

Leukaemia Immunophenotyping
Educational Sample A
All Participant Report

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Introduction

As part of the ongoing developments of this programme an educational sample was issued and laboratories were requested to analyse the sample as per their in-house testing procedures.

This report contains the preliminary findings of the data analysis of this sample and provides detailed analysis of the results returned. However, we would encourage all participants to examine the report fully in order to better understand how their results compare to other laboratories and in order to provide feedback to UK NEQAS.

It is intended that following the release of this report a survey will be issued to all participants that took part in this exercise to provide a forum for feedback on the operation of the exercise and the contents of the report. This will then allow UK NEQAS to continue development of the Leukaemia Immunophenotyping programme and to ensure that it meets the requirements of participants.

Whilst this trial will not be performance monitored, analysis of the data has highlighted several areas that we wish to highlight to participants.

- There were a total of 140 returns of which several were incomplete in parts and have been excluded from some (but not all) of the data analysis. Thus, total numbers will fluctuate due to this.
- This case was a B-CLL, however no two laboratories returning results had identical testing panels in terms of antigens tested.
- Of the 120 laboratories that provided full details of their panel design, when asked stated if they used either a screening tube(s) or a full panel, 91/120 (76%) employed a screening panel.
- Of the 91 that used a screening panel, 6 did no further testing. See flow chart on page 11 for further details.
- Where laboratories used a screening tube(s) only 66/91 (73%) incorporated CD45 in combination with CD19.
- Of laboratories using screening tubes (n=91) none were using the Euroflow consortium LST tube, although one centre did use the Euroflow consortium ALOT tube
- Following the use of screening tube(s) 69 laboratories progressed to using a full panel and 29 laboratories progressed to using a full panel without using a screening tube(s).
- The data shows that a full panel comprised of between 1 and 6 tubes but please note screening tubes and full panels were comprised of varying numbers of fluorochromes/antibodies.
- Results submitted by laboratories covered a total of 41 different antigens.
 - CD19 was the most commonly tested with 98% of laboratories testing for this antigen (n=125/128).
 - CD45 was tested by 90% of laboratories (n=115/128)
 - CD235a (n=1/128) and CD81 (n=1/128) were the least used with 0.8% of laboratories testing for these antigens.
- Despite difference in panel design the consensus results returned showed a high degree of concordance with >95% of laboratories in consensus for the antigens CD19, CD20, CD5, CD45, CD10, CD3, CD4, CD8 and CD200.

Leukaemia Immunophenotyping

Educational Sample A All Participant Report

Distribution – Educational Sample A

Participant - 40823

Date Issued – 11 July 2016

Closing Date – 05 August 2016

PLEASE NOTE- THIS TRIAL WILL NOT BE PERFORMANCE MONITORED

Trial Comments

This trial was issued to 277 participants, with 140 laboratories returning results for analysis. The format for the trial was designed to allow UK NEQAS LI to capture information on laboratory testing protocols for leukaemia investigations with the aim of using the information to redesign the existing programme to better meet the needs of participants. Please note that this trial **will not be scored**. All results and statistical analyses are provided for information purposes only.

Sample Comments

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

Trial Results

n.b. 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 outlined in bold.

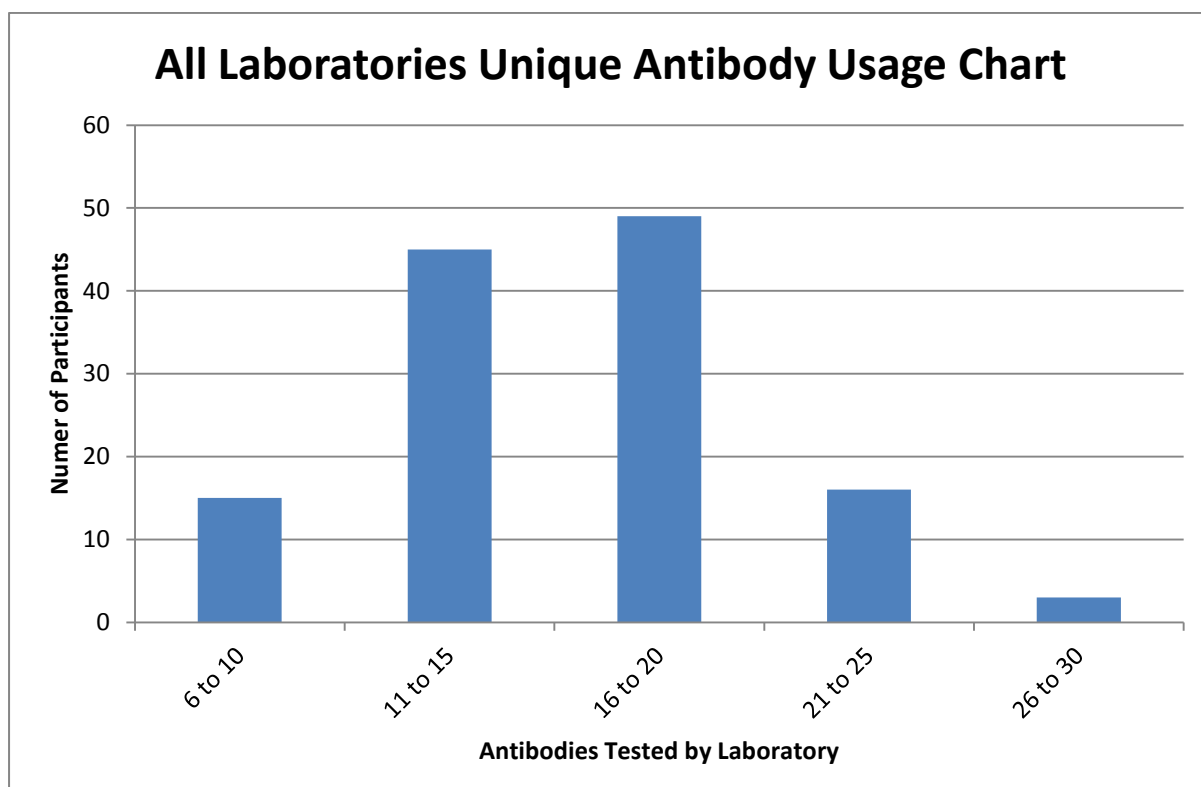
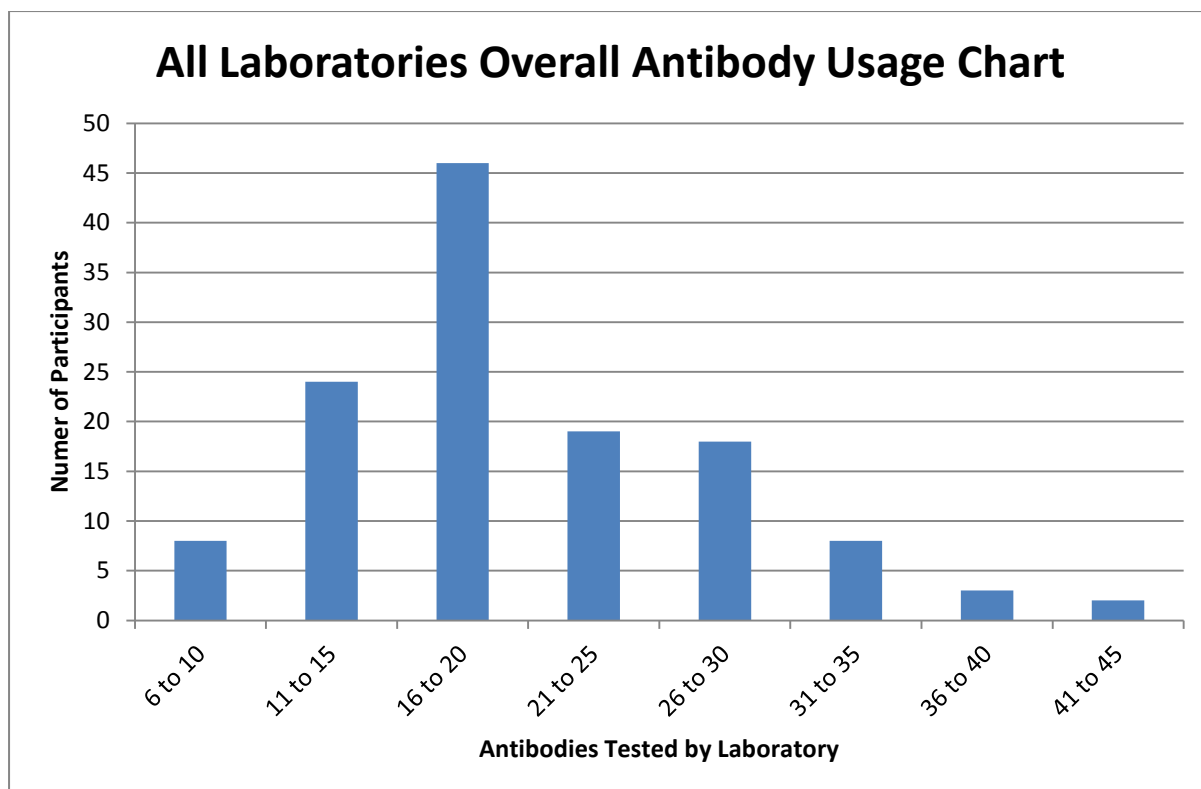
Antigen Tested	Number of Participants Testing (% value in brackets)	Your Result (Positive/Negative)	Consensus Result (Positive/Negative)	Percentage of Participants in Consensus
CD19	125 (98%)	Not Tested	Positive (+)	99%
CD20	119(93%)	Not Tested	Positive (+)	97%
CD5	119(93%)	Not Tested	Positive (+)	97%
CD45	115(90%)	Not Tested	Positive (+)	97%
CD10	104(81%)	Not Tested	Negative (-)	99%
CD23	106(83%)	Not Tested	Positive (+)	85%
CD3	101(79%)	Not Tested	Negative (-)	93%
KAPPA	74(58%)	Not Tested	Negative (-)	58%
LAMBDA	74(58%)	Not Tested	Negative (-)	55%
CD4	76(59%)	Not Tested	Negative (-)	91%
CD8	73(57%)	Not Tested	Negative (-)	93%
CD38	72(56%)	Not Tested	Negative (-)	58%
CD79b	73(57%)	Not Tested	Negative (-)	73%
CD200	71(55%)	Not Tested	Positive (+)	94%

