SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	1. Cell preparation											
Lab Code	Cell preparation method	Cell vol (µl)	Cell concentration	Diluent	Serum vol (µl)	Used as supplied	Diluted	Local negative control	Replicates	Local positive control	No of pos control replicates	No of test replicates
9	Density gradient centrifugation	200	2-3x10^6	RPMI with 10% FCS	50	Yes	1:2	Biosera	5	Pooled serum of sensitised patients	2	4
11		30	5x10^6	PBS/Azide (0.1%)	20	Yes		AB Serum (unsensitised male blood donors)	4	Pooled highly sensitised patient sera	1 weak, 1 strong	2
12	Density gradient centrifugation	25	10x10^6	McCoys 5A +0.1% sodium azide	25	Yes		NIBSC and AB Serum	4	Pooled serum	2	2
14	Lymphoprep	30	10^7	PBS/BSA/Azide	40	Yes		NIBSC	2	In-house	2	2
15		T: 25, B:50	10x10^6	FACS diluent	T: 25, B: 50	Yes		In-house	2	In-house	2	2
19												
20		50	5x10^6	PBS	50	Yes		In-house	4	NIBSC	2	4
23	Lymphoprep	50	5x10^6	PBS/1%FBS	50	Yes		NIBSC	3	In-house	2	3
24	Lymphocyte separation medium (Lympholyte)	50	2.5x10^6	HBSS	50	Yes		NIBSC	2	NIBSC and In-house	1	3
25	Lymphocyte separation medium	50	5M	1% PBSA	50			NIBSC	3	In-House	2	3
28	Lymphocyte density gradient method	30	16x10^6	PBA	50	Yes		Biorad	3	Campath	2	3
34	Carbonyl iron lympholyte + Dextran sulphate	50	6x10^6	TBS	50	Yes		Local	6	Local	2	3
35	Ficoll density gradient	50	5x10^6	PBS+1% FCS	50	Yes		NIBSC	3	In-house	3	3
38	Density gradient centrifugation	30	5M	PBS azide	20	Yes		NIBSC	2	In-House	4	
39	Lympholyte	50	1x10^7	RPMI	50	Yes		NIBSC	3	In-House	3	3
41	Density gradient	25	10x10^6	2% FBS/PBS	50	Yes		Seralab	3	In-house	3	
42		50	4x10^6	1% FCS/PBS	50				3		3	3
45		40	2.5x10^6	TPM	10	Yes		Local and NIBSC	2	2 Local and NIBSC	2	2
48		30	5x10^6	Local flow diluent	20	Yes		NIBSC	2	Local pool	2	2
51	Dendity gradient centrifugation	50	2x10^6	PBS	50	Yes		Local	3	Local	2	2
54	Ficoll Hypaque	100	2x10^6	PBS 0.1% azide 1% normal goat + 1% normal mous serum	50			SLI	6	In-House + Human monoclonal antibodies	1	
58		50	5.7x10^6	PBS	50	Yes		NIBSC	4	In-House poitive pool	3	3
62	Gradient centrifugation	50	5M	1% NBCF in PBS (PBSCS)	50	Yes		NIBSC	3	In-House	2	2
101		100			50				2		2	2
112	Concentartion gradient-lympholyte-H + Pronase Treatment	25	10x10^6	PBS	30	No		Human serum AB	2	Pool patients	2	
114	Density gradient separation	100	2.5x10^5	AB sera	20	No		Pooled AB sera	2	Pooled sensitized patients	2	2
115		250,000			25	Yes		NIBSC	2	NIBSC and In-House	1	2
116	Treatment with RBC lysis bufffer	200		PBS/sodium azide	50	Yes		Local	2	Local	2	2
117	Ficoll		200,000	RPMI with 20% FBS	50	Yes		NIBSC	3	Local pool	3	
118	200	10	1x10^6	PBS		No		Local + NIBSC	2	Local	2	2
119	Density gradient	100-120	200000	PBS	100	Yes		AB+ male	4	Patient pool	3	
120	Cell wash centrifugation suspended in Hanks	100	5M	PBS1x -BSA1%	20	Yes		AB serum	2	Hyperimmunised serum	2	2
122		50	3x10^5	PBS+2% FCS	50	Yes		NIBSC	2	Local	1 weak, 1 strong	2
126												
130			0.25-0.3x10^6		50	Yes		In-House	2	In-house	1 weak, 1 strong	2
133	Buffy coat (without density gradient)	50	Visual check	RPMI	50	Yes	1/2	AB serum	1	PRA positive (>10000MFI or 100%+)	1	2
136	Ficoll hypaque separation	100	2.5x10^6	RPMI	20	No		AB serum	3	Hypersensitized sera pool	2	2
138												
139		50	6000	RPMI-SVF (5%)	50	Yes		AB serum	2	Hyperimmunixed patient pool	1	1
142		2000	6-15	PBS	20	Yes	1:8		3		1	1
143	Ficoll layering (Lymphoprep) and magnetic nanoparticle separation	40	3x10^6	FBS	50	Yes		In-house	2	Local pooled sera	1	2
144	Ficoll separation	25	15x10^6	PBS	25	Yes		Local	1	Local	2	2
145	Ficoll	50	1x10^7	PBS	50	Yes		Local	1	Biorad	1	1
147	Ficoll	100	5000	RPMI	50	Yes		Human serum AB	2	Serum from polcry immunised patients	2	2
154		20	15x10^6	PBS BSA 1%	20	Yes		Commercial	2	Commercial	2	
157		15	1.5x10^5	PBS	50	Yes		In-house	2	Hyperimmunixed patient pool	2	2
159		100	3000	PBS BSA 1%	50	Yes		Local	3	Local	2	2
160	Cell wash centrifugation suspension in RPMI+2%FBS	50	10^6	RPMI+2%FBS	50	Yes	2	Local	2	Biotest	1	2
163	Lymphocyte separation medium	0	0.2x10^6		50	Yes		NIBSC	2	NIBSC	2	2
167												
169												
176	PBMC isolation (Lymphoprep	100	2E6/ml	TBS	100	Yes		AB donor pool	1	Local serum	1	1
186	Ficoll	100	5M		25	Yes		Pool sera for AB donors	1	Local sera from immunized patients	2	1
190	Ficoll	100	200000	NACL	50			SAB male	3	Pool hyperimmunised	2	3
191	Isolated cells sent were used. Washed with PBS	100	3.5x10^5	PBS	20	Yes	1/4	AB+ serum	1	Pooled positive sera	2	2
193	Manual	100	3000-5000	RPMI	20	Yes		Pooled sera	2	2 pooled sera	2	2
194		100	2400-3800	PBS	20	Yes		In-house	2	In-house	1	2
195		25	20000	RPMI	50	Yes		NIBSC	3	Local	1	2
201		50	1-1.5x10^7	20% medium (IMDM+FCS)	50	Yes		Local NC	1	Local PC	1	1
202												
204	Ficoll separation	50	6000	RPMI	50	Yes		Pool of AB sera from healthy blood donors	2	Poll of sera from hypersensitized recipients	1	1
209												
218	Ficoll	400			200			Local	2	Local	1	1
220	Pronase-treatment	15	10000	2% FCS0, 5% NaN3-PBS	15	Yes		In-house	4	In-house	2	2
227	STEMCELL	50	6x10^6	NaCl 0.9%	50	Yes		In-house	2	In-house	2	2
235	Ficoll hypaque	50	600000	RPMI+FCS	50	Yes	1/2	AB non sensitized donor	2	Hypersensitized patient	1	1
238	Lymphocytes Separation Medium	50	10M	RPMI	50	Yes		Pool AB human sera	2	Pool hyperimmunised patient's sera	2	2
245	Whole blood	100	2500	PBS	75	Yes		NIBSC	3	NIBSC	2	2
246	Ficoll	150	400000	RPMI	150	Yes		NIBSC	4	NIBSC+BAG	1	1
252		25			25			AB + Healthy serum		PRA >80% serum		
262	Ficoll	50	500000	RPMI + Human SAB 10%	50			Human SAB	2	Immunised patient	1	2
271	Ficoll hypaque	Dried pellet	250000	PBS 2% FBS	25	Yes		AB NHS Invitrogen	2	In-house	1	2
284	Mononuclear cells isolated by density centrifugation over Ficoll	50	5x10^6	RPMI	50	Yes		NIBSC	3	Local + NIBSC	2	2
297		50			50			Local	2	Local	1	2
341	Density gradient centrifugation using Ficoll	50	5500	PBS+2% FCS	50	Yes	1/16	NC HLA Class I+II	2	PC HLA Class I+II	1	2
351	Ficoll hypaque	200	2-2.5x10^6		20	Yes		Local	1	Local	1	2

