How Are We Measuring Sensitization

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CEOT

CUTTING EDGE OF TRANSPLANTATION

RESOLVING THE ORGAN SHORTAGE

PRACTICE | POLICY | POLITICS

FEBRUARY 25-27, 2016 • PHOENIX, ARIZONA

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Conflict of Interest Disclosure

- Astellas, Advisory Board

- I have no relevant financial relationships to disclose.
- No off-label use of drugs will be discussed

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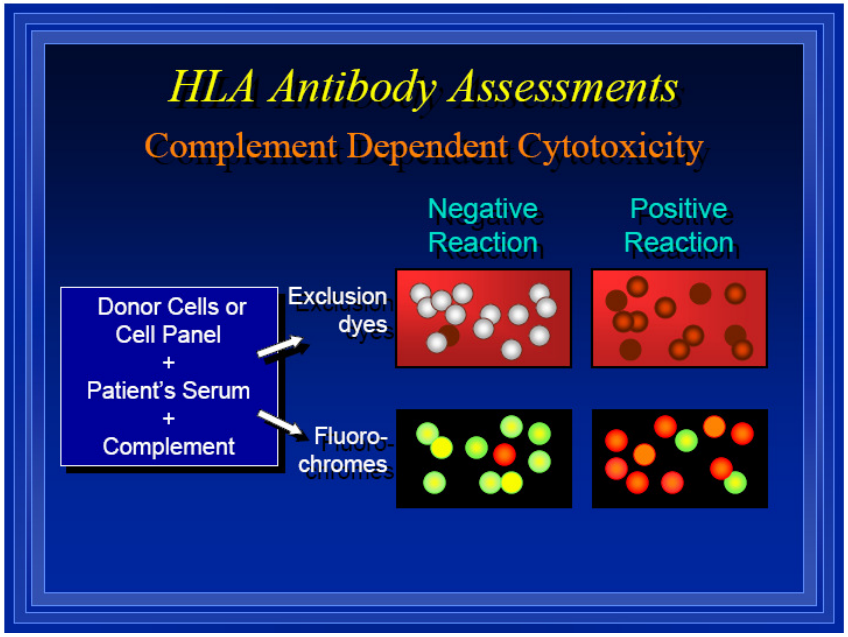
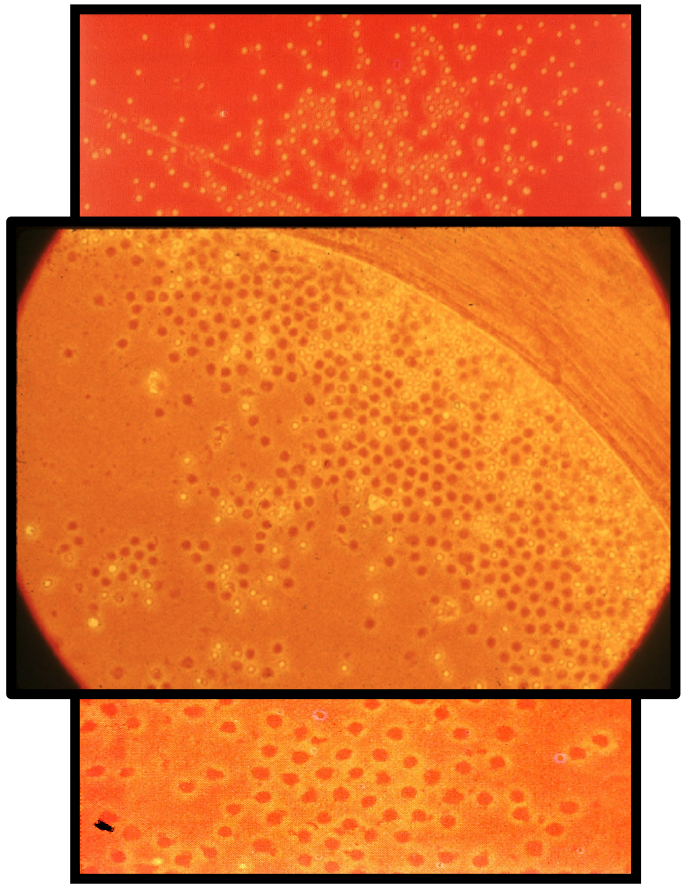
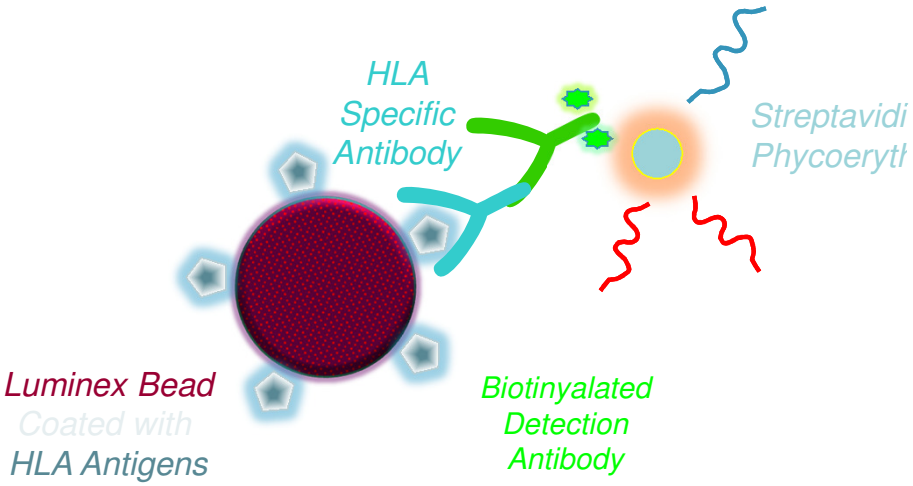
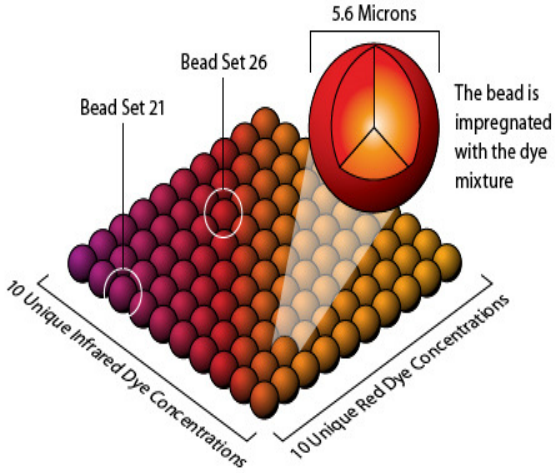




