UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

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Proposed Redesign of the Leukaemia Immunophenotyping Programme

Dear Participant

As part of our commitment to develop the services and External Quality Assessment (EQA) programmes provided by this centre we are redesigning the Leukaemia Immunophenotyping (LI) programme to better reflect current hospital laboratory testing protocols. These changes are based on the format and findings from Educational exercises A and B, previously issued within this programme.

The existing UK NEQAS LI Leukaemia Immunophenotyping programme is based on 6 core antigens (CD2, CD3, CD5, CD13, CD19 and CD20) which are required to be tested by all participants in every exercise for performance monitoring purposes. Participants are also provided with 8 recommended case specific antigens (participants can test any number of these) plus an additional 6 optional antigens. Data for the recommended and optional antigens is not performance monitored but is provided to participants to allow for methodological comparisons.

The redesigned programme will allow participants to test the samples as per their in-house protocols and will be able to use their custom panels whether it be a screening tube, a specific cocktail or commercially produced lyophilized tubes.

There will be no change in the frequency of exercises per year or the sample supplied i.e. 1ml of stabilised whole blood spiked with leukaemic cells. In addition to receiving the sample for analysis participants will also be provided with a digital image and a brief case history (gender, age, presenting symptoms and FBC).

A full outline of the proposed changes and example reports can be seen below, and at this time we are actively seeking feedback and comments from our participants regarding their opinions on these changes and any other features they feel would be useful.

Any feedback on the proposed changes should be sent via email to alison.whitby@ukneqasli.co.uk by 31st October 2019.

Thank you in advance for your time and support in this matter.

Yours sincerely

Alison

Mrs. Alison Whitby

Advanced Biomedical Scientist

Proposed Changes to the Leukaemia Immunophenotyping Programme

Online Results Entry

The results entry form will be redesigned to make this process much less laborious than the current system. To achieve this, panel information will be entered initially by the participant as a new panel and then named and stored for recall on future exercises. Panels will be stored securely and accessible to that participant only.

Additionally, the centralisation of testing has led to laboratories not testing all types leukaemia e.g. a laboratory may only test chronic leukaemia with acute leukaemia cases sent to another laboratory or vice versa. Under the new design, participants will have the option to say that they consider the case to acute/chronic and that this case would be referred to another centre. If their classification is correct this would be classed as satisfactory performance. Incorrect classification would be classed as unsatisfactory performance.

Panel Entry

Participants will be able to enter a new panel, access a saved panel or edit a previously saved panel. These changes can be saved for the current exercise only or an existing panel can be updated and saved for future use.

When entering a panel, the participant will also be asked to enter the testing information relating to the antibody used in terms of manufacturer, fluorochrome and clone. There will be a checkbox for participants to highlight screening tube(s).

Results Entry

For each antigen tested participants will be required to enter whether they consider it to be Positive or Negative and the intensity of expression based on the system published by Dvorak (Dworzak et al., 2018).

Exercise Report

As per the existing report format information will be provided regarding the distribution, such as the issue and closing dates, number of participants in the exercise, how the sample was produced.

Trial Results

This section will display all antigens tested by ≥50% of participants with the top 10 most popular highlighted in bold (these will be used for performance monitoring – see below). The number of participants testing the specific antigens will also be displayed.

Antibody Usage Information

This will show the median, minimum and maximum number of individual antigens tested by all laboratories and the individual number of antigens tested by the participant. Allowing laboratories to compare the size of their testing panel to that of other participants in the exercise

Testing Pathway Information

A flow chart displaying the various testing pathways used by all participants, including information on the use of screening tubes and the total number of tubes were used in the panel will be included in the report to allow laboratories to compare their testing pathway to that of other centres.

Antigen Manufacturer Tables

The report will also feature tables detailing the intensity of staining and positive/negative results broken down in terms of the different reagent manufacturers, to allow participants to compare reagent performance on a like for like basis.

Antigen Fluorochrome Tables

The report will also feature tables detailing the intensity of staining and positive/negative results broken down in terms of the various fluorochromes used, to allow participants to compare reagent performance on a like for like basis.