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	2. Sensitisation Stage							3. Detection Stage:							
Lab Code	Time (mins)	Temp (°C)	Manual Automated washer	Wash medium	Vol / tube (ml)	Washes	Temp (°C)	Manufacturer	Raised in	Labelled with	Dilution	Diluted in	Vol (µl)	Inc time (mins)	Inc temp (°C)
9	30	37	Manual	PBS	100	3	RT	Life Technologies	Goat	FITC/Fab2 fragment	1:20	PBS	50	30	4
11	30	37	Manual	Cold PBS/Azide	4	3	22	Sigma	Goat	FITC/Fab2 fragment	1/10	PBS/Azide	20	20	4
12	30	22	Manual	PBS/BSA/Azide	1.5	2	4	Southern Biotech	Goat	FITC	1:40	McCoys 5A +0.1% sodium azide	50	30	4
14	30	RT	Manual		2	2	RT	Sigma	Goat	FITC/Fab2 fragment	1/23	PBS/BSA/Azide	115	20	4
15	30	RT	Automated	PBS	3	3	RT	Sigma	Goat	FITC/Fab2 fragment	1:180	FACS diluent	100	15	4
19													50	30	4
20	15	37	Automated	PBS	2	2	2-8	Beckman Coulter	Goat	FITC/Fab2 fragment	1:20	CD3-PC5 and CD19-PE (detection cocktail)	5	15	2-8
23	30	37	Automated	PBS/FBS	1	3	4	Sigma	Goat	FITC/Fab2 fragment	1:40	PBS	50	30	4
24	30	37	Automated	BD cell wash	BD LWA	4ml x3	RT	Sigma	Goat	FITC/Fab2 fragment	1/15	PBS	5	30	2-8
25	15	37	Automated	1% PBSA	1	3	RT	Sigma	Goat	FITC/Fab2 fragment	1/50	1% PBSA	5	15	4
28	30	20	Manual	PBA	2.5	2	4	DAKO	Rabbit	FITC/Fab2 fragment	1:20	PBA	100	30	4
34	30	37	Automated	TBS	5	2	4	Sigma	Goat	FITC/Fab2 fragment	1/32	TBS	50	30	4
35	30	37	Manual	PBS+1% FCS	1.5	3	4	Sigma	Goat	FITC	1:300	dH2O	50	30	RT
38	30	22	Manual	PBS azide	4	2	22	Southern Biotech	Goat	FITC/Fab2 fragment	1/50	PBS azide	100	20	4
39	30	37	Manual	1% FBS in PBS buffer	4	3	RT	Sigma	Goat	FITC/Fab2 fragment	1:20	1% FBS in PBS buffer	4	20	4-8
41	20	22	Automated		4	3	22	Sigma	Goat	FITC/Fab2 fragment	1:2000	2% FBS/PBS	90	10	22
42	30	22	Manual	1% FCS/PBS	2	2			Goat	FITC/Fab2 fragment	1/10	PBS	10	15	22
45	30	RT	Manual	PBA	1	2	RT	BioSciences	Goat	FITC/Fab2 fragment	1/50	PBA	50	45	Ice
48	30	21	Manual	Local flow diluent	2	3	21		Rabbit	FITC/Fab2 fragment	Neat		4	30	4
51	30	RT	Manual	PBS	3	2	RT	DAKO	Rabbit	FITC/Fab2 fragment	1:10	PBS	5	30	4
54	30	22	Yes	PBS 0.1% azide	4	2	4	Sigma	Goat	FITC/Whole IgG	1:20	PBS 0.1% azide 1% normal goat + 1% normal mous serum	100	30	4
58	30	37	Manual	PBS	4	3	4	Sigma	Goat	FITC/Fab2 fragment	1:50	PBS	5	30	4
62	30	37	Manual	PBSCS	4	3	22	DAKO	Rabbit	FITC/Fab2 fragment			12	30	22
101	30	Ambient	Manual	PBS	2-3	2	Ambient	Life Technologies	Goat	FITC/Fab2 fragment	Lot dependent	PBS	10	30	4
112	30	4	Automated	PBS	0.8	2	Ambient	Invitrogen	Goat	FITC/Fab2 fragment	1/60	PBS	80	10	4
114	30	RT	Manual	DPBS without Ca++	150	3	RT	DAKO	Rabbit	FITC/Fab2 fragment	1/30	PBS	20	20	4
115	30	4	Manual	PBS+2% FBS	0.4	3	4	Jackson	Goat	FITC/Fab2 fragment	1:160		20	30	4
116	30	RT	Manual	PBS/sodium azide	3	2	Ice	Jackson	Goat	FITC/Fab2 fragment	1:40	PBS	100	30	Ice
117	30	24	Manual	PBS	2	3	24	BD		FITC/Fab2 fragment			10	30	4
118	30	RT	Manual	PBS	3	4	RT	BD	Goat	Strepavidin FITC/Fab2 fragment/ Biotin IgG	1/50, 1/100	PBS	200	1.15, 2.10	RT
119	30	RT	Manual	PBS	2	3	RT	Jackson	Goat	FITC/Fab2 fragment	Optimum dilution after titration	H2O distiled 1ml	10	30	RT
120	30	RT	Manual	PBS1x - BSA1%	2	3	RT		Goat	FITC/Fab2 fragment	1/80	PBS1x - BSA1%	50	30	4
122	30	22		PBS+2% FCS	1	3	22	Invitrogen	Goat	FITC/Fab2 fragment	1:50	PBS+2% FCS	50	30	4
126													50	20	4-8
130	30	RT	Manual	Washing buffer	2	3	RT	Jackson	Goat	FITC/Fab2 fragment	1/80	Washing buffer	25	20	Ice
133	30	22	Manual	PBS	2	2	22	DAKO	Rabbit	FITC/Fab2 fragment	1/20	PBS	50	40	4
136	30	RT	Manual	PBS/azide/FCS 3%	200	4	RT	DAKO	Rabbit	FITC/Fab2 fragment	1/50	Gelatine/PBS/azide	20	20	4
138													10	20	4
139	30	22	Manual	PBS	0.2	3	22	Coulter	Goat	FITC/Fab2 fragment	1/40	PBS	10	30	4
142	30	4	Manual		0.2	3	4		Goat	FITC/Fab2 fragment			0.5	20	4
143	20	24	Manual	BD pharmingen stain buffer	1.5	2	2-4	Jackson	Goat	FITC/Fab2 fragment			10	15	2-4
144	30	RT	Manual		4	3	RT	DAKO	Rabbit	FITC/Fab2 fragment	1:25	PBS	25	30	4
145	30	RT	Manual	PBS	3	2	RT	Sigma	Goat	FITC/Fab2 fragment	1:40	PBS	50	20	4
147	30	4	Manual	PBS	2.5	3	22	Biorad	Goat	FITC/Fab2 fragment	1/500	PBS	100	30	4
154	30	20	Manual	PBS-BSA1%	1	2	20	Beckman Coulter	Goat	FITC/Fab2 fragment	1:250	PBS-BSA1%	50	30	20
157	30	4	Manual	PBS 0.02% NaN3 2% FBS	2	2	4	DAKO	Rabbit	FITC/Fab2 fragment	1:3	PBS	20	20	4
159	30	22	Manual	PBS-BSA1%	1	3	4	Biorad	Sheep	FITC/Fab2 fragment	1/500	PBS-BSA1%	100	30	4
160	30	37	Manual	BD cell wash	2	2	RT	Sigma	Goat	FITC/Fab2 fragment	1:80	PBS	50	30	4
163	30	Ambient	Manual	PBS 1X/BSA 0.5%	2	3	20	Jackson	Goat	FITC/Fab2 fragment	1:200	PBS-BSA1%	20	30	4
167													20	30	4
169															
176	30	20	Manual	TBS	3	3	18	DAKO	Rabbit	FITC/Fab2 fragment			5	20	20
186	30	4	Manual		2	2	4	Beckman Coulter	Goat	FITC/Fab2 fragment	1/200	PBS + FBS 8%	50	30	4
190	30	20	Manual	PBS/BSA/Azide	0.2	4	20	Jackson	Goat	FITC/Fab2 fragment			40	30	4
191	30	RT	Automated	PBS	5	2	RT	Jackson	Goat	Phycoerythrin/Fab2 fragment	1/100	PBS	5	20	RT
193	30	22	Manual	PBS-BSA1%	2	3	RT	Jackson	Goat	FITC/Fab2 fragment	1/60	PBS-BSA1%	50	30	4
194	30	22	Manual	PBS-BSA	2	2	22	Beckman Coulter	Goat	FITC/Fab2 fragment	1/100	PBS	50	30	5
195	30	22	Manual	PBS	4	2	22	DAKO	Rabbit	FITC/Fab2 fragment	1/50	PBS	100	30	22
201	30	22	Manual	PBS	4	3	22	Jackson	Goat	FITC/Fab2 fragment	1:100	PBS	5	30	22
202															
204	30	22	Manual	PBS	0.2	4	22	Beckman Coulter	Goat	FITC/Fab2 fragment	1/400	PBS	10	30	22
209															
218	60	37	Manual	PBS	5	2	RT	Abcam	Goat				5	30	RT
220	30	4	Manual	2% FCS0, 5% NaN3-PBS	200	2	4	Jackson	Goat	Phycoerythrin/Fab2 fragment	512	PBS-CD3-CD19	50	30	4
227	30	22	Manual	PBS	0.2	3	22	Jackson	Goat	Phycoerythrin/Fab2 fragment	1/10	PBS	10	30	4
235	30	4	Manual	PBS	1	2	4	DAKO	Rabbit	Phycoerythrin/Fab2 fragment	1/10	PBS	25	30	4
238	30	22	Manual	PBS	4	2	22	Biorad	Sheep	Phycoerythrin/Fab2 fragment	1/80	PBS	100	20	22
245	30	22-24	Manual	PBS + SVF	3	2	22-24	DAKO	Rabbit	FITC/Fab2 fragment			10	30	22-24