Score	*Interpretation*	*% Dead Cells*
1	*Negative*	*0-10%*
2	*Doubtful Negative*	*11-20%*
4	*Weak Positive*	*21-50%*
6	*Positive*	*51-80%*
8	*Strongly Positive*	*81-100%*
0	*Uninterpretable*	*n/a*

State of the art assays
Specifically Detecting HLA Antibodies





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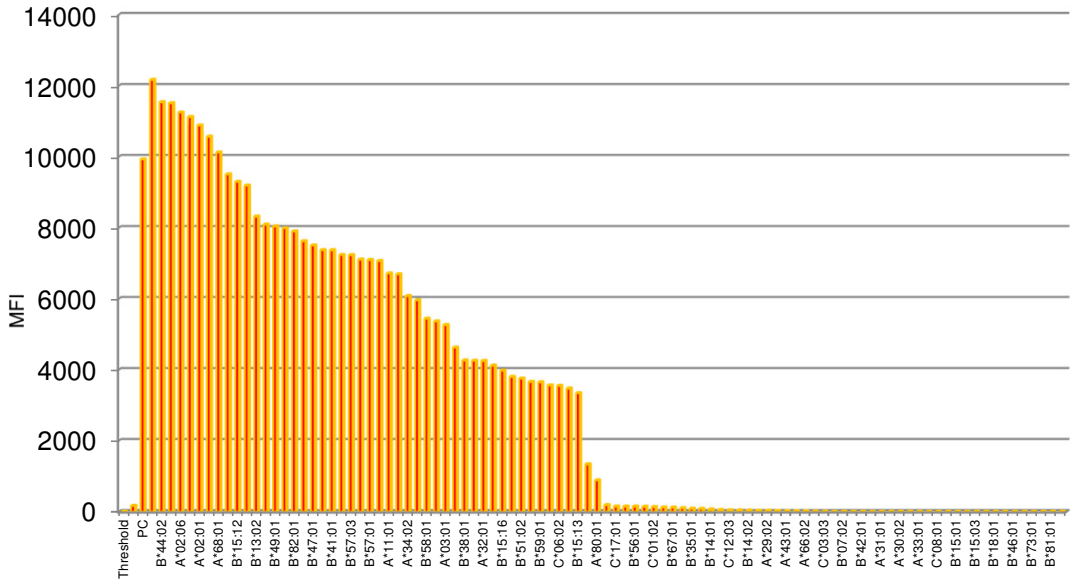
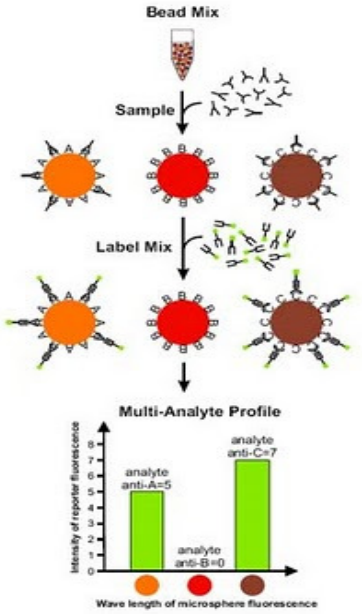
State of the art assays Luminex Technology





