

UK NEQAS for H&I Scheme 2B - Crossmatching by Flow Cytometry

T-CELL AND B-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B06/2018 PC (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 10 JULY 2018

HLA PHENOTYPE OF BLOOD DONOR: HLA-A1, A68; B8, B60; Cw3, Cw7; DR1, DR17; DQ2, DQ5

		Summary of Results										
		T-cells				B-cells						
Total tested	39	41	41	41	36	38	36	37				
Positive	4	0	1	38	5	0	29	36				
Negative	31	41	40	3	30	38	7	1				
Equivocal	4	0	0	0	1	0	0	0				
NT	6	4	4	4	6	4	6	5				
% Positive	10.3%	0.0%	2.4%	92.7%	13.9%	0.0%	80.6%	97.3%				
% Negative	79.5%	100.0%	97.6%	7.3%	83.3%	100.0%	19.4%	2.7%				
% Equivocal	10.3%	0.0%	0.0%	0.0%	2.8%	0.0%	0.0%	0.0%				
Consensus		Negative	Negative	Positive	Negative	Negative	Positive	Positive				
HLA Antibody Specificity (Defined By CDC)	A1	Negative	Cw5 Cw6	Multi	A1	Negative	Cw5 Cw6	Multi				
		T-cells				B-cells						
Lab No.	Assessment T-cells B-cells	Serum 1	Serum 2	Serum 3	Serum 4	Serum 1	Serum 2	Serum 3	Serum 4	Date Received	Date Tested	Comments
112	YES YES	NT	NT	NT	NT	NT	NT	NT	NT	11-Jul	13-Jul	Cells with 0% viability
115	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
117	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	NT	Positive	12-Jul	11-Jul	Serum 3 gave bad replication in B-cells
118	YES	Negative	Negative	Negative	Positive					11-Jul	12-Jul	Cell clumps, low cell count
120	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
122	YES YES	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
130	YES YES	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	11-Jul	13-Jul	
133	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	11-Jul	11-Jul	
138	YES	Negative	Negative	Negative	Positive					11-Jul	11-Jul	
139	YES YES	NT	NT	NT	NT	NT	NT	NT	NT	13-Jul	12-Jul	Technical problem
143	YES	NT	NT	NT	NT					11-Jul	12-Jul	NT due to poor viability. In clinical setting, new samples requested
144	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
145	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
147	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	13-Jul	
149	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	11-Jul	12-Jul	
154	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
157	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	11-Jul	
159	YES YES	Equivocal	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	20-Jul	Equivocal because pos on T-cells and neg on B-cells.
160	YES YES	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
163	YES YES	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	11-Jul	12-Jul	
167	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
169	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
176	YES YES	Negative	Negative	Negative	Positive	NT	NT	NT	NT	11-Jul	16-Jul	Insufficient number of B-cells. Had we reached a sufficient number of cells for robust results, the results would have been reported as follows for B cells: Serum 1 (Equivocal) Serum 2 (Negative) Serum 3 (Positive) Serum 4 (Positive)
189	YES YES	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
190	YES YES	Equivocal	Negative	Negative	Positive	Equivocal	Negative	Positive	Positive	11-Jul	12-Jul	
191	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	11-Jul	12-Jul	
193	YES YES	Equivocal	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
194	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	11-Jul	
195	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	13-Jul	13-Jul	Samples with aggregated cells
201	YES YES	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	11-Jul	12-Jul	
209	YES YES	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
218	YES YES	NT	NT	NT	NT	NT	NT	NT	NT	11-Jul	17-Jul	Very low viability
220	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
235	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	11-Jul	
238	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	Very few number of cells
240	YES YES	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Positive	11-Jul	12-Jul	
246	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	11-Jul	
252	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	12-Jul	12-Jul	
260	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	12-Jul	12-Jul	
262	YES YES	NT	Negative	Negative	Positive	NT	Negative	Positive	Positive	11-Jul	11-Jul	
271	YES YES	NT	Negative	Negative	Positive	NT	Negative	Negative	Positive	11-Jul	13-Jul	Clotted cells. Not able to rule out weak pos
276	YES YES	Negative	Negative	Negative	Positive	Positive	Negative	Positive	Positive	11-Jul	13-Jul	
293	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive	11-Jul	12-Jul	
297	YES YES	Equivocal	Negative	Negative	Positive	Positive	Negative	NT	NT	11-Jul	12-Jul	Low cell viability
351	YES YES	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	11-Jul	14-Jul	
392	YES YES	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive			No results returned