Panel Design and Result Analysis

Number of antigens in top 10 tested within your panel	Panel Design Grade	Antigen results within consensus	Antigen results out of consensus	Antigen Testing Grade
Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable

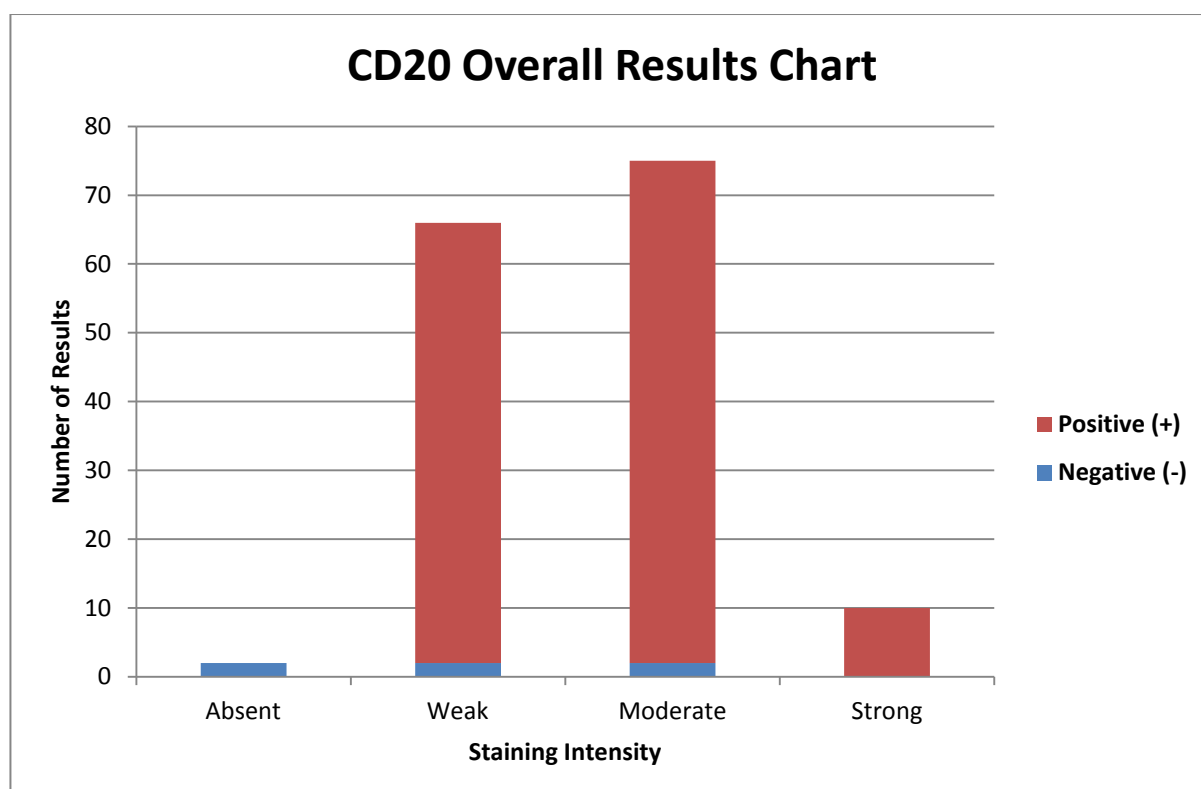
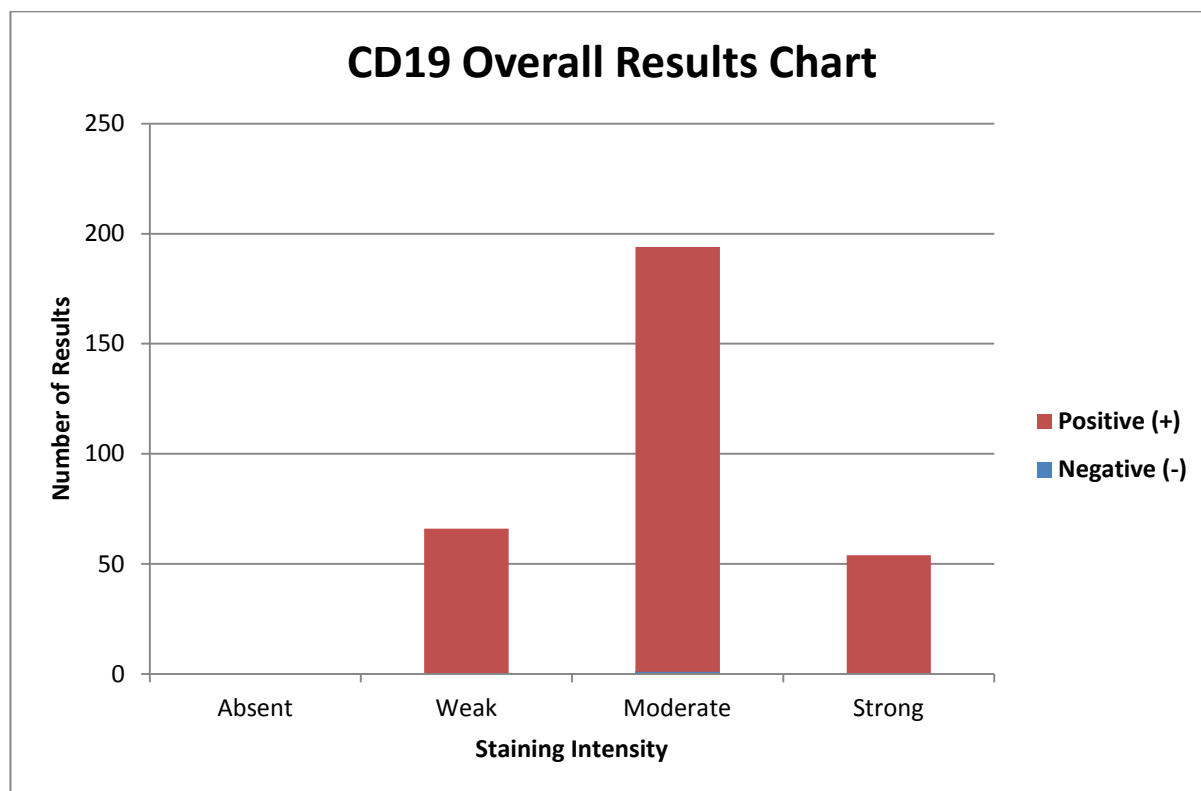
Individual Laboratory Antibody Usage Analysis