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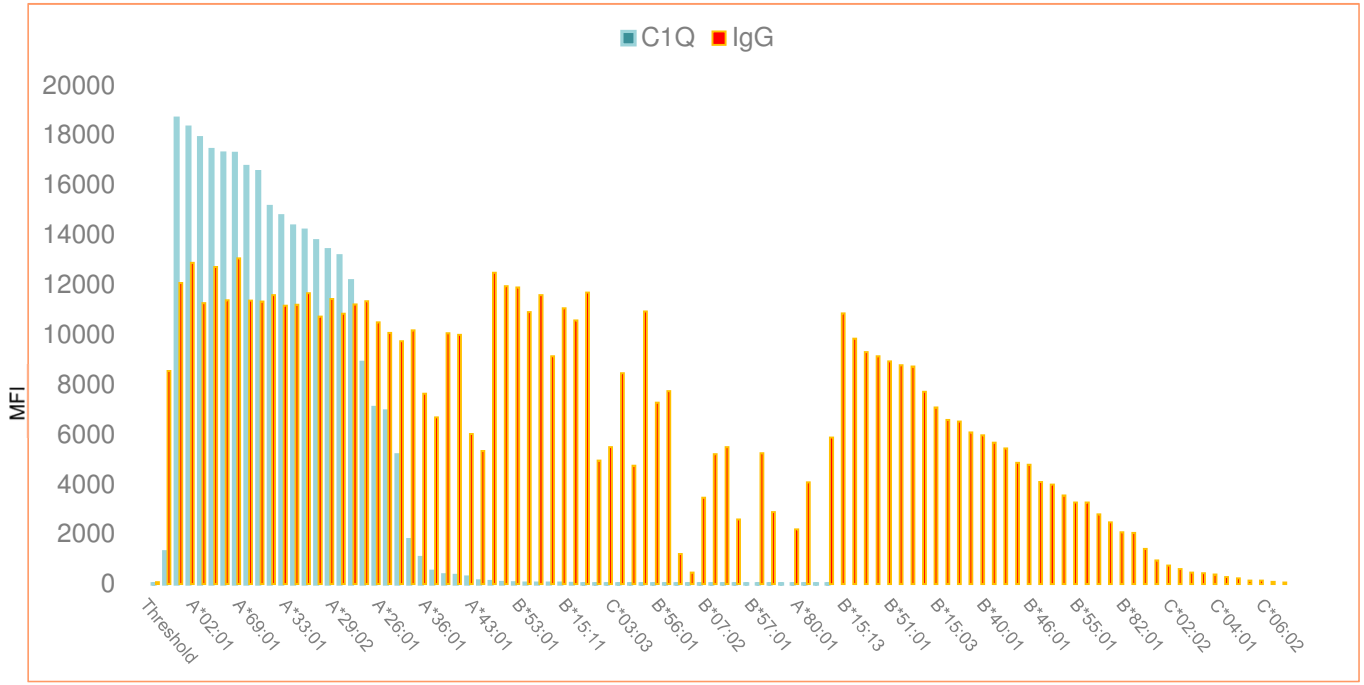
Identification of Complement Binding HLA-Antibodies