UK NEQAS for H&I Scheme 2B - Crossmatching by Flow Cytometry

T-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B06/2018 (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 10 JULY 2018

Lab No.	Serum 1 cytometer reading	Serum 2 cytometer reading	Serum 3 cytometer reading	Serum 4 cytometer reading	Negative control (local)	Positive control (local)	Strong positive (local)	Weak positive (local)	Positive result value	Cytometer reading units	Viability (%)
112	NT	NT	NT	NT	NT	NT				Median log channel	0
115	259	252	279	492	364	408			>40	Median linear channel shift	
117	4.81	4.27	4.74	26.9	3.98	50.06			Ratio (RMF) >1.3	Median log channel	95
118	129	104	125	322	113	712			179 (113+66)	Median linear channel	80
120	1.55	1.01	1.11	6.34	41	1208			ratio >1.6	Median	100
122	11	8	24.5	102	7		76	26	3SD	Geomean linear value	92
130	213	141	145	379	161	302			40	channels	99
133	0.3	0.3	0.4	3.6	0.2	28.5			2sd	MFI	100
138	0.15	0.02	0.11	0.8	0	0.87			>0.20	D-Value Kolmogorov-Smirnov	
139	NT	NT	NT	NT	NT	NT					80
143	NT	NT	NT	NT	NT	NT			40 Channel Shift	Mean channel shift	74.6
144	1.36	1.05	1.38	5.44	1279	2.6			1.6	Mean/Median→Ratio	
145	0.665	0.624	0.444	2.93	0.448	42.5			2SD of the ratio	Median log channel	90
147	143	129	131	256	120	503			>40 linear channel shift	Linear channel	100
149	0.309	0.271	0.276	1.219	0.35	68.3			2SD	Median log channel	88
154	79	56	72	712	56	999			median >1.5 x negative control	Median fluorescence	
157	154	135	138	551	130	3881			2SD (cut off 158)	Median log channel	99
159	238	187	206	407	183	479			40 linear channel shift (223)	Linear channel	50
160	9.4	6.8	7.2	81	6.5	187			>13.1 (Neg +2SD)	Mean linear channel	
163	4.2	1.1	1.5	47.7	1	17.7			2.3 (ratio above)	Geomean linear channel	100
167	262	208	200	1032	204	12609			2SD	Median log channel	96
169	224	207	216	384	237	484			≥50 channel	Median channel	97
176	0.2	0.03	0.13	1	0	0.74			MFI shift ≥0.40	MFI Shift	N/D
189	0.57	0.29	0.66	2.91	0.34	99.2			>5%	Percentage	85
190	67.7	47.3	49	328	45.3	530			ratio >1.5	MFI median	86.2
191	1	-0.9	-4	259	0	549			>30	MFI	0.9
193	10.53	4.4	5.06	87.34	3.87	153.14			negative control mean x 2.4	Geometric mean linear channel	
194	0.68	0.42	0.44	4.3	0.41	13.9			ratio>2.2	Median MFI	90
195	248	240	227	364	231	420			Test/NC ≥1.3	Median log channel	80
201	599	507	903	449	468	36.1			S/NK ≥ 2,0	X-mean	97
209	512 (2.5)	202 (1.0)	255 (1.2)	4454 (21.4)	208	1309 (6.3)			Ratio>1.6	Geometric mean value	98
218	NT	NT	NT	NT	NT	NT				MFI	
220	5708	1611.5	2085.5	95783	1770	136546			6000 above the mean of the NC	Linear acquisition	96
235	3.46	4.45	3.85	17.78	4.83	124.09			2 MLC above the NC = Pos	Median log channel	85
238	1417	1132	1163	3509	1193	20171			MESF seum/MESF NC	MESF	
240	20	12	14	47	12	730			1.2 x NC	MFI	50
246	1.2	1.17	1.11	8.65		2.34			1.75	MFI	93.8
252	1.14	1.31	1.29	2.1	117.57	457.25			Median ratio ±2SD	Median channel ratio	
260	12.51%	5.17%	10.54%	46.17%	18.32%	98.9			>25% shift	% shift	
262		1028	470	3314	804	14506			2SD	Mean channel shift	70
271		193	145	423	182	502			64	Median log channel	0.85
276	165	147	157	1360	148	10421			>1,25x	MFI	93.83
293	2.89	3.08	2.71	17	4.53	134.56			10 channel shift	Channel shift	80
297	156	96	107	887	94	338			1.3x Tneg (grey zone 1.3-1.8)	MFI	
351	207	205	207	232.5	201	429			2.5 SD	MCS	0.56