Your overall antibody usage	All Laboratories Overall Antibody Usage			Unique antibodies used by your laboratory	All Laboratories Unique Antibodies Used		
	Median	Minimum	Maximum		Median	Minimum	Maximum
Not Applicable	19	6	44	Not Applicable	16	6	27

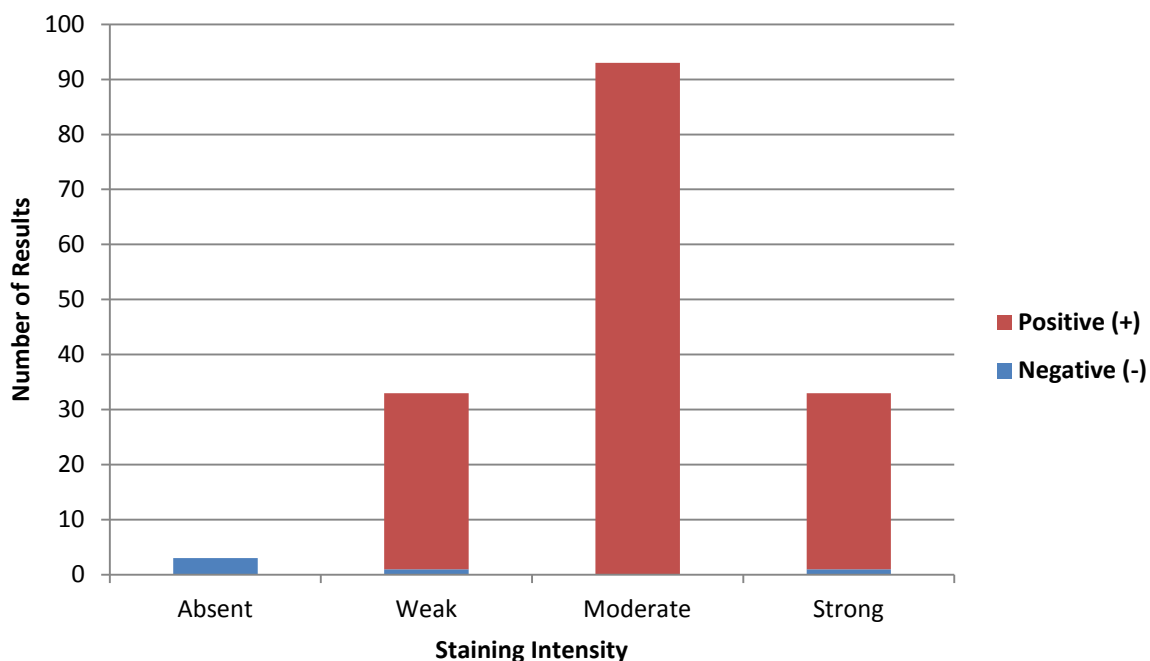


Charts of Antigen Intensity and Staining Result against Number of Participants

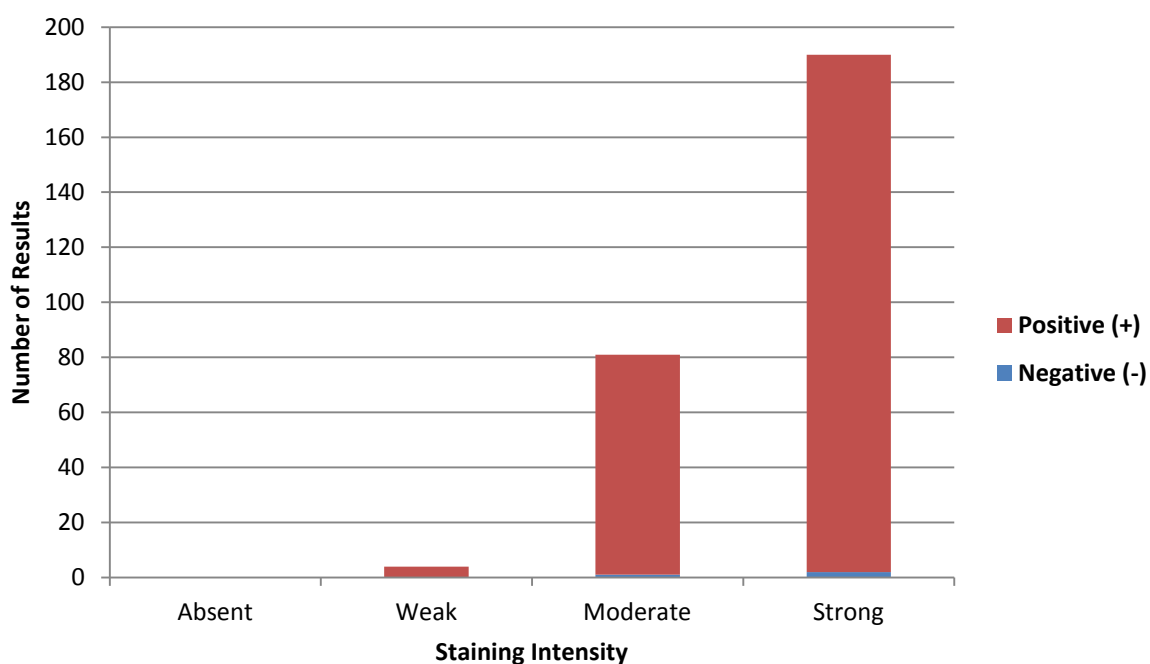
n.b. to allow for concise reports only antigens tested by >50% of participants are shown in the following charts
Please note, numbers in all charts may not equal the number of participants due to not all participants providing all information or to some participants performing multiple combinations of techniques, for example inclusion of the same antibody in multiple tubes.