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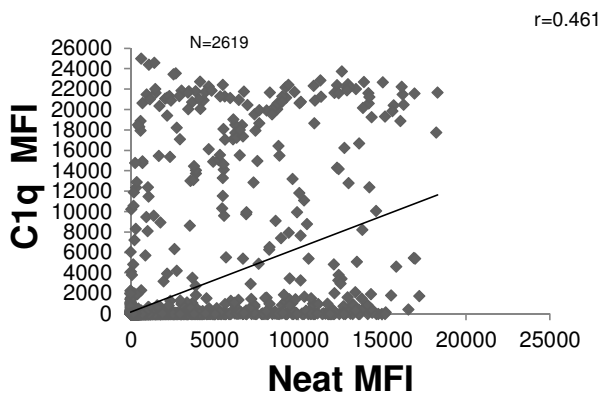
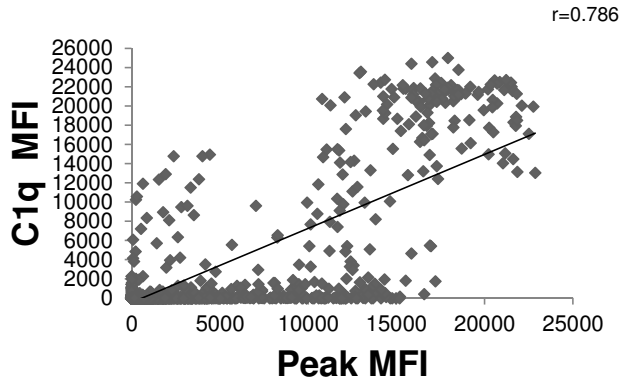
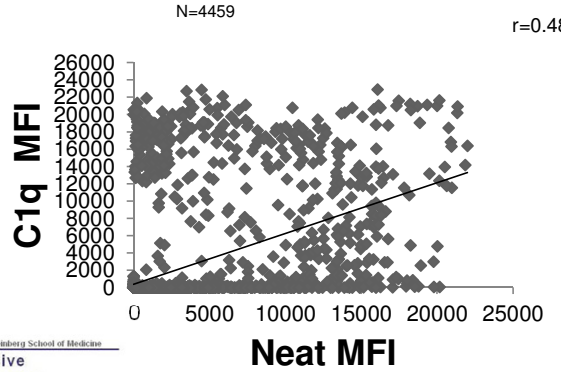
IgG negative C1q positive usually = unmasked prozone

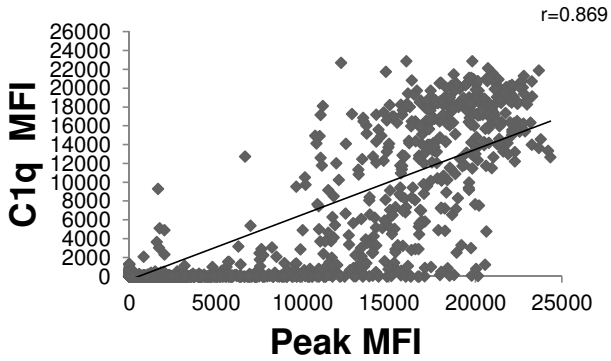
Class I





Class II





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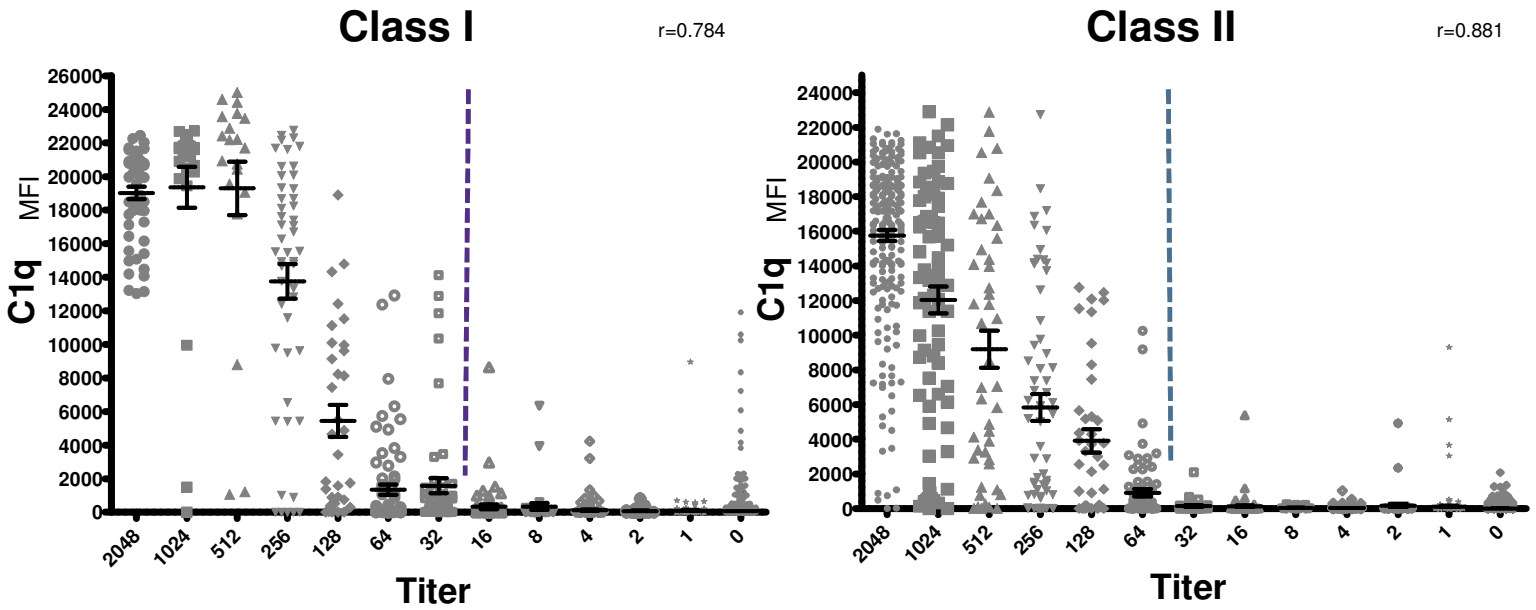
C1q binding is an indication of a stronger antibody - Higher titer