Leukaemia Immunophenotyping Performance Monitoring

Participants will receive 2 performance scores

The Panel Design Grade is based on the number of antigens tested by the participant that are present in the consensus top 10 tested antigens.

The Antigen Testing Grade is based on how many of the antigens tested that are in the top 10 are in consensus in terms of positive/negative.

Panel Design Grade

The Panel Design Grade is based on panel design and the number of antigens tested by the participant matching the 10 most commonly tested antigens from the trial. Where the number of antigens matching is 50% or above, performance is considered satisfactory (performance grades A to C).

Number of consensus top ten antigens tested by the laboratory	Classification
10	Satisfactory
9	
8	
7	
6	
5	
4	Unsatisfactory
3	
2	
1	
0	

Antigen Testing Grade

The Antigen Testing Grade is based on the participant's panel design and the number of antigens tested that are in/out of consensus with the 10 most commonly tested antigens from the exercise. It is proposed that >70% in consensus will be classified as satisfactory performance.

Number of consensus top ten antigens tested by the laboratory	Number of antigens required to be in consensus for satisfactory performance	Classification
10	≥7	Satisfactory
9	≥7	
8	≥6	
7	≥5	
6	≥5	
5	≥4	
4	N/A	Unsatisfactory
3		
2		
1		
0		

Where the number of antigens matching the top 10 is <50% then an Antigen Testing Grade is not possible and therefore performance would be automatically classed as unsatisfactory.

Both performance grades will be used together for performance monitoring as in the table below

Panel design grade	Antigen consensus grade	Overall performance grade
Satisfactory	Satisfactory	Satisfactory
Satisfactory	Unsatisfactory	Unsatisfactory
Unsatisfactory	Unsatisfactory	Unsatisfactory

Performance monitoring will be classed that if a laboratory receives an overall unsatisfactory performance grade two times in a 12-month (6 sample) period then their performance will be upgraded to Persistent Unsatisfactory Performance.

References

Dworzak, M. N., Buldini, B., Gaipa, G., Ratei, R., Hrusak, O., Luria, D., … Basso, G. (2018). AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of Pediatric acute lymphoblastic leukemia. *Cytometry Part B: Clinical Cytometry*, *94B*, 82–93.

Leukaemia Immunophenotyping

All Participants

Distribution – XXXXXX Participant - XXXXX

Date Issued – XX XXXX XXXX Closing Date – XX XXXX XXXX

Trial Comments

This trial was issued to XXX participants

Sample Comments

The sample was manufactured by UK NEQAS using a sample of blood from a leukaemia patient which was stabilised and added to a stabilised unit of whole blood.

Trial Results

Please note - to allow for concise reports only antigens tested by >50% of participants are shown in the following tables and charts. The top 10 most popular antigens are highlighted in bold.

Antigen Tested	Number of Participants Testing (% value in brackets)	Your Intensity of Staining[1]	Consensus Intensity of Staining	Your Result (Positive / Negative)	Consensus Result (Positive / Negative)
CD19	106 (97%)	Strong	Strong	Positive (+)	Positive (+)
CD34	101 (93%)	Strong	Strong	Positive (+)	Positive (+)
CD45	89 (82%)	Strong	Strong	Positive (+)	Positive (+)
CD13	87 (80%)	Weak	Strong	Negative (-)	Positive (+)
CD33	86 (79%)	Strong	Strong	Positive (+)	Positive (+)
CD7	84 (77%)	Absent	Absent	Negative (-)	Negative (-)
CD117	83 (76%)	Absent	Absent	Negative (-)	Negative (-)
HLA-DR	82 (75%)	Strong	Strong	Positive (+)	Positive (+)
CD10	81 (74%)	Absent	Absent	Negative (-)	Negative (-)
CD3	79 (72%)	Absent	Absent	Negative (-)	Negative (-)
Myeloperoxidase	76 (70%)	Strong	Strong	Positive (+)	Positive (+)
CD14	75 (69%)	Strong	Absent	Positive (+)	Negative (-)
CD20	68 (62%)	Absent	Absent	Negative (-)	Negative (-)
CD64	61 (56%)	Strong	Strong	Positive (+)	Positive (+)

Reference:

1. Dworzak, M. N., Buldini, B., Gaipa, G., Ratei, R., Hrusak, O., Luria, D., … Basso, G. (2018). AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of Pediatric acute lymphoblastic leukemia. *Cytometry Part B: Clinical Cytometry*, *94B*, 82–93.