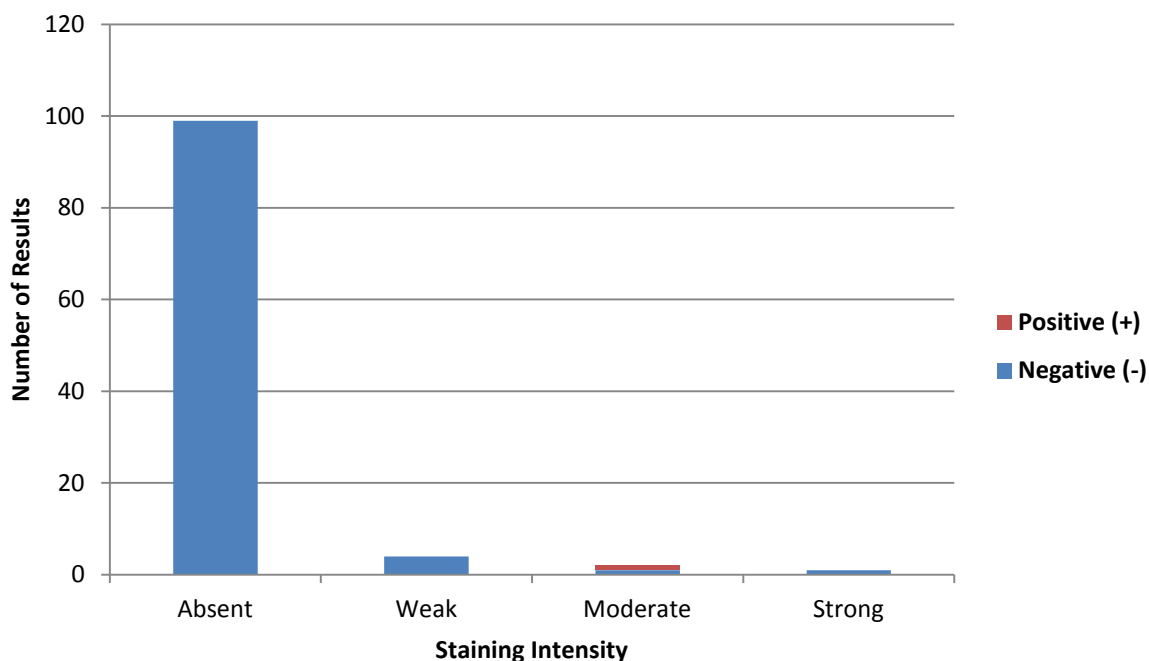
CD5 Overall Results Chart



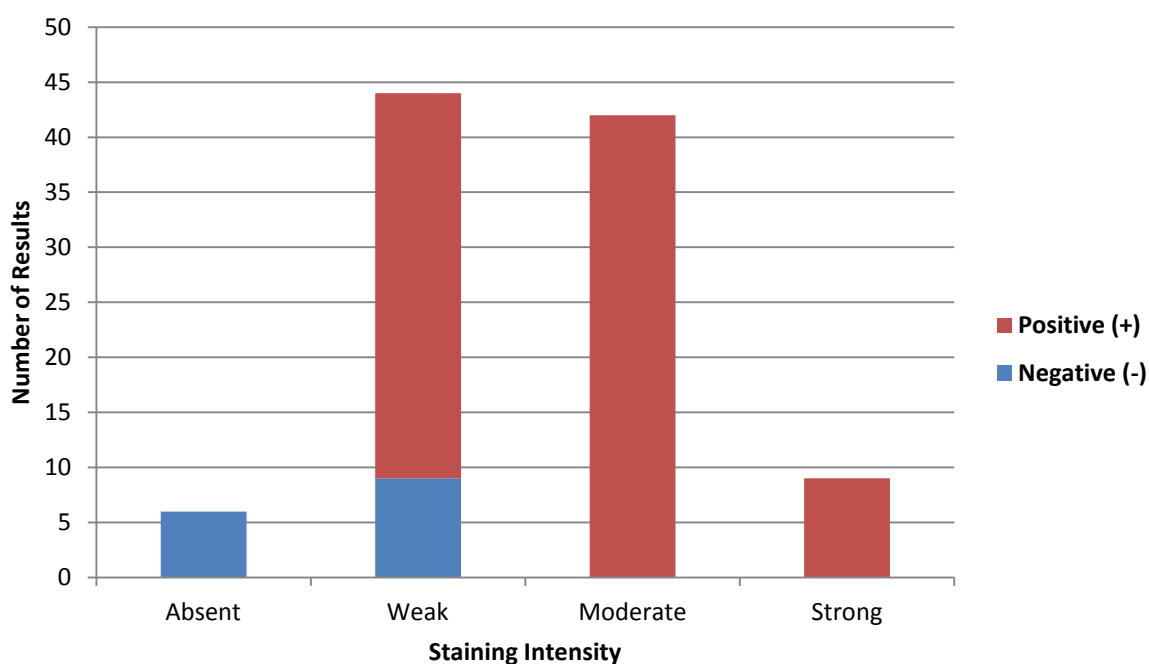
CD45 Overall Results Chart



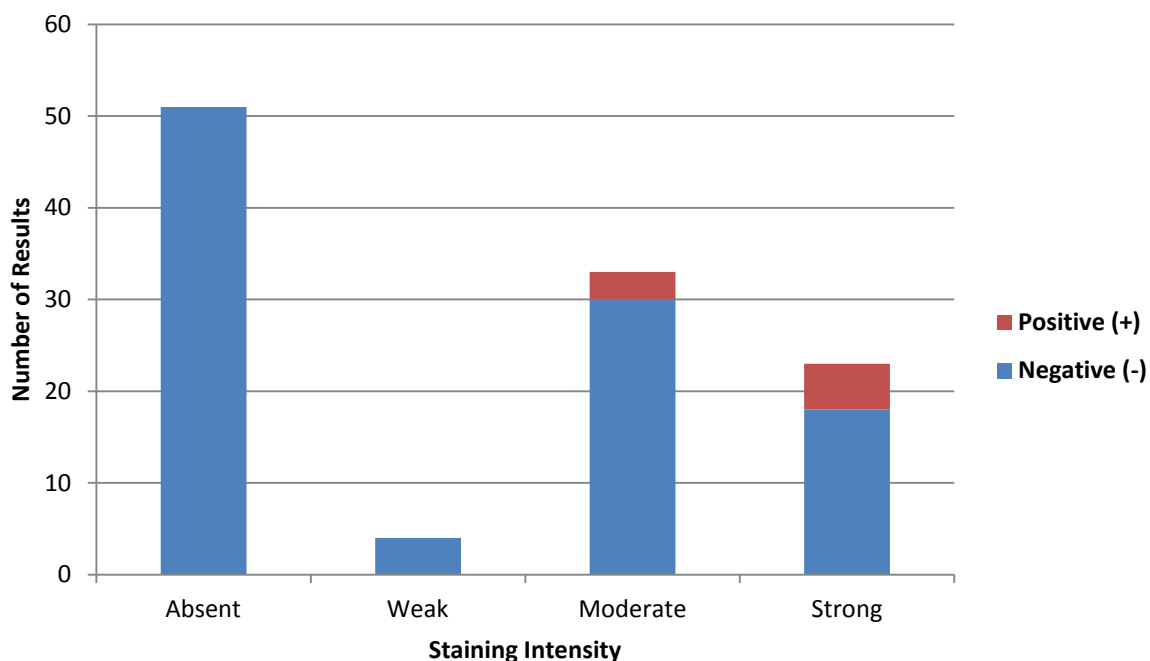
CD10 Overall Results Chart



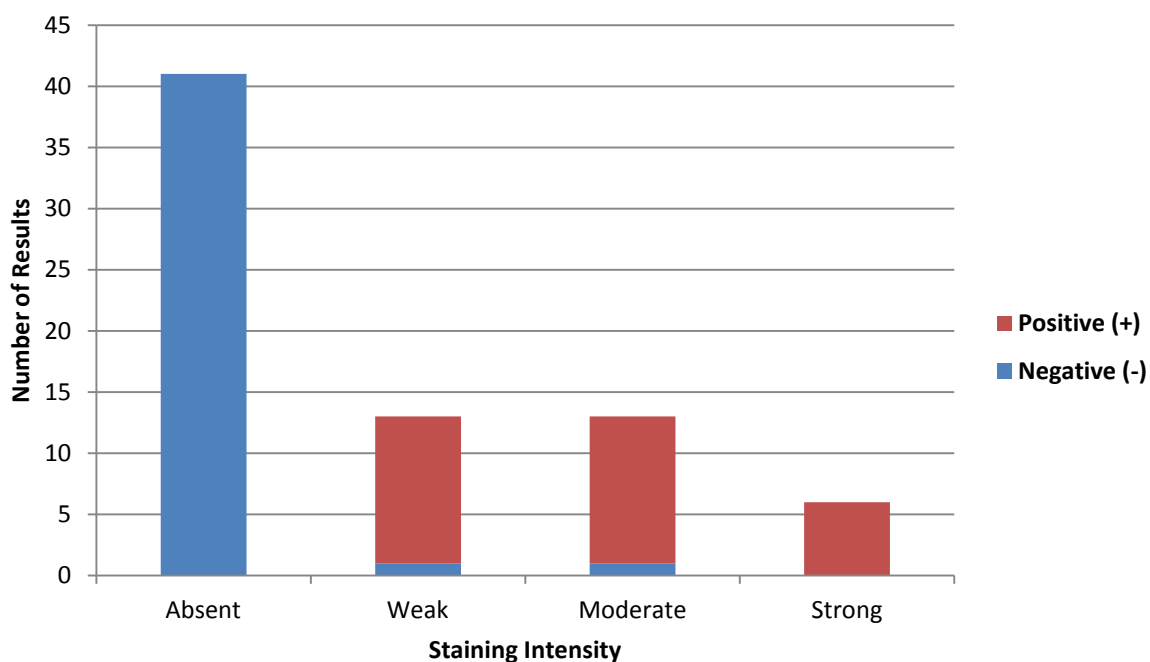
CD23 Overall Results Chart



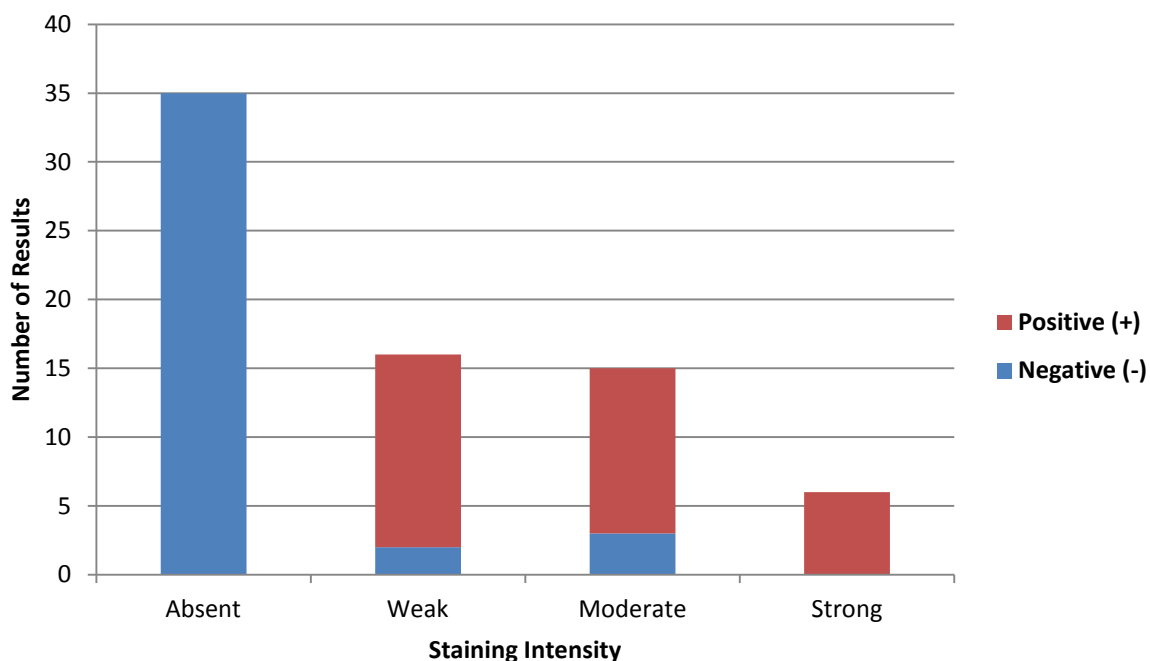
CD3 Overall Results Chart



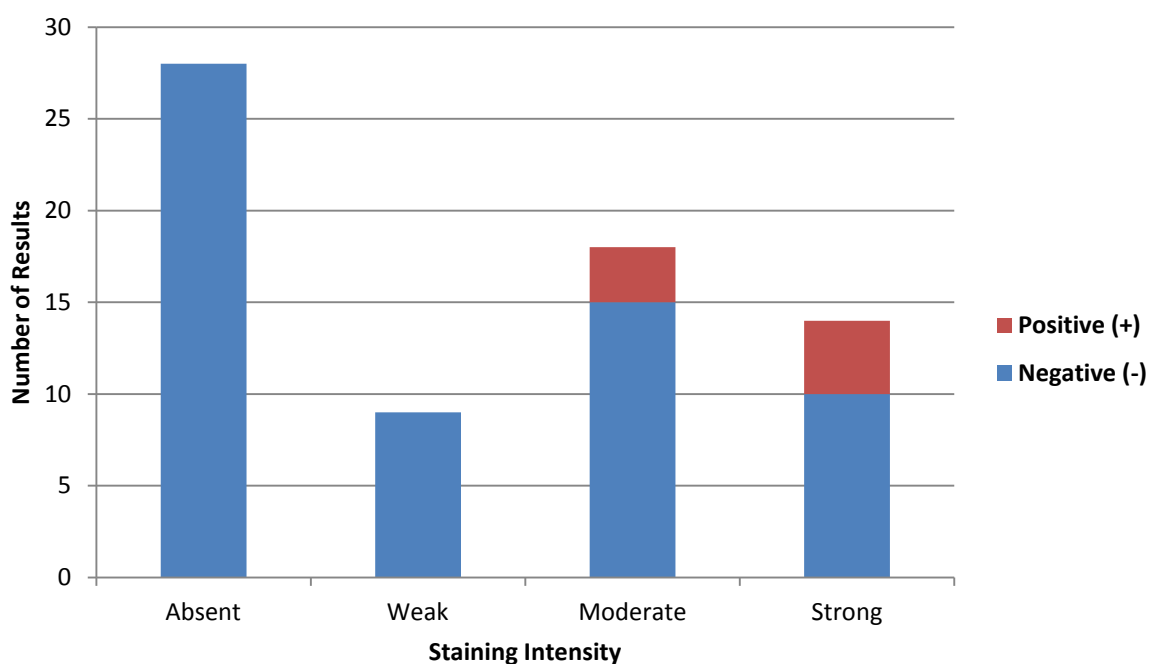
Kappa Overall Results Chart



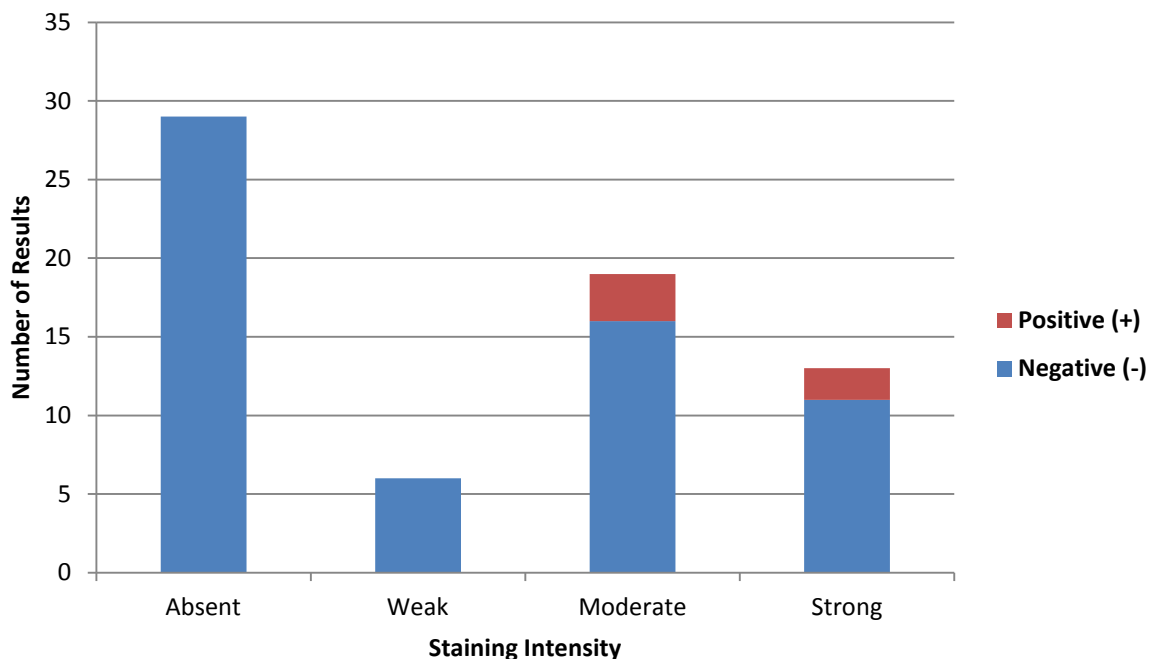
Lambda Overall Results Chart



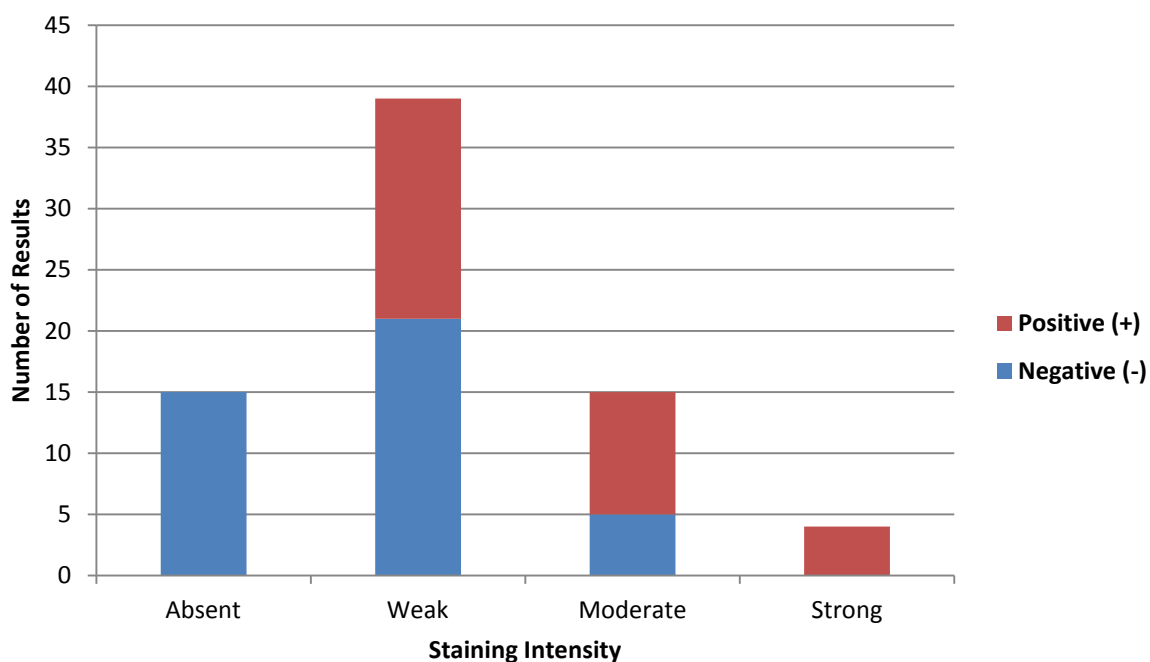
CD4 Overall Results Chart



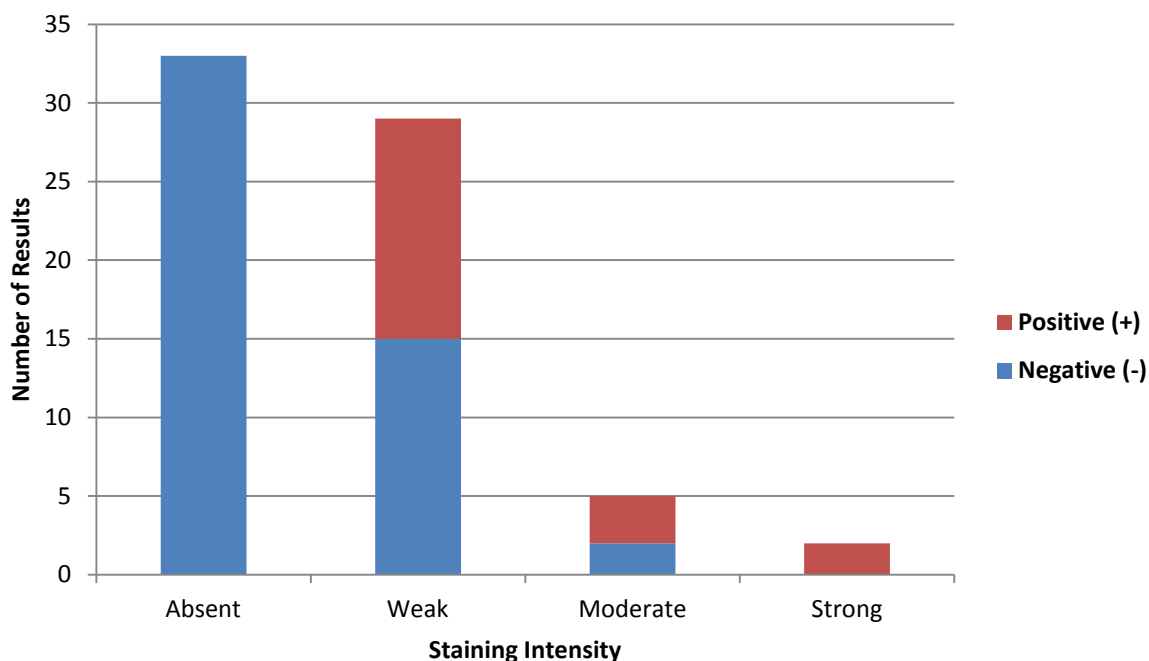
CD8 Overall Results Chart



