Positive (+)	Weak	11	1				6										
		Strong	4				1	2					1					
CD45	Negative (-)	Absent	2					1										
		Weak	1															1
	Positive (+)	Weak	83					3	6			1		1	1		13	11
		Strong	21						6					3			3	1
CD38	Negative (-)	Absent	3						2				1					
	Positive (+)	Weak	8						5	2			1					
		Strong	101	5	1	9	2	7	27	1	1	2	11	1	5	1		
CD20	Negative (-)	Absent	84		1	11	4	2	4	24		20	3	2	1	3		1
		Weak	2			1				1								
		Strong	3									2						
	Positive (+)	Weak	13						1	7		1					1	
		Strong	3															1
CD58	Negative (-)	Absent	18		2	15										1		
		Weak	1			1												
		Strong	1			1												
	Positive (+)	Weak	23	2	7	13												
		Strong	16	3		12	1											
CD123	Negative (-)	Absent	9	5	1		1									2		
	Positive (+)	Weak	9	5	1		1									2		
		Strong	22	20														
CD22	Negative (-)	Absent	6	2	1										2			
	Positive (+)	Weak	21	11	5									1				
		Strong	9	3	1											3		
CD66c	Negative (-)	Absent	1	1														
	Positive (+)	Weak	9	6		3												
		Strong	39	33		4												
CD81	Negative (-)	Absent	7		1	3			3									
	Positive (+)	Weak	27	1	5	17		1	1	1								
		Strong	21	1		14			3	1								

Measurable Residual Disease for ALL by Flow Cytometry Programme

Distribution - 232402 | Sample - 131 | Participant ID - 40003

Date Issued - 14 August 2023 | Closing Date - 11 September 2023 | Machine Used - FACSCanto II

Trial Comments

This exercise was issued to 175 participants of which 152 (87%) returned results at the time of report generation. Of the non returning centres, 10 had requested an extension to the exercise deadline, 12 were non returns and 1 was a pre notified non-return. 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

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 (%)
	0.0330	0.0611	0.0212

Percentage MRD Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
	-1.33	Satisfactory	9	2	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 Present	MRD Present
Total Denominator Events	98,330	430,617
Total Number of MRD Events	32	256
Percentage MRD	0.0330	0.0611
Total Number of Events Acquired	150,640	649,674

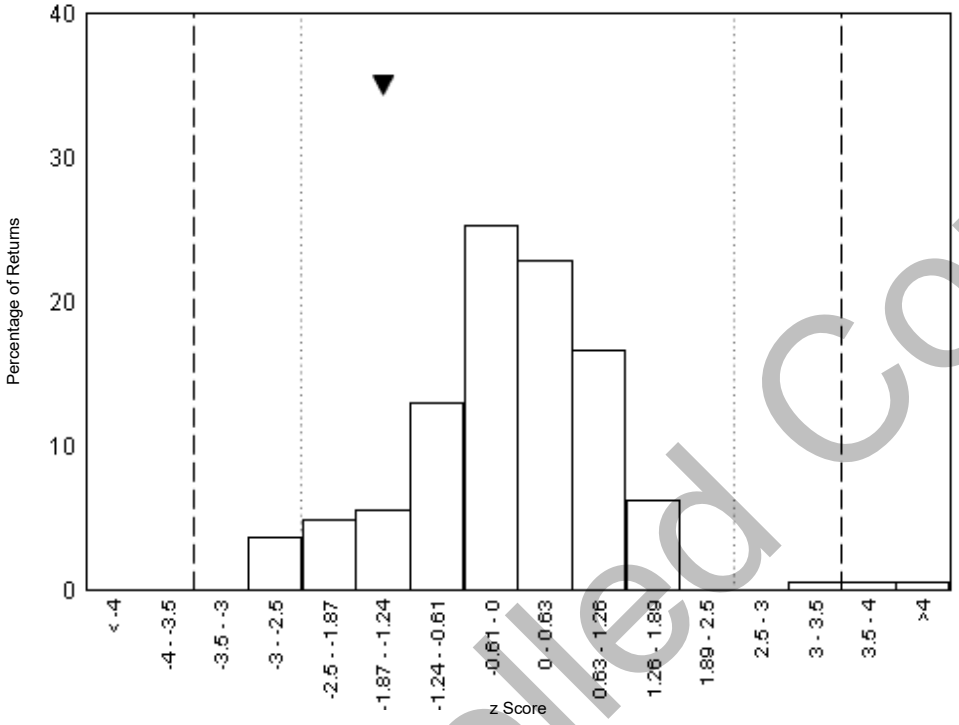
* consensus data shown are median values for events acquired and robust mean for percentage MRD