246	30	20	Manual	FACS Flow	10	3	20		Mouse	FITC/Whole IgG			5	10	20
252	30	RT	Manual	PBS x1	1	3	RT		Rabbit	FITC/Fab2 fragment	1x	PBS x1	50	30	RT
262	30	20-25	Manual		3	2	20-25	Biorad	Sheep	FITC/Fab2 fragment	1/100	PBS	100	20	4
271	30	4	Manual	PBS 2% FBS	0.5	3	20-25	Jackson	Goat	FITC/Fab2 fragment	1/100	PBS 2% FBS	20	20	4
284	30	22	Manual	Cell wash BD	2	2	22	DAKO	Rabbit	FITC/Fab2 fragment			10	30	4
297	30	22	Manual	PBS	0.2	3	22		Goat	FITC/Fab2 fragment	1/40	PBS		30	4
341	30	22-25	Manual	PBS+2%FCS	1	3	22-25	Invitrogen	Goat	FITC/Fab2 fragment	1000	PBS+2%FCS	50	30	4
351	30	4	Manual	Cold PBS Azide	1	5	4	DAKO	Rabbit	FITC/Fab2 fragment			3	30	4

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	4. Anti-T cell reagent																		
Lab Code	Manufacturer	IgG added with anti-T cell reagent	Wash step before adding anti-T cell reag	Wash step	Wash medium	Vol / tube (ml)	Number of washes	Temp (°C)	Anti-T cell reagent added with the anti-B cell reagent	Labelled with	CD3	Other	Diluted	Diluent	Dilution	Vol / tube (µl)	Inc time (mins)	Inc temp (°C)
9	Beckman Coulter	Yes		Manual	PBS	100	3	RT		Phycoerythrin-cyanine 5	Yes		Yes	PBS	1:30	50	30	4
11		No		Manual	Cold PBS/Azide	4	1	22	Yes	PerCp	Yes		No			3	20	4
12	BD	No	Yes	Manual	PBS/BSA/Azide	1.5	2	4	Yes	PE-Cy7	Yes		No			3	20	4
14	BD	Yes							Yes	PE	Yes		Yes		1/11.5	115	20	4
15	DAKO	No	Yes	Automated		3	1	RT	No		Yes		Yes	FACS diluent	1:60	100	15	4
19													No			10	30	4
20	Beckman Coulter	Yes							Yes	Phycoerythrin-cyanine 5	Yes		Yes	Detection cocktail	1:2	5	15	2-8
23	BD	Yes							No	PE	Yes		No			5	30	4
24	BD	Yes							Yes	Phycoerythrin	Yes		No			5	30	2-8
25	BD	No	Yes	Automated	1% PBSA	1	3	RT	Yes		Yes	PE	No			5	15	4
28	BD	Yes									Yes		No			5	30	4
34	BD	Yes							No	Phycoerythrin	Yes		No			5	30	4
35	DAKO	No	Yes	Manual		1.5	3	4	No	R-Phycoerythrin	Yes		No			10	20	2-8
38	Beckman Coulter	No	Yes	Manual	PBS azide	4	1	4	Yes	Phycoerythrin-cyanine 5	Yes		Yes	PBS azide	1/20	100	30	4
39	DAKO	No	Yes	Manual	1% FBS in PBS buffer	4	1	RT	Yes	RPE	Yes		No			4	20	4-8
41	BD	Yes							Yes		Yes	APC	Yes	2% FBS/PBS	1:20	4.75	10	22
42	DAKO	No	Yes	Manual	1% FCS/PBS				Yes	RPE	Yes		No			5	15	22
45	Beckman Coulter	No	Yes	Manual		1	1	RT			Yes		Yes	PBA	1/10	50	15	Ice
48	DAKO	Yes							Yes		Yes	PE				4	30	4
51	Beckman Coulter	Yes		Manual	PBS	3	2	4	Yes	ECD	Yes		Yes	PBS	1:10	5	30	4
54	BD	No	No						No		Yes	PE				5	15	4
58		Yes							Yes	Phycoerythrin-cyanine 5	Yes		No			5	30	4
62	Beckman Coulter	No	Yes	Manual	PBSCS	4	2	22	Yes	Phycoerythrin-cyanine 5	Yes		No			5	15	22
101	Beckman Coulter	Yes							Yes	A750	Yes		No			5	30	4
112	Iotest	No	No						Yes	PE	Yes		No			5	20	4
114	Beckman Coulter	Yes		Manual	DPBS without Ca++	150	1	4	Yes	Phycoerythrin	Yes		No			20	20	4
115	BD	Yes		Manual	PBS+2% FBS	0.4	1	4	Yes	PerCp	Yes		Yes	PBS+2% FBS	1:4	20	30	4
116	Beckman Coulter	Yes							Yes	Allophycocyanin	Yes		No			2	30	Ice
117	BD	No	No						Yes	Phycoerythrin	Yes		No			10	30	4
118	BD	No	No	Manual	PBS	3	2	RT	No	PE	Yes		Yes	PBS	1/20	200	15	RT
119	Beckman Coulter	Yes	No	Manual	PBS	2	3	RT	Yes	Phycoerythrin-PE	Yes		No			10	30	RT
120	Beckman Coulter	Yes		Manual		2	3	RT	Yes		Yes	PC7	No			10	30	4
122	Immunostep	Yes							Yes		Yes	PerCp	Yes	PBS+2% FCS	1:16	50	30	4
126													No			2.5	20	4-8
130	BD	Yes		Manual	Washing buffer	2	2	RT	No		Yes	Phycoerythrin	Yes	Washing buffer	1/4	25	20	Ice
133	BD	Yes							Yes	PerCp	Yes		No			15	40	4
136	DAKO	Yes								PE - Phycoerythrin	No	CD2	No			5	20	4
138													No			10	15	4
139	Coulter	Yes							Yes	APC	Yes		No			10	30	4
142		Yes		Manual		0.2	2	4	Yes	APC-CD3			No			5	20	4
143	BD	No	Yes	Manual	BD pharmingen stain buffer	20	1	2-4	No		Yes	PE	No			20	15	2-4
144	BD	Yes							Yes	PE	Yes		Yes	PBS	1:25	25	30	4
145	BD	Yes		Manual	PBS	3	1	RT	No	PE	Yes		No			10	20	4
147	BD	Yes	No	Manual	PBS	2.5	3	22	Yes		Yes	PE	No			10	30	4
154	Beckman Coulter	Yes		Manual	PBS-BSA1%	1	2	20	Yes	Phycoerythrin	Yes	PE	No			15	30	20
157	BD	No	No	Manual	PBS 0.02% NaN3 2% FBS	2	1	4	Yes	PerCp	Yes		Yes	PBS	1:10	20	20	4
159	Beckman Coulter	Yes		Manual		1	3	4	Yes		Yes	PC7	No			10	30	4
160	BD	No	Yes	Manual	BD cell wash	2	2	RT	No	Phycoerythrin	Yes		Yes	BD cell wash	1:10	50	30	4
163	Beckman Coulter	No	No						Yes	PE	Yes		No			4	30	4
167													No			5	30	4
169																		
176	BD	No	Yes	Manual	TBS	3	1	18	Yes	Phycoerythrin	Yes					10	20	20
186	Beckman Coulter	Yes		Manual	PBS + FBS 8%	2	1	4		PE	Yes		No			10	30	4
190	Beckman Coulter	Yes							Yes	Phycoerythrin-cyanine 5			No			5	30	4
191	BD	Yes		Automated	PBS	5	2	RT	Yes	PerCP	Yes		No			5	20	RT
193	BD	No	Yes	Manual	NH4CL	2	1	4	Yes	Phycoerythrin	Yes		No			20	15	4
194	Beckman Coulter	Yes		Manual		2	2	22	Yes	PE	Yes		No			5	30	5
195		Yes							Yes	V450	Yes		No			5	30	22
201	BD	Yes	No						Yes	PE	Yes		No			5	30	22
202																		
204	Beckman Coulter	Yes	No						Yes	APC	Yes		No			10	30	4
209																		
218	BD	No	Yes	Manual	PBS	5	2	RT	Yes	APC	Yes		No			5	15	RT
220	eBioscience	Yes							Yes	450nm	Yes		Yes	2% FCS0, 5% NaN3-PBS	512	50	30	4
227	Beckman Coulter	Yes							Yes	ECD	Yes		No			10	30	4
235	BD	Yes		Manual	PBS	1	2	4	No	Phycoerythrin	Yes		No			3	30	4
238	Beckman Coulter	Yes							Yes	Phycoerythrin	Yes		No			5	20	22
245	Beckman Coulter	Yes		Manual		3	2	22-24	Yes	APC	Yes		No			5	30	22-24
246	BD	Yes							Yes	PerCp	Yes		No			5	10	20
252	BD	Yes							Yes	Phycoerythrin-cyanine 5	Yes		No			5	30	RT
262	BD Biosciences	Yes		Manual	PBS	3	1	20-25	Yes	Phycoerythrin	Yes		No			5	20	4
271	BD	Yes	No	Manual		0.5	1	RT	Yes	PerCp	Yes		Yes	PBS 2% FBS	1/4	20	20	4
284	BD	Yes		Manual	Cell wash BD	2	2	22	Yes	APC	Yes		No			10	30	22
297		Yes							Yes		Yes	PC7	No			5	30	4
341	BD	Yes							Yes	PerCp	Yes		Yes	PBS+2%FCS	10	5	30	4
351	BD	Yes		Manual	Cold PBS Azide	1	5	4	Yes	Phycoerythrin-cyanine 5	Yes		No			4	30	4

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	5. Anti-B cell reagent																		