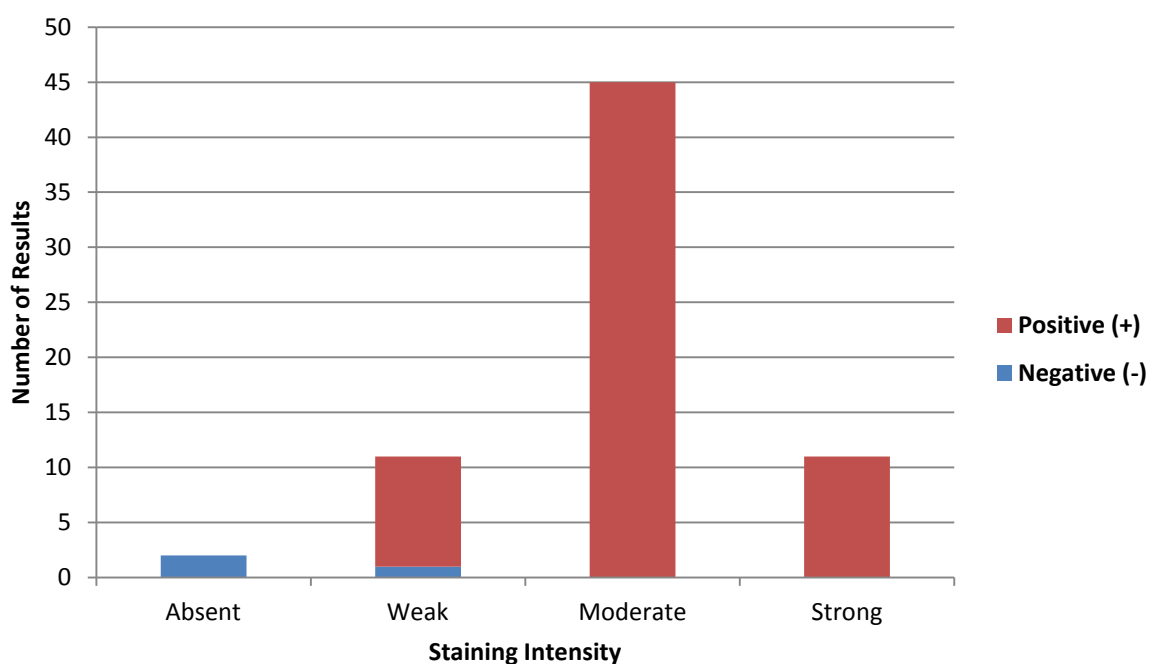
CD38 Overall Results Chart



CD79b Overall Results Chart



CD200 Overall Results Chart



Flow chart of laboratory testing pathways analysis

Report issue Date: 27 October 2016 ;

Version: 1.1.0

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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

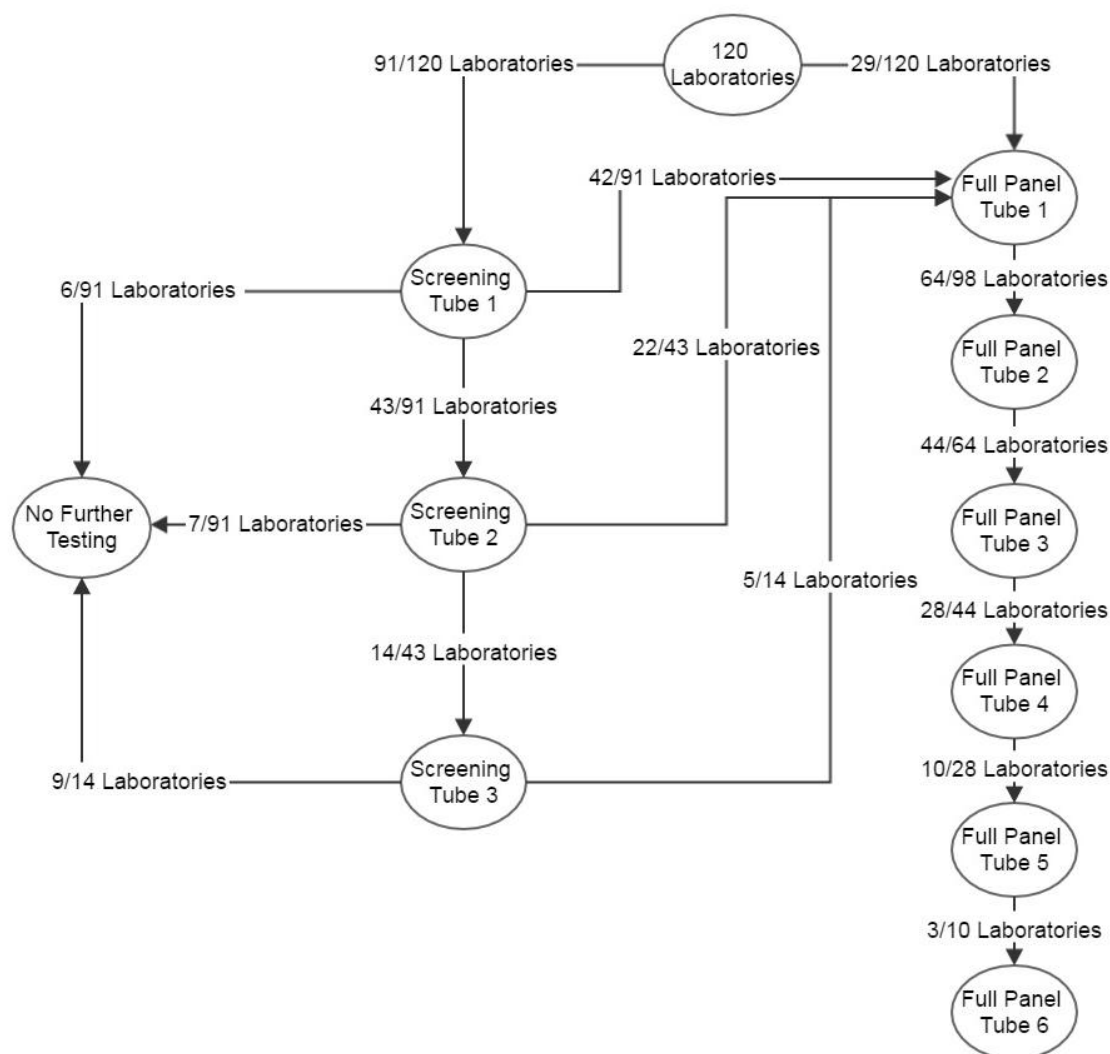


Table showing the breakdown of participant antibody usage per analysis tube

Tube Position in Analysis	Antibodies Per Tube			
	1 to 5	6 to 10	11 to 15	15 to 20
Screening Tube 1	24	53	15	0
Screening Tube 2	14	31	1	0