392

UK NEQAS for H&I Scheme 2B - Crossmatching by Flow Cytometry

B-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B06/2018 (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 10 JULY 2018

Lab No.	Serum 1 cytometer reading	Serum 2 cytometer reading	Serum 3 cytometer reading	Serum 4 cytometer reading	Negative control (local)	Positive control (local)	Strong positive (local)	Weak positive (local)	Positive result value	Cytometer reading units	Viability (%)
112	NT	NT	NT	NT	NT	NT				Median log channel	0
115	331.00	329.00	586.00	675.00	418.00	742.00			>60	Median linear channel shift	
117	8.73	6.37	-	83.51	7.09	174.09			Ratio (RMF) >1.5	Median log channel	95
120	1.33	1.34	3.79	10.68	158.50	2424.00			ratio >2	Median	100
122	41.50	58.20	339.00	848.00	26.50		370.00	126.00	3SD	Geomean linear value	92
130	525.00	249.00	533.00	585.00	244.00	498.00			80-100 channels		99
133	0,2	0,1	4,9	32,9	1,1	76,1			2sd	MFI	100
139	NT	NT	NT	NT	NT	NT					
144	1.32	1.25	4.46	11.79	2601.00	4.56			2.6	Mean/Median→Ratio	
145	0.98	1.07	5.23	22.80	0.94	19.70			2SD of the ratio	Median log channel	90
147	185.00	181.00	290.00	410.00	162.00	655.00			>60 linear channel shift	Linear channel	100
149	0.61	0.41	1.38	16.60	1.65	61.30			2SD	Median log channel	88
154	127.00	128.00	1301.00	3523.00	152.00	1539.00			median > 2 x negative control	Median fluorescence	
157	166	162	389	1824	146	6722			2SD (cut off 182)	Median log channel	99
159	355	296	517	643	287	644			100 linear channel shift (387)	Linear channel	50
160	49.4	42.2	341	558	31.6	284			>65.8 (Neg +2SD)	Mean linear channel	
163	2.4	1.4	10	43.8	1	6.9			1.7 (ratio above)	Geomean linear channel	100
167	791	780	1412	10335	644	25548			2SD	Median log channel	96
169	240	183	420	579	283	626			>=80 CHANNEL	Median channel	97
176	0.21	0.08	1.03	1.52	0	0.83			MFI-shift >= 0.22	MFI Shift	N/D
189	3.06	4.41	8.27	23.19	2.93	99.99			>%8	Percentage	85
190	178	148	635.7	1000	71.7	1000			ratio >2.5	MFI median	86.2
191	-11	-0.3	48	271	0	535			>100	MFI	0.9
193	38.53	35.65	177.92	455.59	23.29	364.5			negative control mean x 2.3	Geometric mean linear channel	
194	1.4	0.9	6.2	14	1	104			ratio>4	Median MFI	90
195	210	207	275	1050	201	2023			Test/NC ≥1.6	Median log channel	80
201	1.65	1.59	6.8	355	995	81.3			S/NK ≥ 2.5	X-mean	97
209	1909 (1.1)	1549 (0.9)	9031 (5.0)	30501(16.9)	1805	4433 (2.5)			Ratio>1.7	Geometric mean value	98
218	NT	NT	NT	NT	NT	NT				MFI	
220	6760	5913	16481,5	222034,5	3766	249325			6000 above the mean of the negative controls	Linear acquisition	96
235	2.17	2.46	15,65	103,66	3,16	513,97			12 MEDIAN LOG CHANNEL ABOVE THE NEGATIVE CONTROL INDICATES A POSITIVE RESULT	Median log channel	85
238	1950	1549	5736	15389	1542	27075			MESF serum/MESF NC	MESF	
240	1100	900	930	1442	700	2000			1.4*NC	MFI	50
246	0.91	0.83	7.43	13.43		21.73			2.5	MFI	93.8
252	1.16	1.14	3.07	4.20	117.57	2267.09			Median ratio ±2SD	Median channel ratio	
260	0.15	0.04	0.31	0.94	0.21	1.00			>25% shift	% shift	
262		1323	5185	17269	990	42961			2SD	Mean channel shift	
271		262	144	647	207	657			90	Median log channel	0.85
276	427	312	2754	13455	295	17913			>1.25x	MFI	93,83
293	14.80	6.15	90.58	182.69	26.18	194.56			20 CHANNEL SHIFT	Channel shift	80
297	190	111	-	-	79	275			1.5x Tneg (grey zone 1.5-2)	MFI	
351	274.5	271.5	299.5	412	270	498			2.5 SD	MCS	0.56
392											