Lab Code	Manufacturer	IgG added with anti-B cell reagent	Wash step before adding anti-B cell reag	Wash step	Wash medium	Vol / tube (ml)	Number of washes	Temp (°C)	Labelled with	CD19	CD20	Other	Diluted	Diluent	Dilution	Vol / tube (µl)	Inc time (mins)	Inc temp (°C)
9																		
11		No	Yes	Manual	Cold PBS/Azide	4	1	22	Phycoerythrin	Yes			No			3	20	4
12	BD	No	Yes	Manual	PBS/BSA/Azide	1.5	2	4	APC	Yes			No			3	20	4
14	DAKO	Yes							Phycoerythrin-cyanine 5	Yes			Yes	PBS/BSA/Azide	1/23	115	20	4
15		No	Yes	Automated		3	1	RT		Yes			Yes	FACS diluent	1:50	100	15	4
19													No			5	30	4
20		Yes							Phycoerythrin	Yes			Yes	Detaction cocktail	1:2	5	15	2-8
23	BD	Yes							Phycoerythrin	Yes			No			5	30	4
24	BD	Yes							Allophycocyanin	Yes			No			5	30	2-8
25	BD	No	Yes	Automated	1% PBSA				APC	Yes			No			5	15	4
28	BD	Yes							Phycoerythrin	Yes			No			5	30	4
34	BD	Yes							Phycoerythrin		Yes		No			5	30	4
35																		
38	Beckman Coulter	No	Yes	Manual	PBS azide	4	1	4	Phycoerythrin	Yes			Yes	PBS azide	1/20	100	30	4
39	DAKO	No	Yes	Manual	1% FBS in PBS buffer	4	1	RT	Phycoerythrin-cyanine 5	Yes			No			4	20	4-8
41	BD	Yes	No						BV241	Yes			Yes	2% FBS/PBS	1:80	0.25	10	22
42	DAKO								Phycoerythrin	Yes			No			5	15	22
45																		
48	DAKO	Yes							Phycoerythrin-cyanine 5	Yes						4	30	4
51	Beckman Coulter	Yes		Manual	PBS	3	2	4	Phycoerythrin	Yes			Yes	PBS	1:10	5	30	4
54	BD	No	No	Manual					Phycoerythrin		Yes		No			10	15	4
58		Yes							Phycoerythrin	Yes			No			5	30	4
62	Beckman Coulter	Yes	No						Phycoerythrin-cyanine 5	Yes			No			5	15	22
101	Beckman Coulter	Yes							ECD	Yes			No			5	30	4
112	Iotest	No	No						Phycoerythrin-cyanine 5	Yes			No			7	20	4
114	BD	Yes		Manual	DPB without Ca++	150	1	4	Phycoerythrin-cyanine 5	Yes			No			5	20	4
115	BD	Yes		Manual	PBS+2% FBS	0.4	1	4	PE	Yes			Yes	PBS+2% FBS	1:4	20	30	4
116	Beckman Coulter	Yes							R-Phycoerythrin-cyanine 7	Yes			No			2	30	Ice
117	BD	No	No						Phycoerythrin-cyanine 5	Yes			No			10	30	4
118	DAKO	Yes		Manual		3	1	RT	Phycoerythrin	Yes			Yes	PBS	1/200	200	15	RT
119	Beckman Coulter	Yes	No	Manual	PBS	2	3	RT	Phycoerythrin-cyanine 5	Yes			No			10	30	RT
120	Beckman Coulter	Yes		Manual		2	3	RT	Phycoerythrin	Yes			No			10	30	4
122	Beckman Coulter	Yes							Phycoerythrin	Yes			Yes	PBS+2% FCS	1:16	50	30	4
126																2.5	20	4-8
130	BD	Yes		Manual	Washing buffer	2	2	RT	Phycoerythrin	Yes			Yes	Washing buffer	1/2	25	20	Ice
133	BD	Yes							Phycoerythrin	Yes			No			15	40	4
136																		
138																		
139	Coulter	Yes							Phycoerythrin	Yes			No			10	30	4
142		Yes		Manual		0.2	2	4	Phycoerythrin	Yes			No			10	20	4
143																		
144	BD	Yes							APC	Yes			Yes	PBS	1:25	25	30	4
145	Immunotech	Yes		Manual	PBS	3	1	RT	Phycoerythrin	Yes			No			10	20	4
147	BD	Yes		Manual		2.5	3	22	APC	Yes			No			10	30	4
154	Beckman Coulter	Yes		Manual	PBS-BSA1%	1	2	20	Phycoerythrin-cyanine 5	Yes			No			10	30	20
157	BD	No	No	Manual	PBS 0.02% NaN3 2% FBS	2	1	4	Phycoerythrin	Yes			Yes	PBS		20	20	4
159	Beckman Coulter	Yes	No	Manual		1	3	4	Phycoerythrin	Yes			No			20	30	4
160	BD	No	Yes	Manual	BD cell wash	2	2	RT	Phycoerythrin	Yes			Yes	BD cell wash	1:10	50	30	4
163	Beckman Coulter	No	No						Phycoerythrin-cyanine 5	Yes			No			2	30	4
167													No			5	30	4
169																		
176	BD	No	Yes	Manual	TBS	3	1	18	PerCp		Yes					10	20	20
186	Beckman Coulter			Manual	PBS + FBS 8%	2	1	4	Phycoerythrin	Yes			No			10	30	4
190		Yes								Yes						5	30	4
191	BD	Yes		Automated	PBS				FITC	Yes			No			5	20	RT
193	BD	No	Yes	Manual	NH4CL	2	1	4	Phycoerythrin-cyanine 5	Yes			No			20	15	4
194	Beckman Coulter	Yes		Manual		2	2	22	Phycoerythrin-cyanine 5	Yes			No			5	30	5
195		Yes							Per-Cp Cy5.5	Yes			No			20	30	22
201	Beckman Coulter	Yes	No						Phycoerythrin-cyanine 5	Yes			No			5	30	22
202																		
204	Beckman Coulter	Yes	No		PBS				Phycoerythrin	Yes			No			10	30	4
209																		
218	Beckman Coulter	No	Yes	Manual	PBS	5	2	RT	Phycoerythrin	Yes			No			10	15	RT
220	eBioscience	Yes							APC	Yes			Yes	PBS-CD3-IgG	512	50	30	4
227	Beckman Coulter	Yes							Phycoerythrin	Yes			No			10	30	4
235	BD	Yes		Manual	PBS	1	2	4	Phycoerythrin	Yes			No			3	30	4
238	Beckman Coulter	Yes							APC	Yes			No			5	20	22
245	Beckman Coulter	Yes		Manual		3	2	22-24	PC7	Yes			No			5	30	22-24
246		Yes							Phycoerythrin	Yes			No			5	10	20
252		Yes							Phycoerythrin	Yes			No			5	30	RT
262	Beckman Coulter	Yes		Manual	PBS	3	1	4	APC	Yes			No			5	20	4
271	BD	Yes	No	Manual	PBS 2% FBS	0.5	1	4	Phycoerythrin	Yes			Yes		1/4	20	20	4
284	BD	Yes		Manual		2	2	22	BV421	Yes			No			2.5	30	4
297		Yes							APC	Yes			No			5	30	4
341	BD	Yes							Phycoerythrin	Yes			Yes	PBS+2%FCS	10	5	30	4
351	BD	Yes		Manual	Cold PBS Azide	1	5	4	R-Phycoerythrin	Yes			No			3	30	4

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	6. Final wash						
Lab Code	Wash step	Wash medium	Vol / tube (ml)	Number of washes	Temp (°C)	Resuspension of cells in	Vol / tube (µl)
9	Manual	PBS	100	3	RT	Phosphate buffered saline	400
11	Manual	PBS/Azide	4	1	22	Phosphate buffered saline	400
12	Manual	PBS/BSA/Azide	1.5	1	4	Fixative	250
14	Manual		2	2	RT	PBS/BSA/Azide	300
15	Automated	PBS	3	1	RT	FACS diluent	200
19							
20	Automated	PBS	2	1	2-8	Phosphate buffered saline	300
23	Automated	PBS/FBS	1	3	4	Fixative	300
24	Automated	BD cell wash	BD LWA	4ml x1	RT	BD cell wash	300
25	Automated		1	3	RT	1% PBSA	500
28	Manual	PBA	2.5	2	4	Sheath fluid	250
34	Automated		5	1	4	TBS	280
35	Manual		2	3	4	Phosphate buffered saline	150
38	Manual	PBS azide	4	1	4	PBS azide	500
39	Manual	1% FBS in PBS buffer	4	1	RT	1% FBS in PBS buffer	250
41	Automated		4	1	22	2% FBS/PBS	300
42	Manual	1% FCS/PBS	2	2	22	1% FCS/PBS	280
45	Manual		1	1	RT	PBA	220
48	Manual		4	1	21	Local flow diluent	500
51							
54	Manual	PBS 0.1% azide	4	1	4	Sheath fluid	100
58	Manual		4	1	4	Phosphate buffered saline	
62	Manual	PBSCS	4	1	22	PBSCS	0.5
101	Manual	PBS	2-3	2	Ambient	Phosphate buffered saline	