Screening Tube 3	7	9	0	0
Full Panel Tube 1	36	57	8	4
Full Panel Tube 2	41	30	0	0
Full Panel Tube 3	37	12	0	0
Full Panel Tube 4	29	6	0	0
Full Panel Tube 5	16	2	0	0
Full Panel Tube 6	7	1	0	0

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. Additionally the 'Total Results' column reflects all results returned however as small user groups are not shown on the table individual row totals may differ from that shown in the 'Total Results' column.

Antigen	Intensity	Positive/Negative	Total Results	Becton Dickinson Biosciences	Beckman Coulter	BioLegend	Cytognos	Dako	eBioscience	Immunotech
CD10	Absent	Negative (-)	99	46	40			5	1	3
	Weak	Negative (-)	4	2	2					
	Moderate	Negative (-)	1		1					
		Positive (+)	1	1						
	Strong	Negative (-)	1		1					
CD19	Weak	Positive (+)	66	20	37			5		4
	Moderate	Negative (-)	1	1						
		Positive (+)	193	103	65	6	2	1	2	10
	Strong	Positive (+)	54	19	21			3	3	8
CD20	Absent	Negative (-)	2							1
	Weak	Negative (-)	2		2					
		Positive (+)	64	35	18	6	2	2		
	Moderate	Negative (-)	2		1	1				
		Positive (+)	73	44	12	7	2			4
	Strong	Positive (+)	10	4	5				1	
CD200	Absent	Negative (-)	2		1					
	Weak	Negative (-)	1							1
		Positive (+)	10	5	2				2	
	Moderate	Positive (+)	45	25	9	1			4	
	Strong	Positive (+)	11	7	1				2	

