

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

T-CELL AND B-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B01/2017 (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 17TH JANUARY 2017

HLA PHENOTYPE OF BLOOD DONOR: HLA-A3, A11; B7, B51; Cw7, Cw14; DR11, DR103; DQ7, DQ-

	Summary of Results							
	T-cells				B-cells			
Total tested	45	50	47	50	35	41	44	45
Positive	37	0	23	1	9	15	37	0
Negative	8	50	24	49	26	26	7	45
NT/Equivocal	12	7	10	7	20	14	11	10
% Positive	82.2%	0.0%	48.9%	2.0%	25.7%	36.6%	84.1%	0.0%
% Negative	17.8%	100.0%	51.1%	98.0%	74.3%	63.4%	15.9%	100.0%
<b>Consensus</b>	<b>Positive</b>	<b>Negative</b>	<b>Not Assessed</b>	<b>Negative</b>	<b>Not Assessed</b>	<b>Not Assessed</b>	<b>Positive</b>	<b>Negative</b>
HLA Antibody Specificity (Defined By CDC)	A11	B44	A2, A28, A9	B57	A11	B44	A2, A28, A9	B57

Lab No.	T-cells				B-cells				Date Received	Date Tested	Viability (%)	Comments
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 1	Serum 2	Serum 3	Serum 4				
101	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Negative	18-Jan	19-Jan		Serum 1 on B-cells is equivocal according to our standard. However, according to the positivity on T-cells for this serum, we choose to interpret B-cells as positive. Results on B-cells were performed after cells-pronase pretreatment.
112	NT	NT	NT	NT	NT	NT	NT	NT	23-Jan	23-Jan	0	Cell viability 0%
114	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative	18-Jan	19-Jan		Serum 1- there is an unexpected T and B cell result discrepancy. This should be repeated to confirm.
115	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan		
117	Positive	Negative	Negative	Negative	Equivocal	Negative	Positive	Negative	19-Jan	19-Jan	95	Low number of B cells
118	NT	NT	NT	NT	NT	NT	NT	NT	19-Jan	19-Jan	NT	The samples were not tested due to a delivery delay as discussed with Luke Gardner
119	Positive	Negative	Negative	Negative	NT	NT	NT	NT	16-Jan	17-Jan	95	B-FCXM not tested due to low number of cells
120	NT	NT	NT	NT	NT	NT	NT	NT	20-Jan	20-Jan	0	Sample delivery delay
122	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative	18-Jan	19-Jan	90	
126	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	19-Jan	20-Jan	90	2B01 serum 1, T cell weak positive, B cell negative, difficult to interpretate, maybe low affinity abs? or HLA-C abs? Normally ab spec data/donor HLA would give us an answer.
130	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Negative	18-Jan	19-Jan	99	
133	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	19-Jan	20-Jan	99	
136	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	18-Jan	19-Jan	99	
138	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	18-Jan	18-Jan	90	
139	Positive	Negative	Equivocal	Negative	Negative	Negative	Positive	Negative	20-Jan	20-Jan	80	
142	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	19-Jan	20-Jan	0	
143	Negative	Negative	Negative	Negative	NT	NT	Positive	Negative	19-Jan	19-Jan	91	
144	Positive	Negative	Positive	Negative	NT	NT	Positive	Negative	18-Jan	19-Jan	92	Cells were clumped. Insufficient B cells and result inconclusive for B cell sera 1 and 2
145	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Negative	18-Jan	19-Jan	92	
147	Equivocal	Negative	Negative	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan	100	Sera 1 T cells displays a weak positive reaction which is discrepant with the negative reaction observed on B cells
149	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Negative	18-Jan	19-Jan	87	