112	Automated	PBS	0.8	2	Ambient	Fixative	450
114	Manual	DPBS without Ca++	150	1	4	Fixative	150
115	Manual	PBS+2% FBS	0.4	1	4	Fixative	300
116	Manual	PBS/sodium azide	3	2	Ice	Phosphate buffered saline	500
117	Manual	PBS	2	1	24	Phosphate buffered saline	650
118	Manual	PBS	3	1	RT	Phosphate buffered saline	200
119	Manual	PBS	2	3	RT	Phosphate buffered saline	500
120	Manual		2	1	RT	PBS1x - BSA1%	400
122	Manual	PBS+2% FCS	1	2	22	Fixative	350
126							
130						Fixative	100
133	Manual		2	2	RT	Phosphate buffered saline	
136	Manual	PBS/azide/FCS 3%	200	1	RT	Phosphate buffered saline	400
138							
139	Manual	PBS	0	3	22	Phosphate buffered saline	450
142	Manual					Phosphate buffered saline	400
143	Manual	BD phatmingen stain buffer	1.5	1	2-4	Stain buffer	600
144	Manual		4	2	4	Fixative	100
145	Manual	PBS	3	1	RT	Fixative	500
147	Manual		2.5	3	22	Phosphate buffered saline	250
154	Manual		1	2	20	Fixative	
157	Manual	PBS 0.02% NaN3 2% FBS	2	1	4	Phosphate buffered saline	100
159	Manual		1	3	4	Phosphate buffered saline	400
160	Manual	BD cell wash	2	2	RT	BD cell wash	350
163	Manual	PBS 1X/BSA 0.5%	2	2	20	Phosphate buffered saline	100/150
167							
169							
176	Manual	TBS	3	1	18	Sheath fluid	500
186	Manual	PBS + FBS 8%	2	1	4	Phosphate buffered saline	300
190	Manual		0.2	4	20	Phosphate buffered saline	300
191	Automated	PBS	5	2	RT	Phosphate buffered saline	100
193	Manual	PBS-BSA1%	2	1	4	Phosphate buffered saline	250
194	Manual		2	2	22	Fixative	300
195	Manual	PBS	4	1	22	Phosphate buffered saline	250
201	Manual	PBS					
202							
204	Manual	PBS	0.2	4	22	Phosphate buffered saline	450
209							
218	Manual		5	1	RT	Phosphate buffered saline	300
220	Manual	2% FCS0, 5% NaN3-PBS	200	2	4	2% FCS0, 5% NaN3-PBS+7-AAD	120
227	Manual		0.2	3	22	Phosphate buffered saline	450
235	Manual	PBS	1	2	4	Phosphate buffered saline	500
238	Manual	PBS	4	1	22	Phosphate buffered saline	200
245	Manual	PBS + SVF	3	3	22-24	Phosphate buffered saline	300
246	Manual	FACS Flow	10	3	20	Sheath fluid	300
252	Manual	PBS x1	1	2	RT	Phosphate buffered saline	500
262	Manual		3	1	20-25	Phosphate buffered saline	250
271	Manual	PBS 2% FBS	0.5	1	20-25	PBS 2% FBS	300
284	Manual		2	2	22	Cell wash BD	500
297	Manual	PBS		3	22	Phosphate buffered saline	500
341	Manual	PBS	1	2	22-25	Phosphate buffered saline	300
351	Manual		1	5	4	Fixative	

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	7. Data Acquisition													
Lab Code	Flow cytometer	Live gated	Calibration beads used	Used for	Type of beads	Events Total events Min	Total events Max	T-cells Min	T-cells Max	B-cells Min	B-cells Max	Parameter used to assess peak performance	Scale used
9	Beckman Coulter	Yes	Yes	Alignment check, Fluorescence check	Flowcheck, Flow set	3000	5000	3000	5000			Median	Log channel
11	BD FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	CST						1000	Median	Log channel
12	BD FACS Canto	No	No	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST			5000		5000		Median	
14	FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	7 colour set-up			3000		3000		Median	Log channel
15	BD FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	CST			300	7500	300	7500	Median	Log channel
19													Log channel
20	Beckman Coulter FC500	No	Yes	Alignment check, Laser check, Fluorescence check	Flowcheck, Flow set, Immunobrite		200000	250	6000	100	5000	Median	Log channel
23	BD FACS Calibur	No	Yes	Alignment check, Laser check, Fluorescence check	Rainbow 8 peak, Calibrite				10000		10000	Median	Log channel
24	BD FACS Canto		Yes	Alignment check, Laser check, Fluorescence check	CST, 7 colour set-up	10000		500		200		Median	Linear channel
25	BD FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST, 7 colour set-up					1000		Median	Log channel
28	BD FACS Calibur	Yes	Yes	Alignment check, Laser check, Fluorescence check	BD Calibrite-3 colour kit			1000		1000		Median	Log channel
34	Beckman Coulter	No	Yes	Alignment check, Fluorescence check	Flowcheck, Immunobrite		10000	100		100		Median	Log channel
35	BD FACS Canto	Yes	Yes	Laser check, Fluorescence check	CST, 7 colour set-up	500		300				Median	Log channel
38	Beckman Coulter 500 MPL	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set					500		The median fluorescence value is converted in to a linear channel value. The mean linear channel value of the negative controls are subtracted from the mean linear channel of each sample to give a linear channel shift.	
39	Beckman Coulter - FC500/Navios	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set, Immunobrite			500	5000	500	5000	Median	Log channel
41	BD		Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST			50	5000	50	500	Median	Log channel
42	BD FACS Canto I	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	7 colour set-up			1000	>1000	1000	>1000		Log channel
45	BD FACS Canto II		Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation				3000	4000			Median	Log channel
48	Beckman Coulter		Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flow check, Flow set			5000		1500		Median	Log channel
51													
54	BD FACS Canto II	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST, DAKO fluorospheres			5000	5000	20000	20000	Median	Log channel
58	Beckman Coulter FC500	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set, Immunobrite			5000			1000	Median	Log channel
62	Beckman Coulter		Yes	Alignment check, Laser check	Fluorospheres	10000						Median	Log channel
101	Beckman Coulter	Yes	No									Mean	Log channel
112	Beckman Coulter FC500	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set, Quantum-MESF					1000		Median	Log channel
114	FACS Calibur	Yes	Yes	Alignment check, Laser check, Fluorescence check	Calibrite 3	5000				1000		Median	Linear channel
115	FACS Calibur	Yes	Yes	Alignment check, Laser check, Fluorescence check	Calibrite + Rainbow Cal. Part.				20000 or 120sec			Median	Log channel