Antigen	Intensity	Positive/Negative	Total Results	Becton Dickinson Biosciences	Beckman Coulter	BioLegend	Cytognos	Dako	eBioscience	Immunotech
CD23	Absent	Negative (-)	6	1	2			1	1	
	Weak	Negative (-)	9	4	3			1		
		Positive (+)	35	15	8	1		8		2
	Moderate	Positive (+)	42	28	9			3		1
	Strong	Positive (+)	9	6	2			1		
CD3	Absent	Negative (-)	51	26	15	1	2	1	3	3
	Weak	Negative (-)	4		3			1		
	Moderate	Negative (-)	30	13	13					1
		Positive (+)	3		3					
	Strong	Negative (-)	18	10	6					1
		Positive (+)	5	3	2					
CD38	Absent	Negative (-)	15	10	4					1
	Weak	Negative (-)	21	9	8			2	1	
		Positive (+)	18	6	7	1	2			1
	Moderate	Negative (-)	5	4						1
		Positive (+)	10	6	4					
	Strong	Positive (+)	4	2					2	
CD4	Absent	Negative (-)	28	16	6		2	2	1	1
	Weak	Negative (-)	9	1	6	1		1		
	Moderate	Negative (-)	15	8	7					
		Positive (+)	3	1	2					
	Strong	Negative (-)	10	5	4	1				
		Positive (+)	4	1	3					
CD45	Weak	Positive (+)	4	3	1					
	Moderate	Negative (-)	1	1						
		Positive (+)	80	47	23					8
	Strong	Negative (-)	2	1	1					
		Positive (+)	188	84	68	2	2	1	6	12

Antigen	Intensity	Positive/Negative	Total Results	Becton Dickinson Biosciences	Beckman Coulter	BioLegend	Cytognos	Dako	eBioscience	Immunotech
CD5	Absent	Negative (-)	3	1	2					
	Weak	Negative (-)	1		1					
		Positive (+)	32	18	13		1			
	Moderate	Positive (+)	93	51	27	1	3	2	1	4
	Strong	Negative (-)	1	1						
		Positive (+)	32	16	10			1	1	4
CD79b	Absent	Negative (-)	33	26	2			4		1
	Weak	Negative (-)	15	11	2			1		1
		Positive (+)	14	6	5	1		1		1
	Moderate	Negative (-)	2	1	1					
		Positive (+)	3		3					
	Strong	Positive (+)	2		1				1	
CD8	Absent	Negative (-)	29	13	7		4	2	1	1
	Weak	Negative (-)	6	1	4	1				
	Moderate	Negative (-)	16	6	9					
		Positive (+)	3		3					
	Strong	Negative (-)	11	7	3		1			
		Positive (+)	2	2						
Kappa	Absent	Negative (-)	41	7	3		4	27		
	Weak	Negative (-)	1					1		
		Positive (+)	12	4	3			4		1
	Moderate	Negative (-)	1	1						
		Positive (+)	12	3	4			2		1
	Strong	Positive (+)	6	2	2				1	1
Lambda	Absent	Negative (-)	35	5	10		1	16	1	2
	Weak	Negative (-)	2		1			1		
		Positive (+)	14	5			2	7		
	Moderate	Negative (-)	3	1	2					
		Positive (+)	12	3			2	7		
	Strong	Positive (+)	6	1	1			4		

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. Additionally the 'Total Results' column reflects all results returned however as small user groups are not shown on the table individual row totals may differ from that shown in the 'Total Results' column

Antigen	Intensity	Positive/ Negative	Total Results	AlexaFluor 750	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 (-)	99	2	15	1	3	2	7			39	4	2	14					
	Weak	Negative (-)	4									2			2					
	Moderate	Negative (-)	1						1											
		Positive (+)	1												1					
	Strong	Negative (-)	1																	
CD19	Weak	Positive (+)	66		3	2	2	26	1			1	7	1	12	1	1	6		
	Moderate	Negative (-)	1															1		
		Positive (+)	193	1	19	4	7	18	1		2	8	11	5	64	6	10	19	1	
	Strong	Positive (+)	54	1	8			7			1	4	2	2	18	1	1	1		
CD20	Absent	Negative (-)	2										1							
	Weak	Negative (-)	2								1				1					
		Positive (+)	64		3	3	2	3	11		11	1	2		5	2		1	16	
	Moderate	Negative (-)	2						1		1									
		Positive (+)	73	1	5	4	5	1	11		7	3	5		4	4	3	5	4	
	Strong	Positive (+)	10		2			1	1				1		2		1		2	
CD200	Absent	Negative (-)	2									1			1					
	Weak	Negative (-)	1									1								
		Positive (+)	10		5							3			1					
	Moderate	Positive (+)	45		13				1			18			5				5	
	Strong	Positive (+)	11		4				1			3			2		1			

Antigen	Intensity	Positive/ Negative	Total Results	AlexaFluor 750	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
CD23	Absent	Negative (-)	6		1							2						1		
	Weak	Negative (-)	9		1				3			2			2					
		Positive (+)	35		7			1	12			10			1		1	1		
	Moderate	Positive (+)	42		8				4			26								
	Strong	Positive (+)	9		1							7								
CD3	Absent	Negative (-)	51	4	16	1	4	3	3		2	1	3			2	1	2	2	1
	Weak	Negative (-)	4					1	1				1							
	Moderate	Negative (-)	30	1	2		4	4	4			1	4	1	1		2	1	3	
		Positive (+)	3										3							
	Strong	Negative (-)	18		4			1	6		2			3			1			
		Positive (+)	5	1	1				2											
CD38	Absent	Negative (-)	15		2	1	3		4			1			1					
	Weak	Negative (-)	21	1	3		4	1	5		1	2								
		Positive (+)	18						1			4	5	1	1			1		
	Moderate	Negative (-)	5			1	2						1		1					
		Positive (+)	10	1	1		1					4		1	1			1		
	Strong	Positive (+)	4		3															
CD4	Absent	Negative (-)	28		1				6		5	5	1		1	1			7	
	Weak	Negative (-)	9		1			1	2		1	4								
	Moderate	Negative (-)	15		2		1	1	4			5			1					
		Positive (+)	3		1							2								
	Strong	Negative (-)	10		2						1	2			5					
		Positive (+)	4								1	1			1					

Antigen	Intensity	Positive/ Negative	Total Results	AlexaFluor 750	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
CD45	Weak	Positive (+)	4				3	1												
	Moderate	Negative (-)	1																	
		Positive (+)	80	4	3	8	8	4	3	4	3		3	2	5	6	2	2		13
	Strong	Negative (-)	2				1			1										
		Positive (+)	188	4	2	6	16	7	7	26	2	1	7	4	25	8	8	2	5	35
CD5	Absent	Negative (-)	3									1						1		
	Weak	Negative (-)	1								1									
		Positive (+)	32		3			1	4			3	1	2	6		5	5		
	Moderate	Positive (+)	93	1	15		1	1	10		3	3	2	2	23		10	9		
	Strong	Negative (-)	1												1					
		Positive (+)	32		4			3	5		2	8	1	3	3		1	1		
CD79b	Absent	Negative (-)	33		10				5			8		1			1	7		
	Weak	Negative (-)	15		2				2			5		1				2		
		Positive (+)	14		2				2			7					1	2		
	Moderate	Negative (-)	2		1									1						
		Positive (+)	3		1							2								
	Strong	Positive (+)	2		1				1											
CD8	Absent	Negative (-)	29				2	2	13			4			2	2		1		1
	Weak	Negative (-)	6		1				3			1			1					
	Moderate	Negative (-)	16			2		3	3			2			1					1
		Positive (+)	3					2	1											
	Strong	Negative (-)	11		1	4			2		1	1	1		1					
		Positive (+)	2			1														

Antigen	Intensity	Positive/ Negative	Total Results	AlexaFluor 750	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
Kappa	Absent	Negative (-)	41		2				21			18								
	Weak	Negative (-)	1						1											
		Positive (+)	12						8			4								
	Moderate	Negative (-)	1									1								
		Positive (+)	12						10											
	Strong	Positive (+)	6						5			1								
Lambda	Absent	Negative (-)	35						7			28								
	Weak	Negative (-)	2						1			1								
		Positive (+)	14						5			9								
	Moderate	Negative (-)	3									3								
		Positive (+)	12						8			4								
	Strong	Positive (+)	6						3			3								