Panel Design and Result Analysis

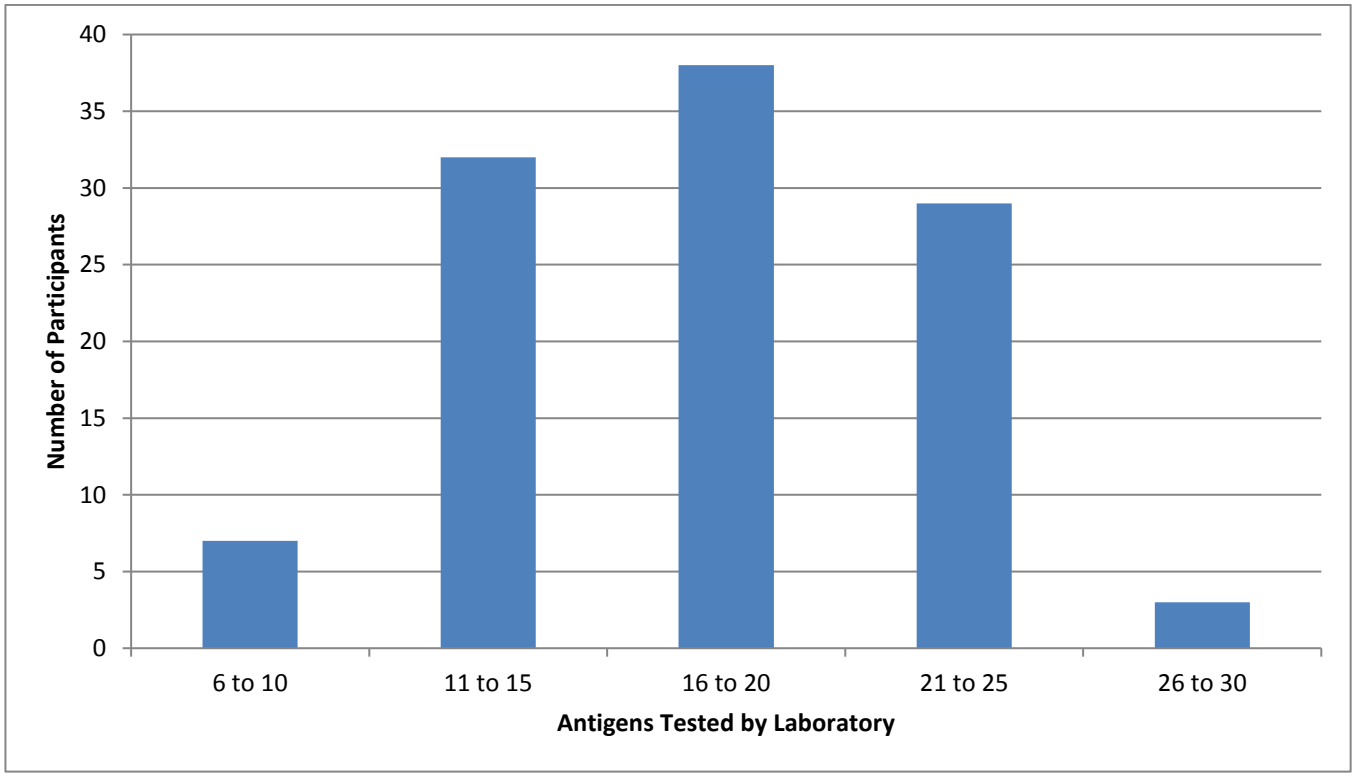
Number of antigens in top 10 tested within your panel	Performance Classification	Antigen results within consensus	Performance Classification	Overall Performance Grade
10	Satisfactory	9	Satisfactory	Satisfactory