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Antibodies with the same MFI value can have different strength

Bead	Antigen	Neat	1:4	1:16	1:64	1:256	1:1024	Titer	C1q
50	DQA1*01:02,-,DQB1*05:02	16349	16490	11793	5503	2171	549	512	22289
53	DQA1*01:01,-,DQB1*06:02	16215	16361	10836	4970	1937	469	256	18203
55	DQA1*01:02,-,DQB1*06:04	16097	16050	11408	5529	2172	608	512	19412
52	DQA1*01:02,-,DQB1*06:02	14990	13744	9011	4087	1472	386	256	13645
51	DQA1*01:03,-,DQB1*06:01	13472	15647	14399	6630	2561	680	512	23810
49	DQA1*01:01,-,DQB1*05:01	13017	15759	16104	8029	3270	910	512	24061
56	DQA1*01:02,-,DQB1*06:09	11650	14011	17963	11974	5304	1557	1024	24479
15	DRB1*08:01	11349	7529	2374	541	156	46	32	1911
23	DRB1*13:01	10819	6231	1996	555	166	46	16	549
54	DQA1*01:03,-,DQB1*06:03	10719	13886	17212	9974	4397	1260	1024	24512
24	DRB1*13:03	10030	4337	1191	267	80	21	16	2225
6	DRB1*03:01	10026	5531	1860	511	163	44	16	428
7	DRB1*03:02	8878	4668	1326	335	107	31	16	1455
25	DRB1*14:01	8518	4607	1356	351	106	31	16	380
22	DRB1*12:02	8323	4248	1208	314	101	29	16	128
21	DRB1*12:01	8286	4146	1173	309	104	26	16	151
26	DRB1*14:02-	7197	3663	1173	339	123	40	16	145
20	DRB1*11:04	6010	3229	962	244	90	30	8	131
19	DRB1*11:01	5855	2964	929	235	74	21	8	156
46	DQA1*03:03,-,DQB1*04:01	5780	2000	461	114	45	23	8	30
27	DRB1*14:54	5148	2753	819	228	83	31	8	118
5	DRB1*01:03	5146	2117	417	79	25	7	8	66
47	DQA1*02:01,-,DQB1*04:02	2731	885	219	60	26	12	2	270
48	DQA1*04:01,-,DQB1*04:02	2548	795	191	55	27	12	2	275
45	DQA1*02:01,-,DQB1*04:01	1881	645	170	54	27	15	1	237
30	DRB1*15:03	1342	665	242	72	29	13	1	45
4	DRB1*01:02	1073	345	86	27	13	7	1	38
8	DRB1*15:01	1027	799	343	107	43	19	1	41

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Applying Antibody Strength to Clinical Decision Making