116	Beckman Coulter	No	Yes	Alignment check, Fluorescence quantitation	Flowcheck, Flow set					2000		Median	Log channel
117	BD	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Calibrite 3					350	1500	Median	Log channel
118	Calibur	Yes		Alignment check, Laser check, Fluorescence check					5000		1000	Median	Linear channel
119	Coulter Epics-XL	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set, Quick-comb, Immunobrite			5000	15000-20000	400	1000	Mean	Log channel
120	FACS Canto	Yes	Yes	Alignment check, Laser check, Fluorescence check	Calibrite 3					3000		Median	Log channel
122	BD FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	7 colour set-up			6000	10000	800	1000	Geomean	Linear channel
126													Linear channel
130	FACS Scan	Yes	Yes	Fluorescence check, Fluorescence quantitation	Sphero rainbow calibration	2000						Mean	Linear channel
133			Yes	Alignment check, Laser check, Fluorescence check	Calibrite 3 beads	20000	50000					Median	Log channel
136	FACS Calibur	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Calibrite			5000	10000			Geomean	Linear channel
138													Log channel
139	Beckman Coulter	Yes	Yes	Alignment check, Laser check, Fluorescence check	Flow check pro, Flow set pro	10000	15000	5000	10000	1000	10000	Mean	Log channel
142	BD FACS Canto II		Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST			50000	100000	50000	100000	Median	Log channel
143	FACS Calibur			Alignment check, Laser check, Fluorescence check	Calibrite 3 and APC	5000	10000	3000	5000			Median	Linear channel
144	BD	Yes	Yes	Alignment check, Laser check, Fluorescence check				1000		500		Median, Mean	Log channel
145	Beckman Coulter	Yes	Yes	Alignment check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set, Immunobrite	10000	15000	5000	10000	500	1000	Median	Log channel
147	FC500	Yes	Yes	Alignment check, Laser check, Fluorescence check	Fluorocheck, Fluoroset		100000		Unlimited		2000	Median	Linear channel
154	FACS Canto II	No	Yes	Alignment check, Laser check, Fluorescence check	CST	≥2000		>2000		>2000		Median	Log channel
157	FACS Calibur	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Calibrite		10000			1000		Median	Linear channel
159	BD		No	Alignment check, Laser check, Fluorescence check	Calibrite 3	12000		10000		1000		Mean	Linear channel
160	BD FACS Calibur	No	Yes	Alignment check, Laser check, Fluorescence check	Calibrite	3000	30000	1000	5000	300	1000	Mean	Log channel
163	BD FACS Canto II	No	Yes	Alignment check, Laser check, Fluorescence check	CST		20000					Geometric mean	Linear channel
167													Log channel
169													
176	CD Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST, Rainbow			200		200		Median	Log channel
186		Yes	Yes	Alignment check, Laser check, Fluorescence check	Flowcheck, Flow set			3500		1500		Mean	Log channel
190	Beckman Coulter Navios	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation		12000						Mean	Linear channel, Log channel
191	BD FACS Calibur	Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Calibrite	>50000		15000		2000		Mean	Log channel
193	FACS Calibur	No	Yes	Alignment check, Laser check, Fluorescence check	Calibrite	10000		250		250			
194			Yes	Alignment check, Laser check, Fluorescence check	Flowcheck, Flow set	3000						Median	Log channel
195	BD	Yes	Yes	Alignment check, Laser check, Fluorescence check	Rainbow	20000	50000					Median	Log channel
201	FC500		Yes	Laser check	Flow check	10000		1000		100		Mean	Log channel
202													
204	Beckman Coulter FC500	Yes	Yes	Alignment check, Laser check, Fluorescence check	Flowcheck, Flow set		10000		3000			Median/Mean	Linear channel, Log channel
209													
218	LSR II	No	Yes	Alignment check, Laser check, Fluorescence check	CST		300000					Mean	Log channel
220	BD FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	CST					2000		Median	Linear scale
227	Beckman Coulter	Yes	Yes	Alignment check, Laser check, Fluorescence check	Flow set	1000	20000	500		500		Mean	Log channel
235		Yes	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	BD Calibrite	30000	100000	10000		1000		Median	Log channel
238		Yes	Yes	Alignment check, Fluorescence check	Immuno-brite	40000	100000					Median	Log channel
245	Beckman Coulter Navios	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	Flowcheck, Flow set			2500	5000	500	2000	Median	Linear channel
246	BD FACS Canto		Yes					100	1000	100	1000	Median	Log channel
252			Yes	Alignment check, Laser check, Fluorescence check	BD Calibrite bead							Median	Log channel
262	3000 events on B-cells	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST, Immunobrite					500	3000	Median	Log channel
271	FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	CST					250	1000		Log channel
284	BD FACS Canto II	No	Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST					1000		Median	Log channel
297			Yes	Alignment check, Laser check, Fluorescence check, Fluorescence quantitation	CST			1000	10000	300	10000	Median	Log channel
341	FACS Canto II	Yes	Yes	Alignment check, Laser check, Fluorescence check	CST, 7 colour set-up			2000	10000	500	1000	Geomean	Linear channel
351	BD FACS Canto II	Yes	Yes	Fluorescence check, Fluorescence quantitation	Fluorospheres	5000	10000	5000	10000	5000	10000	Median	Log channel

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	8. Data Analysis					9. Use of XM by flow cytometry
Lab Code	Parameter used to assess difference in fluorescence of anti-human IgG reagent	Minimum value for valid positive control T-cell	Minimum value for valid positive control B-cell	Discriminator value taken to distinguish negative from positive	Discriminator value to distinguish clinically significant binding	
9	Median difference	pos/neg trimmed mean value >3		>1.5 x trimmed mean		Pre-transplant crossmatching
11	Median difference	>Mean AB +2SD	>1.6 RMF			Clinical/Diagnostic, Pre-transplant crossmatching