Please note – Participants testing 4 antigens or less will automatically receive an Unsatisfactory Antigen Grading Score as the panel design is classed as Unsatisfactory

Individual Laboratory Antibody Usage Analysis

Antigens Tested by Your Laboratory	All Laboratories Antigens Tested		
	Median	Minimum	Maximum
30	18	10	29

All Laboratories Antigens Tested



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Flow Chart of Laboratory Testing Pathways Analysis

Please note, numbers in the following chart may not equal the number of participants due to not all participants providing all information required for production

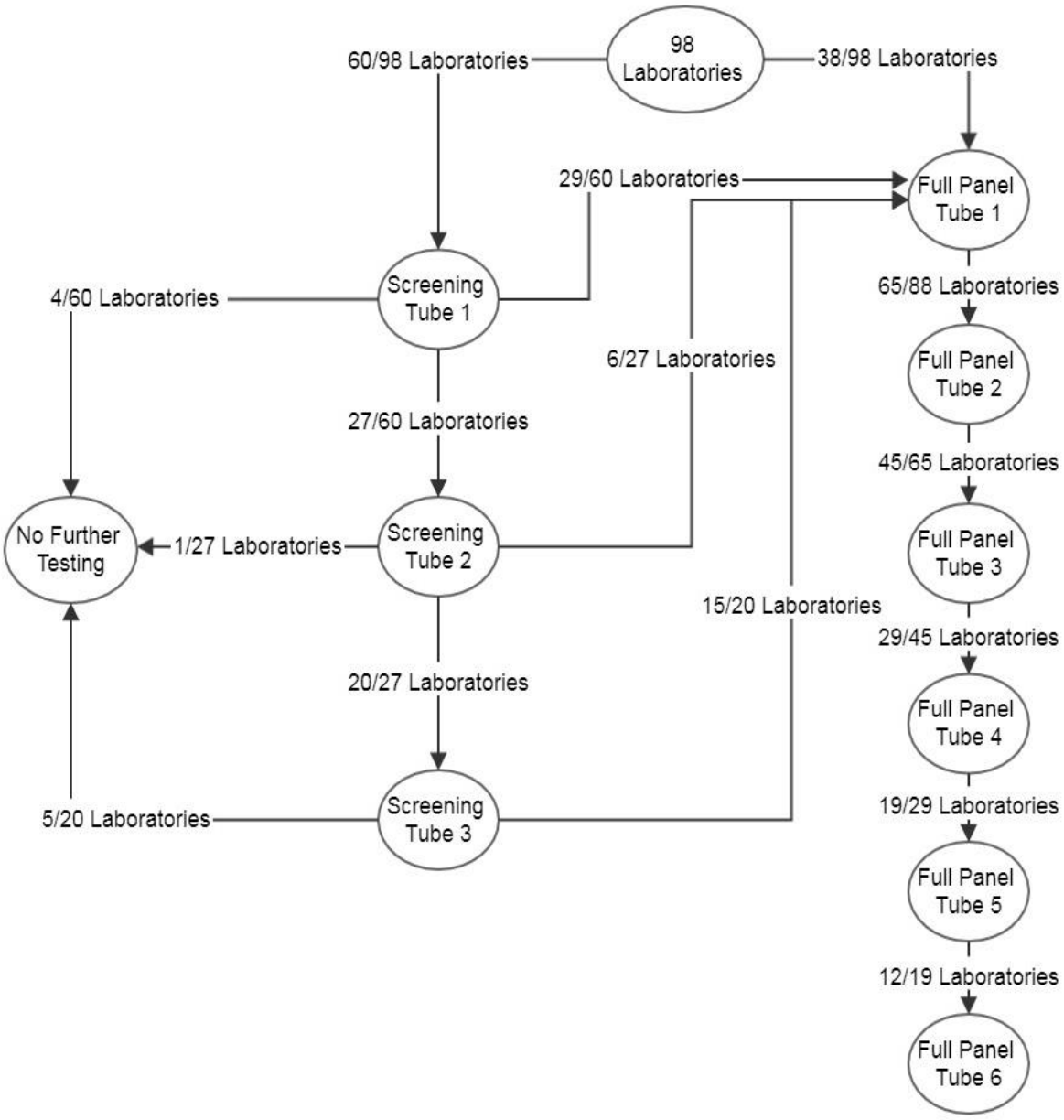

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Table showing the breakdown of participant returns according to antibody manufacturer