	C1q MFI							Bead	Titer						
	Pre-Rx	Post 1X Rx	Pre 2X Rx	Post 2X Rx	F/U 1M	F/U 3M	F/U 6M	Nmb	Pre-Rx	Post 1X Rx	Pre 2X Rx	Post 2X Rx	F/U 1M	F/U 3M	F/U 6M
DQA1*01:03/DQB1*06:01	17950	27129	26244	22326	17200	17488	5672	1	4096	1024	1024	256	256	256	128
DQA1*01:02/DQB1*06:09	17538	26971	25469	20675	14940	21603	10082	2	4096	1024	1024	256	128	256	128
DQA1*01:01/DQB1*05:01	17458	27219	25390	22122	16991	20502	8076	3	4096	1024	1024	256	512	256	128
DQA1*03:03/DQB1*04:01	15308	27086	25971	19981	14182	19034	6956	4	2048	512	512	256	256	128	64
DQA1*02:01/DQB1*03:03	14912	23282	18138	575	0	2	2	5	512	128	256	16	4	8	4
DQA1*01:01/DQB1*06:02	14173	25936	22327	12447	6813	14828	1778	6	2048	512	512	256	256	256	128
DQA1*03:02/DQB1*03:02	13953	20790	12606	83	99	1	2	7	256	64	64	16	16	8	4
DQA1*01:02/DQB1*06:02	13392	25554	21124	12263	6249	7401	817	8	4096	512	512	128	256	64	64
DQA1*03:02/DQB1*03:03	13192	21654	13094	77	26	1	1	9	256	64	64	4	16	16	4
DQA1*02:01/DQB1*03:02	2978	1177	967	221	7	2	1	10	128	32	32	16	4	4	4
DQA1*03:01/DQB1*03:02	2963	909	322	36	21	1	1	11	64	16	32	2	16	2	1
DQA1*03:01/DQB1*03:03	2876	1022	262	26	27	2	1	12	128	16	32	4	16	2	1

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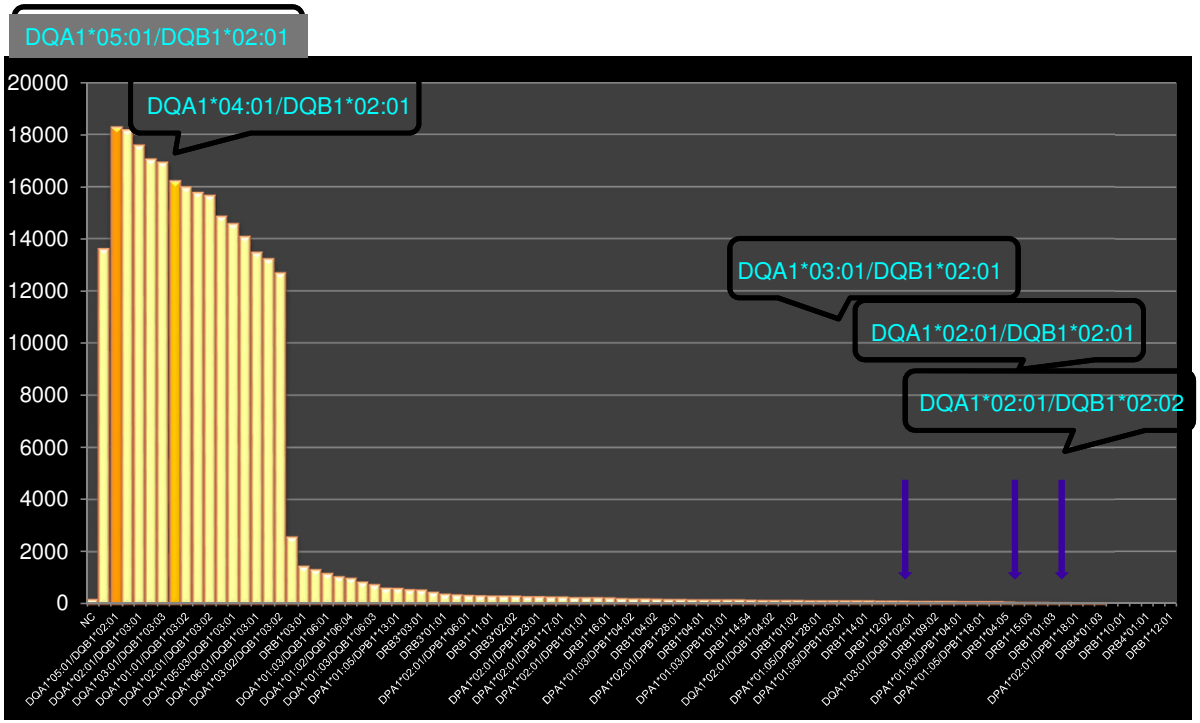
Do we have Donor-Specific Antibodies (HLA-DQ2) ?