	Your Technique	Returns (n)
Lysis Method	Stain Lyse Wash	89
Doublets Excluded	Yes	30
Number of MRD Events to Define a Population	20	54
Denominator Used	Total Leucocytes	60
Stated Limit of Detection of Assay	1.0000%	
Calculated Limit of Detection Based on Events Collected	0.0203%	

Measurable Residual Disease for ALL by Flow Cytometry Programme

Histograms of Participant z Scores

Percentage MRD Population -
Please note ▼ denotes your result

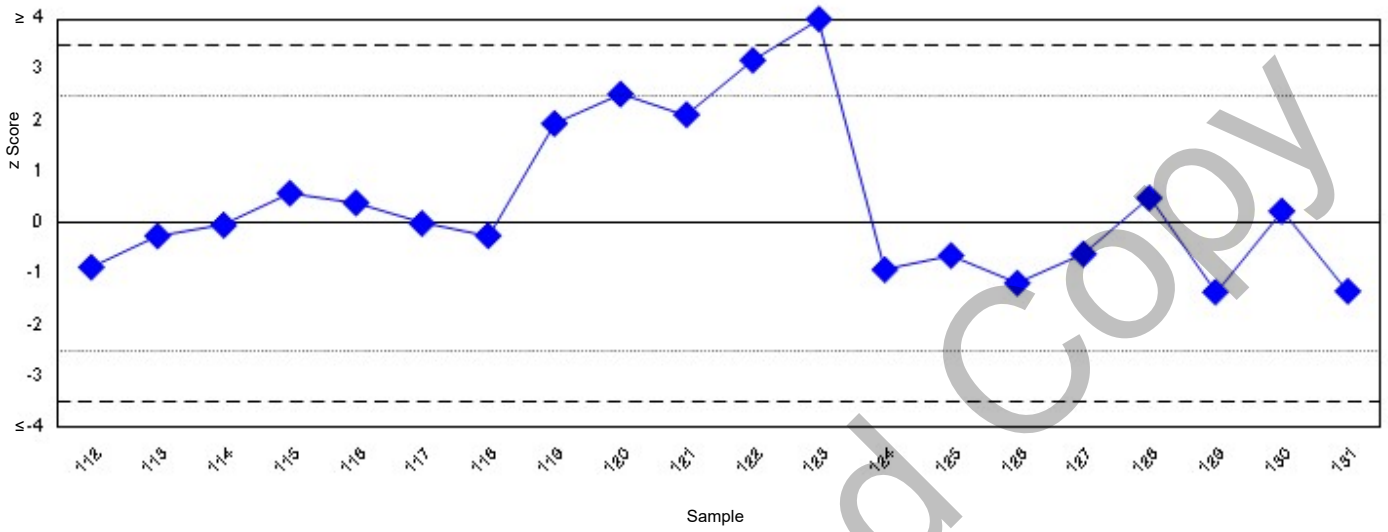


Measurable Residual Disease for ALL by Flow Cytometry Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