12	Peak shift	50 MCS	50 MCS	50 MCS	50 MCS	Clinical/Diagnostic, Pre-transplant crossmatching
14	Median difference	4x neg	4x neg	1.5x mean neg	T-cells: 2.4x mean neg B-cells: 2.45x mean neg	Clinical/Diagnostic, Pre-transplant crossmatching
15	Median difference	20x neg	10x neg	NEQAS RMF >1.5	OTX RMF >4 Prev. Tx RMF >2.3	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching
19						
20	Median difference	60	60	40	40	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching
23	Median difference	+3SD for mean of AB	+2SD	T: +3SD for mean of AB, B: +2SD	T: +3SD, B: +2SD	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring, Renal transplant
24	Median difference	1.5x neg	2x neg	T: 1.5x neg, B: 2x neg	T: 1.5x neg, B: 2x neg	Clinical/Diagnostic, Pre-transplant crossmatching
25	Median difference			RMF >1.3	RMF >1.3	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
28	Ratio of median fluorescence of test sample to negative control	ratio ≥3.1	ratio ≥3.6	T-cell ≥1.6 B-cell ≥2.0	T-cell ≥1.6 B-cell ≥2.0	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring (if antibody screening is not possible)
34	Mean difference	2x neg	2x neg	+2SD	+2SD	Clinical/Diagnostic, Pre-transplant crossmatching
35	Median difference			Mean +3SD	Mean +3SD	Clinical/Diagnostic, Pre-transplant crossmatching
38	Linear channel shift	>46 channels	>63 channels	T-cells: >46 channels B-cells: >63 channels	T-cells: >46 channels B-cells: >63 channels	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
39	Peak shift, Median difference	>1.5 RMF	>2.0 RMF		>1.5 RMF/ >2.0 RMF	Pre-transplant crossmatching
41	Mean difference	10x neg	10x neg	T: 2SD, B:3SD	T: 2SD, B:3SD	Pre-transplant crossmatching
42	Mena difference	MCS ~30, RMF >1.3	MCS ~45, RMF >1.5	T: RMF <1.3, B: RMF<1.5	T: RMF <1.3, B: RMF<1.5	Pre-transplant crossmatching
45		40 channel shift		40 channel shift	40 channel shift	Pre-transplant crossmatching
48	Relative median fluorescence		4x neg	1.3x neg	2.3x neg	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
51						
54	Test of neg control MESF ratio	a test/neg MESF ratio of >10		≥1.2 MESF ratio	≥1.5 MESF ratio	Pre-transplant crossmatching
58	Median difference	1.3x mean	1.3x mean	1.3x mean	1.3x mean	Pre-transplant crossmatching
62	Median difference	RMF >1.5	RMF >3.0			Pre-transplant crossmatching
101	Mean difference					Pre-transplant crossmatching
112	Ratio	>1.5 ratio	>2.0 ratio	T-cell: >1.5 ratio B-cell: >2.0 ratio	Not determined	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
114	Median difference	40 channel	70 channel	50	T: 60 channel, B: 90 channel	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
115	Median difference	>40	>60	>40 channel shift		Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
116	Median difference			>1.7	Ratio >1.7	Clinical/Diagnostic, Post-transplant monitoring
117	Median difference		RMF >4	T: RMF >1.3, B: RMF >1.5	T: RMF >1.3, B: RMF 1.5-1.7	Clinical/Diagnostic, Post-transplant monitoring
118	Median difference			T: Med neg +66 ch, B: Med neg +25.5 ch	T: Med neg +66 ch, B: Med neg +25.5 ch	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
119	Mean difference	ratio >1.285	ratio >1.7	T-cells: ratio >1.285 B-cells: ratio >1.7	T-cells: ratio >1.285 B-cells: ratio >1.7	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
120	Median difference	ratio >13	ratio >12	T-cells: ratio >1.6 B-cells: ratio >2		Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
122	Fluorescence index	FI >1.5	FI>2.0	3SD	3SD	Clinical/Diagnostic, Research/Development, Antibody screening, Pre-transplant crossmatching, Post-transplant monitoring
126						
130	Mean difference			T: >40 channels, B: >80 channels		Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
133	Peak shift, Median difference, Mode difference	>x4 of NC	>x4 of NC		>+50% of neg, >+100% of neg	Pre-transplant crossmatching
136					NR >200	Pre-transplant crossmatching, Post-transplant monitoring
138						
139	Mean difference			1.5 or 2 (T or B cells) of neg mean log channel		Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching
142	Peak shift				Mean +3SD	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
143	Mean difference, Median difference	40		>500	40	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
144	Mean difference, Median difference	1.76	2.5	Ratio	Ratio	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
145	Ratio median log channel test/Median log channel neg	Mean ratio +2SD	Mean ratio +2SD	Mean ratio +2SD	Mean ratio +2SD	Clinical/Diagnostic, Pre-transplant crossmatching
147	% shift	Tpos-Tneg=200		t: >40 channel, B: >60 channel	None	Pre-transplant crossmatching, Post-transplant monitoring
154	Mean difference			150% of neg control and shape of the curve	150% of neg control and shape of the curve	Clinical/Diagnostic, Pre-transplant crossmatching
157	Median difference	150 channel shift	250 channel shift	2SD from neg control	>10 channel shift over cutoff	Pre-transplant crossmatching, Post-transplant monitoring
159	Mean difference	100 channel shift	100 channel shift	T: 40 channel shift, B: 100 channel shift	T: 40 channel shift, B: 100 channel shift	Pre-transplant crossmatching, Post-transplant monitoring
160	Mean difference	100 mean log channel	170 mean log channel	>Mean of neg sera +2SD	>Mean of neg sera +2SD plus anti-HLA antibody	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
163	Ratio of geometric mean	2.3	1.7	T: 2.3, B: 1.7	T: 2.3, B: 1.7	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
167						
169						
176	MFI of test serum/MFI of neg control serum	0.5	0.22	T: 0.50, B:0.22	T: 0.50, B:0.22	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
186	Mean difference			T-cells: ratio >1.5 x the local neg B-cells: ratio >2 x the local neg	NA	Pre-transplant crossmatching
190	Mean difference				T <1.2Neg >1.5Pos B <1.2Neg >2.5Pos	Clinical/Diagnostic, Pre-transplant crossmatching