154	Positive	Negative	Positive	Negative	Negative	Equivocal	Positive	Negative	19-Jan	20-Jan	NT	
157	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Negative	18-Jan	18-Jan	98	
159	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative	19-Jan	20-Jan	100	
160	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Negative	18-Jan	19-Jan		
163	NT	NT	NT	NT	NT	NT	NT	NT	19-Jan	NT	NT	Sample delivery delay
167	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan	98	
169	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan	98	
176	Positive	Negative	Positive	Negative	Equivocal	Negative	Positive	Negative	19-Jan	20-Jan		
186	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	18-Jan	18-Jan	99	
190	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	18-Jan	19-Jan	78.4	
191	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan	100	
193	Positive	Negative	Positive	Negative	Equivocal	Negative	Positive	Negative	18-Jan	18-Jan		
194	Equivocal	Negative	Equivocal	Negative	Equivocal	Equivocal	Positive	Negative	19-Jan	20-Jan		Few cells
195	NT	NT	NT	NT	NT	NT	NT	NT	18-Jan	19-Jan	97	Sample delivery delay
201	Positive	Negative	Negative	Negative	NT	NT	NT	NT	18-Jan	19-Jan	NT	B cell NT because of low cell count
202	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	18-Jan	18-Jan	60	
204	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative	18-Jan	18-Jan		
206	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	18-Jan	19-Jan		both readings for the Serum1 are positive on T-cells but results are negative on B-cells both readings for the Serum2 are really close to our positive cut-off on T-cells (but results are negati
209	Equivocal	Negative	Positive	Negative	Equivocal	Negative	Positive	Negative	17-Jan	19-Jan	90	
218	NT	NT	NT	NT	NT	NT	NT	NT	27-Jan	NT	NT	Sample delivery delay
220	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	19-Jan	20-Jan	92	
227	NT	NT	NT	NT	NT	NT	NT	NT	18-Jan			Samples were not tested
235	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	20-Jan	20-Jan	80	Cell count and viability rate are low. Not enough cells were counted while evaluating B FCXM.
238	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	19-Jan	20-Jan	100	
245	Positive	Negative	Negative	Negative	Equivocal	Positive	Positive	Negative	18-Jan	19-Jan	100	
246	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	19-Jan	20-Jan	98	
252	Negative	Negative	Negative	Negative	Negative	Positive	NT	Negative	19-Jan	19-Jan	75	
260	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	19-Jan	19-Jan	N/A	
262	Equivocal	Negative	Negative	Negative	Equivocal	NT	Positive	Negative	19-Jan	20-Jan	52	samples arrived after three days, completely cold and the viability of cells was very low - some results cannot be interpreted due to low cell count
271	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	22-Jan	23-Jan	97	Samples were received 5 days after shipping and cells were clumped
284	Equivocal	Negative	Equivocal	Negative	Equivocal	Positive	Positive	Negative	18-Jan	18-Jan		
293	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	19-Jan	19-Jan	80	
297	Positive	Negative	Positive	Negative	Equivocal	Negative	Positive	Negative	18-Jan	19-Jan		Not enough cells to test duplicates
341	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative	18-Jan	19-Jan	98	
351	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	21-Jan	21-Jan	94.8	
358	Positive	Negative	Negative	Positive	Negative	Positive	Positive	Negative	18-Jan	18-Jan		