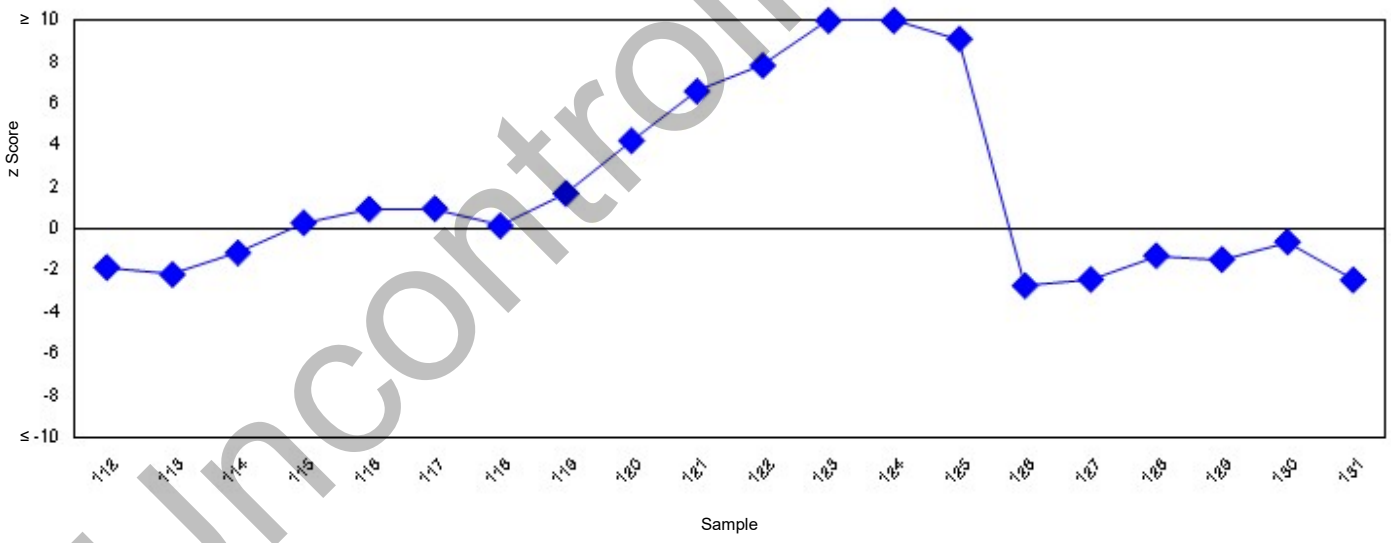
Values (Percentage MRD Population)



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	0.0638	0.0275	0.0676	0.0417	0.0818
FACSCanto II	42	0.0612	0.0126	0.0614	0.0544	0.0689
FACSLyric	44	0.0681	0.0144	0.0725	0.0556	0.0784
Navios	35	0.0531	0.0294	0.0553	0.0341	0.0700

MRD Group Specific Statistics

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
IBFM	29	0.0619	0.0196	0.0600	0.0504	0.0743
Non-Affiliated	85	0.0585	0.0222	0.0600	0.0400	0.0720
NOPHO	14	0.0682	0.0167	0.0660	0.0581	0.0818
Other	18	0.0634	0.0205	0.0600	0.0503	0.0800

Technique Specific Statistics

Lysis Technique

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
Lyse Stain Wash	53	0.0556	0.0224	0.0600	0.0350	0.0700
Stain Lyse Wash	89	0.0661	0.0174	0.0680	0.0557	0.0790

Doublet Exclusion

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
No	119	0.0612	0.0198	0.0600	0.0485	0.0750
Yes	30	0.0600	0.0220	0.0618	0.0408	0.0729

Denominator

Method	Percentage MRD					
	Returns	Robust Mean	Robust SD	Median	Lower Quartile	Upper Quartile
All BM Nucleated Cells	79	0.0645	0.0159	0.0660	0.0544	0.0747
Total Leucocytes	60	0.0563	0.0215	0.0583	0.0398	0.0700

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	2	1.6	56	45.2	64	51.6	124
CD19	3	2.5	24	20.2	90	75.6	119
CD34	93	80.9	15	13.0	5	4.4	115
CD38	3	2.7	7	6.4	99	90.0	110
CD45	2	1.9	82	77.4	21	19.8	106
CD20	80	76.9	17	16.4	6	5.8	104
CD58	18	31.6	21	36.8	17	29.8	57
CD81	7	12.5	29	51.8	20	35.7	56
CD66c	1	2.0	10	20.0	39	78.0	50
CD123	9	22.0	10	24.4	22	53.7	41
CD22	6	17.1	20	57.1	9	25.7	35
CD24	0	0.0	1	5.6	17	94.4	18
CD73	12	70.6	3	17.7	2	11.8	17
CD33	12	85.7	1	7.1	1	7.1	14
CD304	8	57.1	3	21.4	3	21.4	14
CD9	0	0.0	3	27.3	8	72.7	11
CD13	8	80.0	1	10.0	1	10.0	10
CD66c/CD123	0	0.0	3	30.0	7	70.0	10
CD3	8	100.0	0	0.0	0	0.0	8
CD73/CD304	2	33.3	3	50.0	1	16.7	6
HLADR	0	0.0	1	16.7	5	83.3	6

