

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

T-CELL AND B-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B04/2017 (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 14TH MARCH 2017

HLA PHENOTYPE OF BLOOD DONOR: HLA-A1, A2; B8, B44; Cw5, Cw7; DR11, DR13; DQ6, DQ7

Summary of Results															
T-cells					B-cells										
Total tested	57	57	54	47	49	50	45	43							
Positive	0	55	50	15	45	46	39	2							
Negative	57	2	4	32	4	4	6	41							
NT/Equivocal	4	7	14	6	5	10	12								
% Positive	0.0%	96.5%	92.6%	31.9%	91.8%	92.0%	86.7%	4.7%							
% Negative	100.0%	3.5%	7.4%	68.1%	8.2%	8.0%	13.3%	95.3%							
Consensus															
Negative		Positive		Positive		Not Assessed		Positive		Positive		Positive		Negative	
HLA Antibody Specificity (Defined By CDC)															
DR4 DR9 A2 A28 A9 B44 A11				DR4 DR9 A2 A28 A9 B44 A11											
T-cells															
B-cells															
Lab No.	Serum 1	Serum 2	Serum 3	Serum 4	Serum 1	Serum 2	Serum 3	Serum 4	Date Received	Date Tested	Viability (%)	Comments			
9	Negative	Positive	Positive	Equivocal					15-Mar	16-Mar	95%				
11	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	16-Mar					
14	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	15-Mar					
15	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	15-Mar		Blood that was sent for crossmatching was very small. Under normal circumstances, for clinical crossmatches, there is more blood and cells.			
19	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	16-Mar					
20	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	15-Mar	16-Mar	90	Serum 4 T/B cell result was reported as equivocal as the result falls within the uncertainty of measurement and we would await HLA antibody results if this was a patient sample.			
24	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Equivocal	16-Mar	17-Mar	95%				
25	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Equivocal	15-Mar	15-Mar					
28	Equivocal	Positive	Positive	Equivocal	Positive	Positive	Positive	Negative	13-Mar	15-Mar	99%				
35	Negative	Positive	Positive	Negative					15-Mar	16-Mar	90%				
38	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	15-Mar	90%				
39	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	15-Mar		HLA-A1 DSA was detected in serum 4, however the level of antibody was not sufficient to cause positivity in either the T or B cell Flow Cytometric Crossmatches.			
41	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	15-Mar					
42	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Equivocal	15-Mar	15-Mar	90%	Insufficient samples. Therefore, equivocal result reported for serum 3 (2B03) and Serum 4 (2B04) as there was not enough cells for repeat testing.			
45	Negative	Positive	Positive	Positive					15-Mar	15-Mar	95				
48	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	15-Mar		Lower than ideal cell counts			
51	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	16-Mar					
112	Negative	Positive	Equivocal	Negative	Positive	Positive	Equivocal	Negative	17-Mar	17-Mar	95	Serum 3 equivocal, not concord between LT and LB reading			
115	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	17-Mar					
117	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	16-Mar		95				
118	Negative	Positive	Positive	Negative					15-Mar	15-Mar	90				
119	Negative	Positive	Positive	Negative	NT	NT	NT	NT			90	B-XM impossible to be done because of less number of B cells/			
120	Negative	Positive	Equivocal	Negative	Positive	Positive	Equivocal	Negative	15-Mar	15-Mar	100	Serum 3 gave incoherent results on B-cells: Positive on T-cells and Negative on B-cells. Not enough cells to repeat the test			
122	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	16-Mar	75	Cells were clumped			
126	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	15-Mar	50-90	Low cell viability for B-cells			
130	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	16-Mar	100				
138	Negative	Positive	Positive	Negative					15-Mar	15-Mar	90				
139	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative	17-Mar	17-Mar	0.8				
143	Negative	Positive	Positive	Negative					16-Mar	17-Mar	87				
144	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	16-Mar		Second time in row the cells were clumped on arrival.			
