2017 CST-Astellas Canadian Transplant Fellows Symposium

HLA – Part I: for the Clinician

James Lan, MD, FRCPC, D(ABHI)

Dr. Lan completed his nephrology training at the University of British Columbia. He then joined the Clinician Investigator Program to cross-train in histocompatibility and immunogenetics under the mentorship of Dr. Elaine Reed at the University of California, Los Angeles Immunogenetics Center. He is currently Assistant Professor at the University of British Columbia and Staff Transplant Nephrologist at the Vancouver General Hospital. He also serves as a Clinical Consultant for the Human Leukocyte Antigen Immunology Laboratory in BC. His clinical and research interests include the translation of next-generation sequencing to solid organ and hematopoietic stem cell transplantation, and application of novel solid-phase assays to risk stratify donor-specific antibodies.

THE UNIVERSITY OF BRITISH COLUMBIA

HLA Part I – HLA for the Clinician

James H Lan, MD, FRCP(C), D(ABHI)
Assistant Professor, University of British Columbia
Nephrology & Kidney Transplantation, Vancouver General Hospital
Transplant Immunology Consultant, BCTS

CST Fellows Symposium
September 26, 2017

Disclosure

- I hold an Astellas/TRFBC grant
- I will discuss off-label and investigational use of drugs

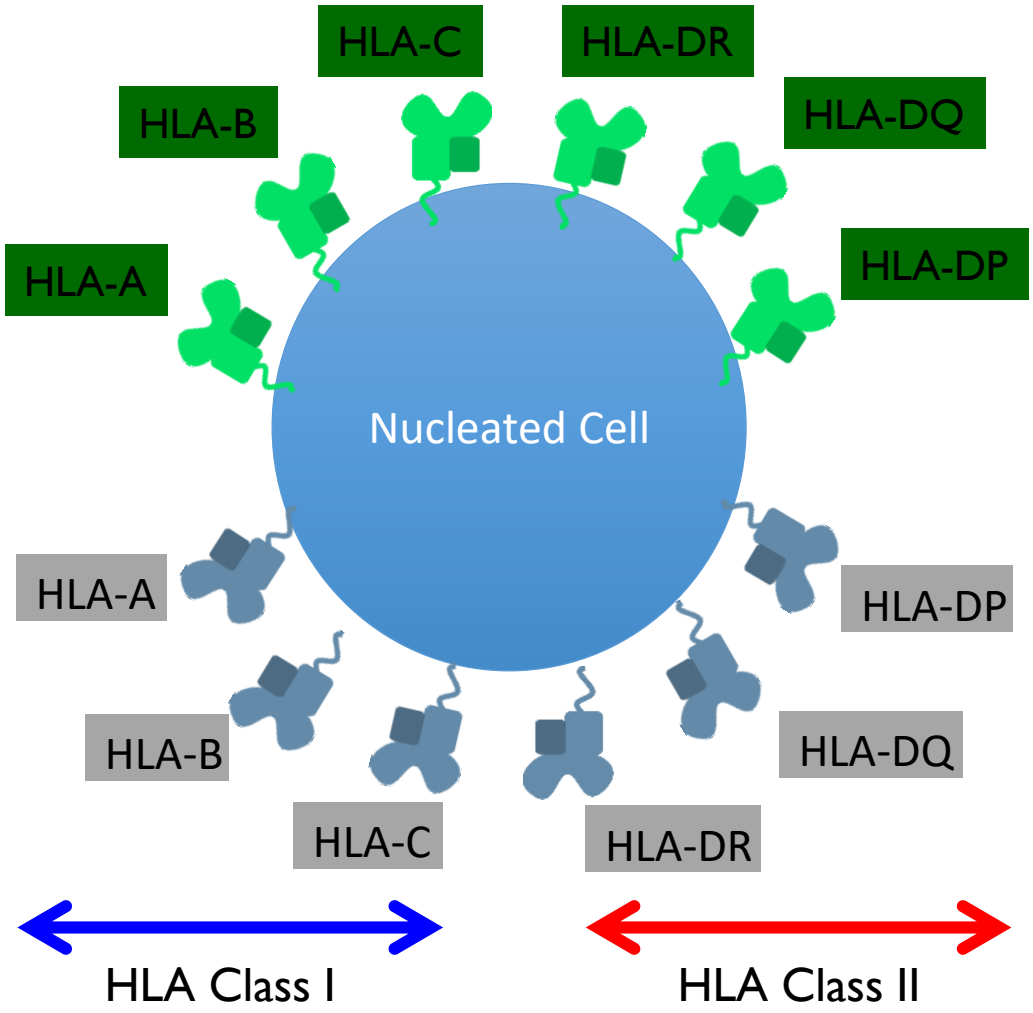
Objectives

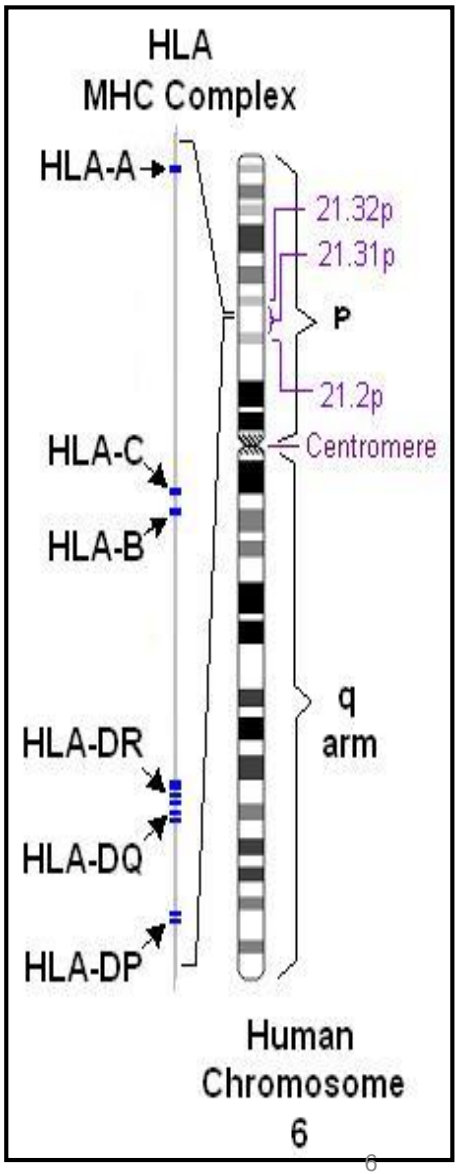
- HLA gene, structure, nomenclature
- Methods of antibody detection and their limitations
- Application: cPRA, virtual crossmatch
- DSA risk stratification

Part I. HLA Gene, Structure, Nomenclature

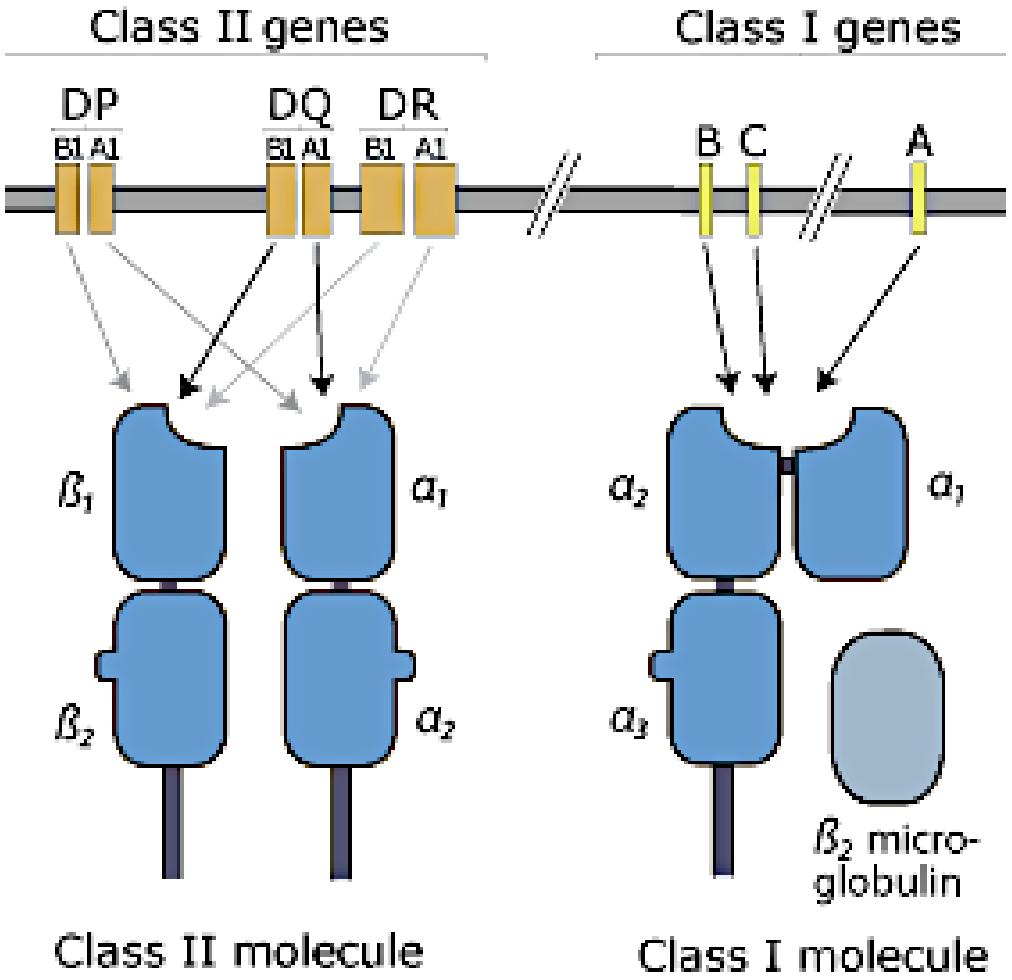
FACULTY OF MEDICINE

HLA (Human Leukocyte Antigen)