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	Other	eBioscience	Dako	invitrogen	Life Technologies	Immunostep	Sysmex	Immunotech
CD10	Negative (-)	Absent	2	2														
	Positive (+)	Weak	56	35	17	1	2	1										
		Strong	64	28	18	7	2	4		1	1		3					
CD19	Negative (-)	Absent	3	2	1													
		Weak	1		1													
	Positive (+)	Weak	23	8	13	1		1										
		Strong	90	31	42	7	6	1	2		1							
CD34	Negative (-)	Absent	93	55	26	5	1	5			1							
		Weak	4	4														
		Strong	1	1														
	Positive (+)	Weak	11	8	1	1	1											
		Strong	4	1	1	2												
CD45	Negative (-)	Absent	2	1											1			
		Weak	1	1														
	Positive (+)	Weak	81	34	26	7	4	2			1			2	2	2	1	
		Strong	21	12	5	1	1							1	1			
CD38	Negative (-)	Absent	3	2	1													
	Positive (+)	Weak	7	5	2													
		Strong	99	49	32	9	4	2		1	1	1						
CD20	Negative (-)	Absent	80	39	21	6	11	2					1					
		Weak	3	2			1											
		Strong	3	2					1									
	Positive (+)	Weak	14	7	2	2	2				1							
		Strong	3	3														
CD58	Negative (-)	Absent	18	8	8					1		1						
		Weak	1	1														
		Strong	1	1														
	Positive (+)	Weak	20	6	8					4	1	1						
		Strong	16	5	7		1	1		2								
CD123	Negative (-)	Absent	9	3	3		2		1									
	Positive (+)	Weak	10	7	2		1											
		Strong	22	11		1			9		1							
CD22	Negative (-)	Absent	6	1	4			1										
	Positive (+)	Weak	20	8	5			2	1		1	2		1				
		Strong	9	4	4		1											
CD66c	Negative (-)	Absent	1		1													
	Positive (+)	Weak	10	2	7													1
		Strong	39	12	24	1				1	1							
CD81	Negative (-)	Absent	7	7														
	Positive (+)	Weak	29	14	3	5	2	2		3								
		Strong	20	13	3	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	PC7	V450	APC-Alexa 750	ECD	APC-Alexa 700	BV421	V500	V500-C
CD10	Negative (-)	Absent	2	1	1													
	Positive (+)	Weak	56	5	26		4		1		1		3	1	2	5		
		Strong	64	11	32	1	4				3			1	2			
CD19	Negative (-)	Absent	3		1		1				1							
		Weak	1								1							
	Positive (+)	Weak	23		2		10	2			2		1	1	1			
		Strong	90	1	12		50	2			15		2	4	2	1		
CD34	Negative (-)	Absent	93	6	8	1	8	38			5		1	7	4			
		Weak	4				1	3										
		Strong	1					1										
	Positive (+)	Weak	11	1				6										
		Strong	4				1	2					1					
CD45	Negative (-)	Absent	2					1										
		Weak	1															1
	Positive (+)	Weak	81					3	5			1		1	1		14	11
		Strong	21						6					3			3	1
CD38	Negative (-)	Absent	3						2				1					
	Positive (+)	Weak	7						5	2								
		Strong	99	5	1	9	2	6	27	1	1	2	11	1	4	1		
CD20	Negative (-)	Absent	80		1	10	3	2	4	24		19	3	2		3		1
		Weak	3			1				1		1						
		Strong	3									2						
	Positive (+)	Weak	14						1	7		2					1	
		Strong	3															1
CD58	Negative (-)	Absent	18		2	15										1		
		Weak	1			1												
		Strong	1			1												
	Positive (+)	Weak	20	2	6	11			1									
		Strong	16	3		12	1											
CD123	Negative (-)	Absent	9	5	1		1									2		
	Positive (+)	Weak	10	6	1		1									2		
		Strong	22	20														
CD22	Negative (-)	Absent	6	2	1										2			
	Positive (+)	Weak	20	10	5									1				
		Strong	9	3	1											3		
CD66c	Negative (-)	Absent	1	1														
	Positive (+)	Weak	10	7		3												
		Strong	39	33		4												
CD81	Negative (-)	Absent	7		1	3			3									
	Positive (+)	Weak	29	1	5	19		1	1	1								
		Strong	20	1		13			3	1								