145	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	22-Mar	22-Mar	0.94				
147	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	16-Mar	16-Mar	100				
149	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	16-Mar	16-Mar	0.79				
154	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Negative	16-Mar	16-Mar		Clot and a lot of platelets were present in this sample			
157	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	16-Mar	16-Mar	95				
159	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Negative	15-Mar	16-Mar	99	Serum 4 - There is a discrepancy between T and B-cells			
160	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	16-Mar		Cell suspension clotted. Low number of LyT and LyB			
167	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	15-Mar	16-Mar	98				
169	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Negative	15-Mar	16-Mar	97				
176	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	NT	15-Mar	16-Mar		Poor samples quality. Low number of cells			
180	Negative	Positive	Positive	Positive	Positive	Positive	Positive	NT	16-Mar	16-Mar	79	Not enough cells in sample			
191	Negative	Positive	Negative	Negative	Positive	Positive	Negative	Negative	16-Mar	17-Mar	75	Samples delivered late			
193	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Negative	15-Mar	15-Mar					
194	Negative	Equivocal	Positive	Negative	Positive	Positive	Equivocal	Negative	15-Mar	16-Mar	98				
195	NT	NT	NT	NT	NT	NT	NT	NT	17-Mar	17-Mar	98	Same problem as 2B03			
201	Negative	Negative	Positive	Negative	Positive	Negative	Positive	Negative	15-Mar	16-Mar	98				
209	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Negative	14-Mar	16-Mar	90				
218	NT	NT	NT	NT	NT	NT	NT	NT				As discussed, we were unable to resuspend the cell pellet for this trial. We attempted agitating and warming the pellet. We were advised to return a report of not tested for this trial.			
220	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	15-Mar	16-Mar	93				
235	Negative	Positive	NT	Negative	NT	Positive	NT	Negative	16-Mar	16-Mar	80				
238	Negative	Positive	Positive	Negative	NT	NT	NT	NT	15-Mar	16-Mar		Not enough events for B cells (only 200 events). Protocol needs at least 500 events.			
240	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Negative	17-Mar	17-Mar	60				
246	NT	NT	NT	NT	NT	NT	NT	NT	15-Mar	15-Mar		Donor cell sample contained only cell clumps which is a sign of apoptosis i.e bad material quality of the donor cell sample. Therefore, it was not possible to acquire enough cells and analyse them.			
252	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative			75				
260	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	17-Mar	17-Mar					
262	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Equivocal	15-Mar	15-Mar	90	Cell clustering in the vial. Serum 4 is equivocal. Ratio was lower than 2 but shift was around 10%			
271	Negative	Positive	NT	Negative	Positive	Positive	NT	Negative	21-Mar	21-Mar	93	Samples received 7 days later than normal. Clumped cells			
293	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Negative	16-Mar	16-Mar	70				
297	Negative	Positive	Positive	Equivocal	Positive	Positive	Positive	Equivocal	15-Mar	16-Mar		Answer of S4 is "Equivocal" on T and B cells because the T cell XM is weakly positive and the B cell XM is weakly negative.			
341	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative	15-Mar	16-Mar	97				
351	Negative	Positive	Negative	Negative	Positive	Positive	Negative	Negative	18-Mar	18-Mar	100				

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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
9	0.77	2.92	2.25	1.52	1.32	38.40			>1.5 x Trimmed mean	Median x (log)