N.B. To allow for concise reports only antigens tested by >50% 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	Intensity	Positive/Negative	Total Results (All Data)	BD Biosciences	Beckman Coulter	BioLegend	Dako	eBioscience	Immunotech
CD19	Absent	Negative (-)	9	2	5		1		1
	Weak	Negative (-)	3		3			1	
		Positive (+)	117	57	466	5	2		3
	Strong	Positive (+)	23	10	10			1	
CD34	Absent	Negative (-)	13	7	6				
	Weak	Negative (-)	9	2	5		1	1	
		Positive (+)	165	108	42	3			3
	Strong	Positive (+)	14	7	7				
CD45	Absent	Negative (-)							
	Weak	Negative (-)	3	2	1				
		Positive (+)	264	153	69	5		1	1
	Strong	Positive (+)	44	21	17			3	3
CD13	Absent	Negative (-)	7	1	5				
	Weak	Negative (-)	9	2	7				
		Positive (+)	71	41	22	1	4		1
	Strong	Positive (+)	3	3					
CD33	Absent	Negative (-)	2		2				
	Weak	Negative (-)							
		Positive (+)	64	39	18	1	2		3
	Strong	Positive (+)	34	16	14			1	2

Antigen	Intensity	Positive/Negative	Total Results (All Data)	BD Biosciences	Beckman Coulter	BioLegend	Dako	eBioscience	Immunotech
CD7	Absent	Negative (-)	71	41	17	1	1	7	1
	Weak	Negative (-)	6	1	4				1
		Positive (+)	6	2	4				
	Strong	Positive (+)							
CD117	Absent	Negative (-)	98	49	42		4		3
	Weak	Negative (-)	4		2	2			
		Positive (+)	9	7	1			1	
	Strong	Positive (+)							
HLA-DR	Absent	Negative (-)	2		2				
	Weak	Negative (-)	1		1				
		Positive (+)	77	46	18	8	1	1	2
	Strong	Positive (+)	33	19	6	7		1	
CD3	Absent	Negative (-)	73	43	21		1	2	1
	Weak	Negative (-)	6	2	4				
		Positive (+)	1	1					
	Strong	Positive (+)							
CD20	Absent	Negative (-)	53	30	14	5		1	
	Weak	Negative (-)	5	1	4				
		Positive (+)	4	3		1			
	Strong	Positive (+)							
Myeloperoxidase	Absent	Negative (-)	2	2					
	Weak	Negative (-)	3	1			2		
		Positive (+)	49	22	11		12		
	Strong	Positive (+)	18	1	10		4		1

Antigen	Intensity	Positive/Negative	Total Results (All Data)	BD Biosciences	Beckman Coulter	BioLegend	Dako	eBioscience	Immunotech
CD10	Absent	Negative (-)	69	34	26		5	2	2
	Weak	Negative (-)	9	4	4	1			
		Positive (+)							
	Strong	Positive (+)	1		1				
CD14	Absent	Negative (-)	40	26	11		1	1	1
	Weak	Negative (-)	11	10	1				
		Positive (+)	12	15	8				
	Strong	Positive (+)	1	1					
CD64	Absent	Negative (-)	13	9	3				
	Weak	Negative (-)	1		1				
		Positive (+)	37	15	11		5	1	1
	Strong	Positive (+)	5	2	1		1	1	

Table showing the breakdown of participant returns according to antibody fluorochrome