191	Mean difference	30	100	T: 30, B: 100	T: 30, B: 100	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
193	Geometric mean			Neg geometric mean x2.5		Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
194	Median difference	ratio >0.7	ratio >1			Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
195	% shift			ratio sample/neg control, T:1.3, B: 1.6		Clinical/Diagnostic, Pre-transplant crossmatching
201	Ratio x-mean sample/NC	2	2.5	NA	NA	Clinical/Diagnostic, Pre-transplant crossmatching, Post-transplant monitoring
202						
204		1.5x	2x	T-cells: 1.5x the local neg B-cells: 2x the local neg	Depend on autocrossmatch done in parallel	Clinical/Diagnostic, Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
209						
218	Mean difference	CTL x1.2	CTL x1.7	T-cell: CTL x1.2 B-cell: CTL x1.7	T-cell: CTL x1.2 B-cell: CTL x1.7	Clinical/Diagnostic, Pre-transplant crossmatching
220	8000 - mean of neg control	8000 - mean of neg control	8000 - mean of neg control	8000 - mean of neg control	8000 - mean of neg control	Pre-transplant crossmatching
227	Mean difference	1.5x neg	2x neg	T: 1.5x neg, B: 2x neg	T: 1.5x neg, B: 2x neg	Clinical/Diagnostic, Pre-transplant crossmatching
235	Median difference			>2 of neg median log channel		Research/Development, Pre-transplant crossmatching, Post-transplant monitoring
238	MESF Serum/MESF neg control			T: ratio >1.8, B: >2.2		Pre-transplant crossmatching, Post-transplant monitoring
245	Median difference	7	15			Clinical/Diagnostic, Pre-transplant crossmatching
246	Median log. FI ratio	1.75	2.5			Clinical/Diagnostic
252	Median ratio	Median ratio ±2SD	Median ratio ±3SD			Clinical/Diagnostic, Antibody Screening, Pre-transplant crossmatching
262	% shift	>12%	>12%	Median RFU sample/Median RFU neg >2		Pre-transplant crossmatching, Post-transplant monitoring
271	Median channel shift	64	90			Pre-transplant crossmatching, Post-transplant monitoring
284	Median difference	ratio >1.2	ratio >1.2	ratio >1.2	ratio >1.2	Clinical/Diagnostic, Pre-transplant crossmatching

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

	10. Reporting results	
Lab Code	Is Positive or Negative adequate?	Reason
9	Yes	
11	No	"Ok to proceed" - If positivem discussion with clinician considering patient history, sensitisation and clinical need.
12	Yes	
14	Yes	
15	Yes	
19		
20	Yes	
23	Yes	
24	No	Immunological risk. Would need to know HLA mismatch and antibody levels.
25	Yes	
28	No	An equivocal range is a good idea.
34	Yes	
35	Yes	
38	Yes	
39	Yes	
41	Yes	
42	No	The positive result should be explained in relation to the prescence of antibodies.
45	Yes	
48	No	Clinically significant?
51		
54	Yes	We also use the term weakly positive if the test/neg MESF value is between 1.2 and 1.5.
58	Yes	As no other clinical data is available to review with the flow crossmatch results then pos/neg along with equivocal is adequate.
62	Yes	
101	Yes	
112	Yes	
114	Yes	
115	Yes	
116	Yes	
117	Yes	
118	Yes	
119	Yes	
120	Yes	
122	Yes	
126		
130	Yes	
133	Yes	
136	Yes	
138		
139	Yes	
142	No	Low positive would also be useful
143	Yes	
144	Yes	
145	Yes	
147	No	The results seen to be equivocal because they are close to the positive threshold. In general our clinical rules consist of investigating the whole historical medical patient file.
154	Yes	
157	Yes	
159	Yes	
160	No	A high proportion of samples sent are marginally positive. In practice such results are evaluated after consideration of other laboratory and clinical sata.
163	Yes	
167		
169		
176	No	It would be useful to include a "Weakly reactive/Borderline" result option. In clinical terms, there is a big difference between "definitely no reaciton" and "probably something"
186	Yes	
190	Yes	
191	Yes	
193	Yes	
194	Yes	
195	Yes	The option "Equivocal result" is interesting.
201	Yes	
202		
204	Yes	
209		
218	No	Sometimes results are indeterminare or borderline. Insufficient samples is sent so they cannot be tested in duplicates as the patient samples are.
220	Yes	
227	Yes	
235	Yes	
238	Yes	
245	No	
246	Yes	
252	Yes	
262	Yes	
271	Yes	
284	Yes	

SCHEME 2B - CROSSMATCHING BY FLOW CYTOMETRY - METHODOLOGY 2016

Lab Code	Other comments
9	
11	
12	
14	
15	
19	
20	
23	
24	
25	With the introduction of uncertainty of measurement by UKAS, how does NEQAS plan to assess potential weak positive/negative results? Will you be taking MoU into account?
28	It might be relevant to show replication of samples and controls -> How the result was obtained as this may show the integrity of the test and the ability of the scientist.
34	
35	
38	
39	
41	
42	
45	I think for the purpose of an EQA scheme you can only apply a positive/negative result to achieve meaningful results. To reflect the clinical situation is not possible. Perhaps measurement uncertainty somehow needs to be included in the future.
48	
51	
54	
58	
62	
101	
112	
114	
115	
116	The amount of borderline samples has been too high. T-cell and B-cell crossmatches should be assessed seperately. Clinically it is crucial to find positivity in general levels, thus it is bigger mistake to have both T and B-cell crossmatches as a false negative than to have either of them false negative.
117	
118	
119	
120	Please provide more cells for crossmatching. IS it possible to have HLA typing of cells before results?
122	
126	
130	
133	
136	
138	
139	
142	
143	
144	
145	
147	
154	
157	
159	
160	For labs that perform T and B-cell crossmatch test in separate tubes, the amount of total cell provided is often too low.
163	Doesn't reflect a real clinical lab situation where you have knowledge of patients historic antibody status and donor/recipient HLA typing.
167	
169	
176	
186	
190	
191	
193	
194	
195	
201	
202	
204	
209	
218	Samples are degenerate when they arrive. Insufficient samples is sent so they cannot be tested in duplicates as the patient samples are.
220	
227	
235	
238	Add the possibility of equivocal
245	
246	
252	
262	
271	
284	