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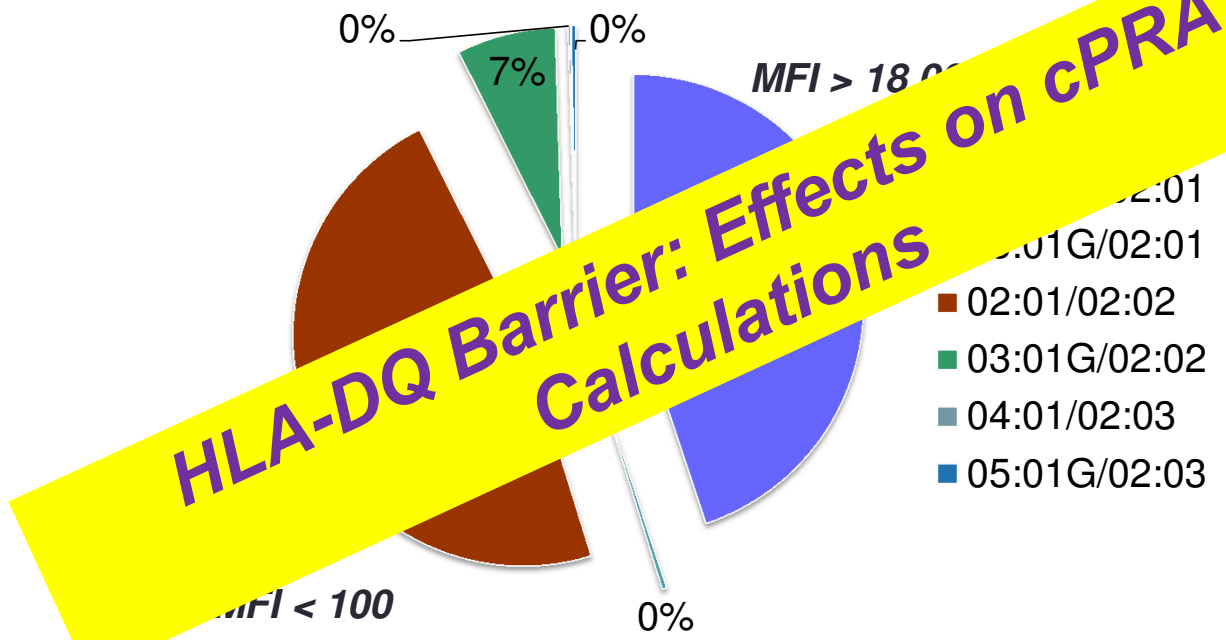
Tambur et al. Transplantation 2013; 96 : 1065

Frequency of HLA-DQ2 alleles

NU-CTC population



HLA-DQ Barrier: Effects on cPRA Calculations

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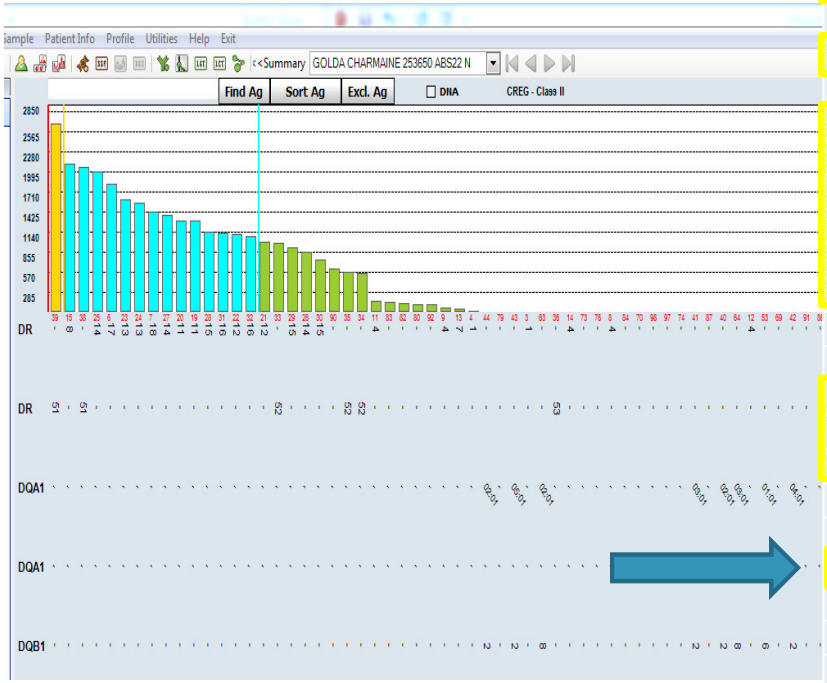
Tambur et al. Transplantation 2013; 96 : 1065