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T-CELL FLOW CYTOMETRY RESULTS OF SAMPLE 2B01/2017 (COMPARED TO LOCAL NEGATIVE CONTROL)

DISPATCHED ON 17TH JANUARY 2017

Lab No.	Serum 1		Serum 2		Serum 3		Serum 4		MoU range	Negative control (local)	Positive control (local)	Strong positive (local)	Weak positive (local)	Positive result value	Cytometer reading units
	cytometer reading	within MoU	cytometer reading	within MoU	cytometer reading	within MoU	cytometer reading	within MoU							
101	0.40	No	0.21	No	0.27	No	0.20	No	MFI samples/MFI negative control near 1.5	0.22	1.34			1.5 SD (0.32)	MFI
112	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
114	343.00	Yes	168.00	Yes	250.00	Yes	161.00	Yes	50 +/- 10	171.00	302.00			50 linear channel shift	Median channel
115	423.00		178.00		325.00		251.00			256.00	426.00			>40	
117	8.2	No	3.92	No	5.06	No	3.87	No	5.46 - 5.88	4.2	71.05			Ratio (RMF) >1.3	MniX
118	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
119	0.657	No	0.168	No	0.281	Yes	0.15	No	1.289 - 1.45	0.146	2.65			Ratio >1.289	MniX
120	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
122	62.3	No	11.6	No	37.8	No	11.9	No	13.85			198.8	66.6	3 SD	Geomean linear values
126	192.00		84.00		131.00		91.00		?	90.00	522.00			>80 linear channel shift	Median linear channel
130	219.00		133.00		196.00		129.00			126.00	250.00			>40	Mean channel shift
133	10		6		8		6			8	90			> x1.5 of neg control	MFI
136	14.1		4.2		6		4			4	29 (NR = 725)			NR >200	Geomean channel
138	0.22		0.03		0.12		0.04			0	0.8			> 0.2	D value Kolmogorov-Smirnov Statistic
139	3.3		0.278		1.01		0.296			0.321	70			0.48	Median
142	252	No	125	No	14	No	74	No		153	4253			170 (153 + 3SD)	Median log channel
143	2.00		23.00		1.00		18.00		N/A	2.00	69.00			>40 channel shift	Mean channel shift
144	2.73		1.08		2.19		1.25			954	12.88			1.7	Median -> Ratio
145	1.68		0.307		0.871		0.342			0.345	68.3			2SD of the ratio	Median log channel
147	157		107		138		109			104	432			>40 linear channel shift	Linear channel
149	1.27	No	0.283	No	0.586	No	0.321	No		0.388	29.1			2SD	Median log channel
154	277.00		70.00		159.00		71.00			72.00	1614.00			median>150% of negative control and shape of the curve	MFI
157	172.00		117.00		148.00		119.00			126.00	492.00			2SD (+30)	Median linear channel
159	292		131		232		132			135	441			40 MLC shift	Mean linear channel (MLC)
160	30.50		7.00		28.70		9.10			6.30	334.00			>13.6 (NEGATIVE + 2SD)	Mean linear
163	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
167	738		190		561		185			210	31066			2SD	Median log channel
169	354.00		258.00		315.00		249.00			283.00	590.00			=>50	Median channel
176	7883.00		816.00		2845.00		880.00			787.00	10436.00			MFI-shift >= 0.32	MFI Shift
186	3.36	No	0.4	No	2.25	No	0.45	No		0.44	28.1			1.5 fold the local negative control	MFI - log channel
190	54.47		47.47		52.37		49.13		ND	51.13	68.20			Ratio sample/negatif>1,5	Median log channel
191	106.00	No	-10.00	No	71.00	No	-14.00	No	25-35	0.00	192.00			0	Mean
193	28.32		4.83		16.64		5.18			4.45	508.24			Negative control mean x 2.4	Geometric mean linear channel
194															Median log channel
195	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
201	1.1		0.48		0.78		0.44			0.442	17.8			S/NK ≥ 2.0	X-mean
202	0.01	No	0.00	No	0.00	No	0.01	No	4%+Neg% < TCell% < 5%+Neg%	0.01	1.00			0.05	Cell Percentage
204	2.38		0.41		0.99		0.38			0.39	43.20			0.579 = 1.5 x loc neg control	Mean log channel
206	4.58		3.89		4.20		3.66			3.45	23.47			Ratio MFI(Serum)/ MFI (Neg Control)	MFI
209	Equivocal		1		2.8		1.2			111	586			>1.07	Geometric mean log channel (ratio)
218	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
220	15530,50		1633,00		12076,50		1818,00			2552,00	261827,00			6000 above the mean of the negative controls	Linear acquisition, linear values
227	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
235	1,78		2,13		2,37		1,53			1,60	74,99			>2 of negative control serum median log channel	Median log channel
238	1356.00		919.00		898.00		1010.00			1140.00	23579.00			MESF serum/MESF negative control	MESF
245	6.30	Yes	-0.20	No	2.30	No	-0.70	No	5-7	mean linear chan	14.30			7	Linear channel shift
246	3.50		0.97		2.50		1.06				12.18			1.75	Median log FI
252	1.00		0.68		0.66		0.71			60.43	791.48			Median ration ± 2SD	Median channel ratio
260	407.72	No	175.50	No	229.70	No	103.16	No	1.8 x MFI (neg) > uncertainty < 2 x MFI	128.60	843.92			2 x negative control MFI	MFI
262	1698.00		863.00		1134.00		828.00			884.00	20243.00			2SD	Median log channel
271	386.00	No	120.00	No	300.00	No	133.00	No		132.00	476.00			64	Median log channel
284	896.00	Yes	705.00	No	823.00	Yes	688.00	No	1,10 < ratio < 1,20	750.00		4187.00	846.00	sample serum / negative control: ratio >	median log channel
293	2.65	Yes	0.74	Yes	1.95	Yes	0.88	Yes	4.80	0.79	45.80			10 CHANNEL SHIFT	Channel shift
297	391.00	No	47.00	No	162.00	No	51.00	No	1.3 - 1.8 x T neg	50.00	479.00			1.3 x T neg	Median MFI
341	6.05	No	3.43	No	4.96	No	3.40	No		3.62	18.67			3SD	Geomean linear values
351	387.00	No	210.00	No	278.00	No	216.00	No	500 + negative results	205.00	11556.00			500+NEGATIVE RESULTS	MFI
358														3x neg	