11	133,147	574,641	474,441	205,201	112, 124, 136, 134	434, 1627			151.8 1.2RMF	Median log channel
14	616	1345.5	1342.5	834	548	9605.5			1.5 x mean NEG	Median log channel
15	0.96	5.87	5.71	2.50	5.49	2.86			RMF >1.5	Median
19	0.174	1.043	0.871	0.272		8.709			1.48 x average negative control	Log Median X
20	0.349	2.245	1.892	0.496	0.314	1.095			40 linear channel shift	Linear Channel Shift
24	110	567	444	175	119	1569,284			1.5 x negative	MFCN
25	50	304	224	102	56	2701			RMF >1.3	Median log channel
28	69	127	130	84	43	684			≥1.6-3: Equivocal, ≥3.1: Positive	MFI
35	1.03	3.43	3.02	1.59	1.07	5.96			mean of negative control +3SD	Median log channel
38	3.5	231.6	220.1	103.1	0.0	358.4			33	Linear Channel Shift
39	0.20	0.61	0.54	0.27	0.20	9.77			>1.5 RMF	Median Log Fluorescence
41	196	890	826	382	205	9436			RMF >1.6	RMF
42	0.97	3.10	2.24	1.54	1	49.96			1	RMF
45	171	321.5	298.5	252.5	180	656			220 MCV	Median Channel Value
48	0.49	1.15	1	0.62	0.51	9.41			>1.3 RMF	Median Log Channel
51	5.05	12.49	15.12	6.35	4.97	128				
112	0.345	1.14	-	0.472	0.386	2.78			Ratio >1.5	Median Log Channel
115	247	427	470	325	264	340			>40	Median Linear Channel Shifts
117	3.27	7.3	7.43	3.8	3.28	56.23			Ratio (RMF) >1.3	NT
118	119	200	190	139	112	715			178(112+66)	Median Linear Channel
119	0.158	0.619	0.591	0.287	0.162	3.4			Ratio Pos >1.289	NT
120	1.03	2.44	-	1.37	63	5645.5			Ratio >1.6	Median
122	6	33	30	10	6		39	12	3SD	Geo mean linear values
126	168	439	450	304	151	625			>80 linear channel shift	Median Linear Channel
130	160	278	279	164	167	279			40	Mean Channel Shift
138	0.05	0.37	0.27	0.12	0	0.92			>0.2	D Value Kolmogorov-Smirnov Statistic
139	0.37	1.28	1.18	0.42	0.3	33			>1.5 of negative mean log channel	Mean log channel
143	1	22	29	5	4	15			>15 linear channel shift	Mean Channel Shift
144	1.11	2.12	2.30	1.47	1215	5.57			1.70	Median - Ratio
145	0.452	1.76	1.76	0.775	0.561	69			2SD of the ratio	Median log channel
147	109	279	284	178	117	520			>40 linear channel shift	Linear channel
149	0.395	1.68	1.92	0.573	0.499	152			2SD of the ratio	Median log channel
154	68	431	437	171	69	259			Median >150% of control and shape of the curve	MFI
157	126	169	163	135	130	470			160 (2SD)	Median Linear Channel
159	146	279	267	193	140	428			40 Linear channel shift	Mean Fluorescence
160	8.0	50.7	40.3	14.1	9.8	260			>19.7 (Negative + 2SD)	Mean linear channel
167	132	1036	1104	408	143	11042			2SD	Median log channel
169	246	336	290	268	261	427			≥50	Median Channel
176	-0.09	0.85	0.85	0.33	0	1.04			0.4	MFI Shift
190	67	211	181	NT	66	567			Ratio sample/negative >1.5	Median log channel
191	13.5	143	144.4	18	0	441			>30	Delta MFI
193	4.9	34.51	34.2	12.56	4.62	145.94			NC mean x2.4	Geo Mean Linear Channel
194	0.4	1	1.2	0.7	0.45	12			Ratio >1.2	Median Channel Log
195	NT	NT	NT	NT	NT	NT			NT	NT
201	0.525	0.969	1.08	0.645	0.478	29		NT	S/NK ≥2.0	X-MEAN
209	84	568	497	-	86	370			1.5 (ratio)	Geometric Mean Log Channel (Ratio)
218	NT	NT	NT	NT	NT	NT			NT	NT
220	1274	16150	10798	3625	1685.5	116609			6000 above the mean of the NC	Linear Acquisition, Linear Values
235	1	4.3		1.9	1	55.3			>2 of Neg median log channel	Median Log Channel
238	585	1617	1588	984	810	26717			MESF serum/MESF negative control	NT
240	72	95	91	75	71	703			1.2x NC	MFI
246	NT	NT	NT	NT	NT	NT			NT	NT
252	1.1	3.55	2.05	1.05	71.69	649.38			Median ratio ±2SD	
260	142.39	462.16	372.81	217.49	303.10	5127.39			2 X Negative control MFI	MFI
262	415	1143	817	745	466	17790			>2 neg	Median Channel Log
271	127	260	-	152	136	427			64	Median Log Channel
293	0.647	2.36	2.04	1.03	0.76	57.8			10 channels hift	Channel Shift
297	29	360	374	128	38	206			1.3x MFI Tneg	MFI
341	3.47	6.28	5.91	3.88	3.62	20.66			3SD	Geo mean linear values
351	224	442	361	240	281	12158			NC +500	MFI