N.B. To allow for concise reports only antigens tested by >50% 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	Intensity	Positive/Negative	Total Results (All Data)	APC	APC-Cy7	APC-H7	ECD	FITC	Krome Orange	Pacific Blue	PE	PE-CY5	PE-CY5_5	PE-CY7	PerCP	PerCP-CY5	PerCP-CY5_5	V450	V500
CD19	Absent	Negative (-)	9		1			4						2				1	
	Weak	Negative (-)	3								1			2					
		Positive (+)	117	16	1	3	6	4		1	8	4	2	45	2	4	6	1	
	Strong	Positive (+)	23	5		1		1			1	1	4	8			1		
CD34	Absent	Negative (-)	13	2				1						1	1		4		
	Weak	Negative (-)	9	1							2	1		3	1				
		Positive (+)	165	33			5	9			13	2		31	2	222	40		
	Strong	Positive (+)	14	3							1	2		2			2		
CD45	Absent	Negative (-)																	
	Weak	Negative (-)	3		1	1						1							
		Positive (+)	246	5	15	32	11	3	32	1		11		9	23	6	13	4	53
	Strong	Positive (+)	44	1					4				2		19		2		6
CD13	Absent	Negative (-)	7	1							2		1	2			1		
	Weak	Negative (-)	9	1			2				4	1							
		Positive (+)	71	1			2	4			54	1		7			1		
	Strong	Positive (+)	3	1							2								

Antigen	Intensity	Positive/Negative	Total Results (All Data)	APC	APC-Cy7	APC-H7	ECD	FITC	Krome Orange	Pacific Blue	PE	PE-CY5	PE-CY5_5	PE-CY7	PerCP	PerCP-CY5	PerCP-CY5_5	V450	V500
CD33	Absent	Negative (-)	2																
	Weak	Negative (-)																	
		Positive (+)	64	18				5			22	2	3	11		1	1		
	Strong	Positive (+)	34	10				1			6	3	4	3		2	1		
CD7	Absent	Negative (-)	70	21			2	19		2	14	3		1				1	
	Weak	Negative (-)	6					3		2								1	
		Positive (+)	8					5		1				1				1	
	Strong	Positive (+)	2					2											
CD117	Absent	Negative (-)	98	11			1				19	6	5	49			2		
	Weak	Negative (-)	4								1			2					
		Positive (+)	9								6					2	1		
	Strong	Positive (+)																	
HLA-DR	Absent	Negative (-)	3																
	Weak	Negative (-)	1																
		Positive (+)	76	8	4	5	3	14	1	15	3			2	1	1	3	15	
	Strong	Positive (+)	33	6			1	4		7	1			2	1			10	
CD3	Absent	Negative (-)	73	14	1	10	6	17		1		2		3	1		5	1	2
	Weak	Negative (-)	6			1	1	1		1		2							
		Positive (+)	1	1															
	Strong	Positive (+)																	

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Antigen	Intensity	Positive/Negative	Total Results (All Data)	APC	APC-Cy7	APC-H7	ECD	FITC	Krome Orange	Pacific Blue	PE	PE-CY5	PE-CY5_5	PE-CY7	PerCP	PerCP-CY5	PerCP-CY5_5	V450	V500
CD10	Absent	Negative (-)	67	22	1	3	2	8			22	3		6					
	Weak	Negative (-)	9	1		1		3			3			1					
		Positive (+)	1											1					
	Strong	Positive (+)									1								
Myeloperoxidase	Absent	Negative (-)	2					1			1								
	Weak	Negative (-)	3	1				1			17								
		Positive (+)	49					29			6								
	Strong	Positive (+)	18					12			5								
CD14	Absent	Negative (-)	29	1	2	14	1	12						2	1			1	
	Weak	Negative (-)	10	2		5					1					1	1		
		Positive (+)	23	1	1	2	3	12			1	1		2					
	Strong	Positive (+)	1		1														
CD20	Absent	Negative (-)	53	8	2	2	1	9		4	6	2		4		4	1	8	
	Weak	Negative (-)	5					3				2							
		Positive (+)	4					2		1	1								
	Strong	Positive (+)																	
CD64	Absent	Negative (-)	13	1		1	1	4			4						2	1	
	Weak	Negative (-)	1																
		Positive (+)	37	1				8			21	2		2					
	Strong	Positive (+)	5	1			1	1			2								

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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
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@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/