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	cytometer reading	within MoU	cytometer reading	within MoU	cytometer reading	within MoU	cytometer reading	within MoU							
101	1.03	Yes	0.68	No	2.30	No	0.58	No	MFI samples/MFI negative control near 2	0.60	6.28			2SD (1 to 1.2)	MFI
112	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
114	566.00	Yes	670.00	Yes	630.00	Yes	522.00	Yes	60 +/- 10	547.00	649.00			60 linear channel shift	Median channel
115	476.00		331.00		543.00		436.00			434.00	680.00			>60	
117	Equivocal	Yes	7.97	No	19.81	No	9.02	No	12.65 - 14.33	8.43	158.49			Ratio (RMF) >1.5	MnIX
119	NT	NT	NT	NT	NT	NT	NT	NT		NT	NT	NT	NT	NT	NT
120	NT	NT	NT	NT	NT	NT	NT	NT		NT	NT	NT	NT	NT	NT
122	157.30	No	246.20	No	351.10	No	54.70	No	90.70			560.60	205.10	3 SD	Geomean linear values
126	150.00		98.00		374.00		131.00		?	82.00	714.00			>120 linear channel shift	Median linear channel
130	507.00		440.00		491.00		221.00			188.00	452.00			>80	Mean channel shift
133	10.00		18.00		19.00		6.00			14.00	215.00			> x2 of neg control	MFI
139	7.61		8.48		19.40		4.20			5.61	162.00			11.22	Median
142	536	No	799	No	791	No	405	No		651	11878			1104 (651 + 3SD)	Median log channel
144	NT		NT		2.99		0.94			4914	5.83			2.5	Median -> Ratio
145	2.26		2.91		5.17		0.903			1.65	69.6			2SD of the ratio	Median log channel
147	180		218		293		171			171	180			>60 linear channel shift	Linear channel
149	2.59	No	2.53	Yes	6.83	No	0.689	No		1.66	41.3			2SD	Median log channel
154	539.00		856.00		1393.00		393.00			379.00	9641.00			median>250% of control and shape of the curve	MFI
157	211.00		151.00		225.00		124.00			157.00	532.00			2SD (+42)	Median linear channel
159	348		390		442		266			262	572			100 MLC shift	Mean linear channel (MLC)
160	120.00		198.00		246.00		46.00			26.30	800.00			>56 (NEGATIVE + 2SD)	Mean linear
163	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
167	1694		1625		3133		1272			1625	75895			2SD	Median log channel
169	373.00		387.00		515.00		293.00			381.00	680.00			=>80	Median channel
176	10230.00		8194.00		27308.00		5775.00			6939.00	32900.00			MFI-shift >= 0.22	MFI Shift
186	4.29	No	3.71	No	8.7	No	2.61	No		2.8	31.6			2 fold the local negative control	MFI - log channel
190	57.27		41.4		87.93		49.03		ND	79.20	435.00			Ratio sample/negatif>2,5	Median log channel
191	99.00	Yes	63.00	No	213.00	No	14.00	No	101-120	0.00	330.00			0	Mean
193	86.2		63.52		213.1		72.42			50.28	802.42			Negative control mean x 1.9	Geometric mean linear channel
194															Median log channel
195	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
201	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.925	44.2			S/NK ≥ 2.5	X-mean
202	0.03	No	0.06	No	0.06	No	0.01	No	7%+Neg% < BCell% < 8%+Neg%	0.01	1.00			0.08	Cell percentage
204	8.95		27.30		18.50		5.61			7.58	59.20			15.2 = 2 x loc neg control	Mean log channel
206	6.50		6.69		8.64		5.12			6.50	38.13			Ratio MFI(Serum)/ MFI (Neg Control) >1.17	MFI
209	Equivocal		1.2		4		1.3			667	1738			1.9	Geometric mean log channel (ratio)
218	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
220	10954,50		3001,00		36774,50		4036,50			4843,25	259850,00			6000 above the mean of the negative controls	Linear acquisition, linear values
227	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
235	9,22		156,89		15,33		10,27			5,57	1333,5			>18 of negative control serum median log channel	Median log channel
238	2557.00		4204.00		3363.00		2344.00			3407.00	30179.00			MESF serum/MESF negative control	MESF
245	9.70	No	20.20	No	16.20	No	2.20	No	13-15	35.30	61.70			15	Linear channel shift
246	1.57		1.40		5.43		0.97				11.19			2.5	Median log FI
252	0.46		1.47		NT		0.30			205.35	2864.38			Median ration ± 2SD	Median channel ratio
260	890.80	No	2333.11	No	1210.00	No	717.20	No	1.8 x MFI (neg) > uncertainty < 2 x MFI	792.46	3614.96			2 x negative control MFI	MFI
262	3058.00		LOW CELLS		5485.00		1109.00			1581.00	53041.00			2SD	Median log channel
271	344.00	No	205.00	No	530.00	No	217.00	No		193.00	540.00			90	Median log channel
284	934.00	Yes	2121.00	No	1478.00	No	705.00	No	1,10 < ratio < 1,20	845.00		11347.00	892.00	sample serum / negative control: ratio > 1,2	Median log channel
293	8.57	Yes	2.25	Yes	7.88	Yes	2.34	Yes	4.38	2.21	178.70			20 CHANNEL SHIFT	Channel shift
297	681.00	No	497.00	No	1718.00	No	343.00	No	1.5 - 2 x T neg	431.00	1122.00			1.5 x T neg	Median MFI
341	7.37	No	18.31	No	19.73	No	5.74	No		9.77	257.65			3SD	Geomean linear values
351	513.00	No	374.00	No	1004.00	No	399.00	No	2500 + negative results	398.00	13843.00			2500+NEGATIVE RESULTS	MFI
358														3x neg	