You think this is confusing? Wait until we start talking about HLA (B-cell) epitopes

MFI values can be artificially low due to sharing of same target (epitope) on multiple beads



MFI	DRB1	DRB3/ 4/5	DR CREG	Allele
2588		DR51		-,-,DRB5*02:02,-,-,-,-,-,-,-,-
2045	DR8		52	DRB1*08:01,-,-,-,-,-,-,-,-,-,-
1992		DR51		-,-,DRB5*01:01,-,-,-,-,-,-,-,-
1941	DR14		52	DRB1*14:01,-,-,-,-,-,-,-,-,-,-
1774	DR17			DRB1*03:01,-,-,-,-,-,-,-,-,-,-
1550	DR13		52	DRB1*13:01,-,-,-,-,-,-,-,-,-,-
1505	DR13		52	DRB1*13:03,-,-,-,-,-,-,-,-,-,-
1380	DR18		52	DRB1*03:02,-,-,-,-,-,-,-,-,-,-
1343	DR14		52	DRB1*14:54,-,-,-,-,-,-,-,-,-,-
1267	DR11		52	DRB1*11:04,-,-,-,-,-,-,-,-,-,-
1257	DR11		52	DRB1*11:01,-,-,-,-,-,-,-,-,-,-
1109	DR15			DRB1*15:01,-,-,-,-,-,-,-,-,-,-
1095	DR16			DRB1*16:01,-,-,-,-,-,-,-,-,-,-
1079	DR12		52	DRB1*12:02,-,-,-,-,-,-,-,-,-,-
1042	DR16		52	DRB1*16:02,-,-,-,-,-,-,-,-,-,-
974	DR12		52	DRB1*12:01,-,-,-,-,-,-,-,-,-,-
950		DR52		-,-,DRB3*01:01,-,-,-,-,-,-,-,-,-
893	DR15			DRB1*15:02,-,-,-,-,-,-,-,-,-,-
842	DR14		52	DRB1*14:02,-,-,-,-,-,-,-,-,-,-
725	DR15			DRB1*15:03,-,-,-,-,-,-,-,-,-,-
610				-,-,-,-,-,-,-,-,DPA1*02:01,-,DPB1*14:01,-
562		DR52		-,-,DRB3*03:01,-,-,-,-,-,-,-,-,-
549		DR52		-,-,DRB3*02:02,-,-,-,-,-,-,-,-,-

Donor Name: DR1, **DR14 (52 CREG**)

ID#:

Crossmatch Date: 08/19/2015

Initial Flow Crossmatch

			Channel Shift		
Serum Date	Dilution	Plt Abs	T*	B**	Comments
08/05/2015	1:1	N	0	+67	
	1:2		-3	+43	
	1:8		-6	+14	
08/05/2015	1:1	N	0	+67	

Comments:

The T cell crossmatch is negative. There is a significant shift of B cell fluorescence.

*A channel shift >+4 channels is 2 standard deviations above the median fluorescence of the negative controls. A channel shift >+7 channels is 3 standard deviations above the median fluorescence of the negative controls.

**A channel shift >+7 channels is 2 standard deviations above the median fluorescence of the negative controls. A channel shift >+10 channels is 3 standard deviations above the median fluorescence of the negative controls.

Donor Name: DR7, DR9

ID#:

Crossmatch Date: 08/19/2015

Initial Flow Crossmatch

Serum Date	Dilution	Plt Abs	Channel Shift T*	Channel Shift B**	Comments
08/05/2015	1:1	N	-3	+12	
	1:2		-7	-3	
	1:8		-7	-4	
08/05/2015	1:1	N	-4	+11	

Comments:

The T cell crossmatch is negative. There is a significant shift of B cell fluorescence.

*A channel shift >+4 channels is 2 standard deviations above the median fluorescence of the negative controls. A channel shift >+7 channels is 3 standard deviations above the median fluorescence of the negative controls.
**A channel shift >+7 channels is 2 standard deviations above the median fluorescence of the negative controls. A channel shift >+10 channels is 3 standard deviations above the median fluorescence of the negative controls.

A matter of perception
A little information can be misleading (dangerous)