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11	6608,7906	5518,6076	14057,13813	2530,2513	3291,2711,2174,2625	19104,41043			3240 1.2RMH	Median log channel
14	8031	5802	11300	2673.5	548	9605.5			1.5 x mean NEG	Median log channel
15	5.49	2.86	5.09	1.28	1882.5	44367.5			RMF >1.5	Median
19	10.520	9.402	18.228	3.682		48.976			1.88 x average negative control	Log Median X
20	24.12	15.46	25.797	3.64	1.983	7.114			40 linear channel shift	Linear Channel Shift
24	834	2540	3070	444	384	9252,793			2 x negative	MFCN
25	2558	2222	*700	810	651	18833			RMF >1.3	Median log channel
28	621	388	703	146	82	825			≥2.0-3.5: Equivocal, ≥3.6: Positive	MFI
38	149.2	85.2	75.9	18.9	0.0	259.8			40	Linear Channel Shift
39	4.53	3.78	9.49	1.02	0.53	19.40			>1.5 RMF	Median Log Fluorescence
41	4475	3332	4432	903	813	14593			RMF >1.6	RMF
42	4.72	4.62	4.21	1.25	1	49.96			>1.3	RMF
48	5.1	4.38	6.05	1.39	1.41	15.35			>1.3 RMF	Median Log Channel
51	72.1	41.6	30.23	10.6	8.14	203.5				
112	4.17	2.71	-	0.551	0.604	8.95			Ratio >2.0	Median Log Channel
115	704	628	635	540	535	759			>60	Median Linear Channel Shifts
117	27.18	36.03	25.95	11.37	11.45	173.5			Ratio (RMF) >1.5	NT
118	159	159	160	159	141	429			166.5(141+25.5)	Median Linear Channel
119	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
120	3.93	2.12	-	1.16	545	8681.5			Ratio >2.0	Median
122	293.5	203	237.5	64.5	70		205	74	3SD	Geo mean linear values
126	603	562	504	382	348	834			>120 linear channel shift	Median Linear Channel
130	524	421	484	317	280	495			80	Mean Channel Shift
139	33	10.8	10.3	1.77	7.41	142			>2 of negative mean log channel	Mean log channel
143	-	-	-	-	-	-	-	-	-	Mean Channel Shift
144	5.28	3.01	3.74	1.38	4113	5.36			2.50	Median - Ration
145	6.77	6.19	4.64	3.04	3.21	10.5			2SD of the ratio	Median log channel
147	347	498	490	365	248	645			>60 linear channel shift	Linear channel
149	21.8	11.9	13.9	7.08	7.21	176			2SD of the ratio	Median log channel
154	3454	2653	2837	949	973	18502			Median >250% of control and shape of the curve	MFI
157	202	227	248	162	159	315			201 (2SD)	Median Linear Channel
159	449	447	459	330	265	602			100 linear channel shift	Mean Fluorescence
160	571	300	700	99	77	764			>173 (Negative + 2SD)	Mean linear channel
167	132	1036	1104	408	2161	66992			2SD	Median log channel
169	462	508	437	383	427	733			≥80	Median Channel
176	0.76	0.59	0.49	-	0	0.66			0.22	MFI Shift
190	592	532	423	NT	192	726			Ration sample/Negative >2.5	Median log channel
191	145	173	-24	1.2		0			370	Delta MFI
193	451.68	239.81	170.42	107.8	87.57	381.35			NC mean x1.9	Geo Mean Linear Channel
194	18.5	19	8.4	5.5	3.5	80			Ratio >4	Median Channel Log
195	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
201	16.6	7.05	7.5	3.69	3.2	71.7			S/NK ≥2.5	X-MEAN
209	3968	2455	1745	871	806	1697			1.7 (ratio)	Geometric Mean Log Channel (Ratio)
218	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
220	25336	27725	9884	3600	3836.75	146618			6000 above the mean of the NC	Linear Acquisition, Linear Values
235	-	37.9	-	8.35	8.13	1669.77			>18 of Neg median log control	Median Log Channel
238	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
240	1174	1682	2502	1089	788	3501			1.4 xNC	MFI
246	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
252	3.11	2.89	1.91	0.99	171.54	973.38			Median ratio ±2SD	
260	1285.71	2037.00	1978.39	1053.76	1285.71	7838.37			2 X negative control MFI	MFI
262	3609	4282	3748	1691	1031	45338			>2 neg	Median Channel Log
271	391	354	-	195	211	720			90	Median Log Channel
293	2.37	13.1	12.1	2.73	2.35	70.3			20 channel shift	Channel Shift
297	3338	2129	2573	873	726	1369			1.5 x MFI Tneg	MFI
341	21.61	19.57	22.07	8.32	18.83	247.48			3SD	Geo mean linear values
351	2186	1702	1055	671	1234	21118			NC +2500	MFI