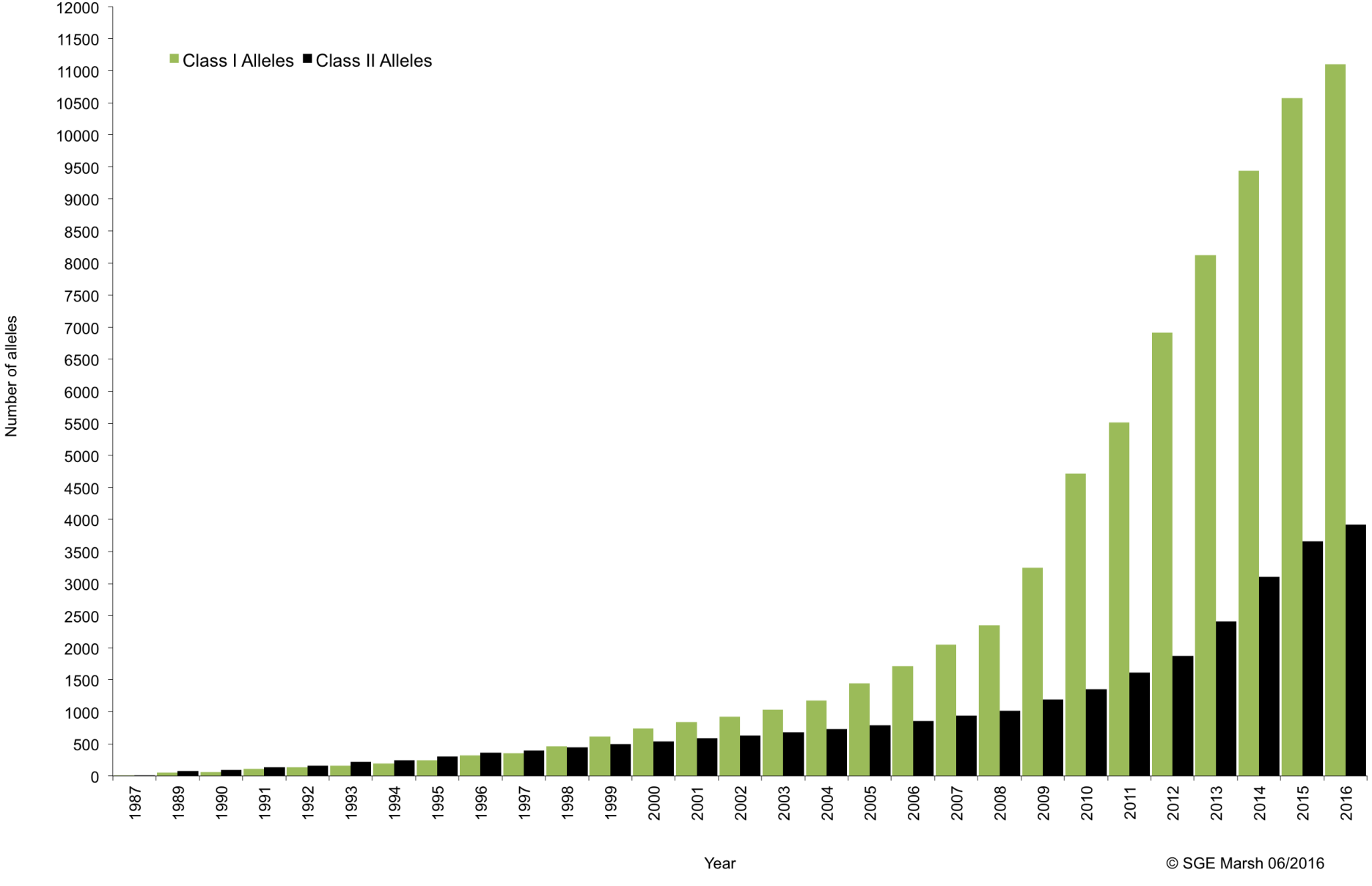
HLA Structure



Expressed on B cells, antigen-presenting cells, activated microvascular endothelium

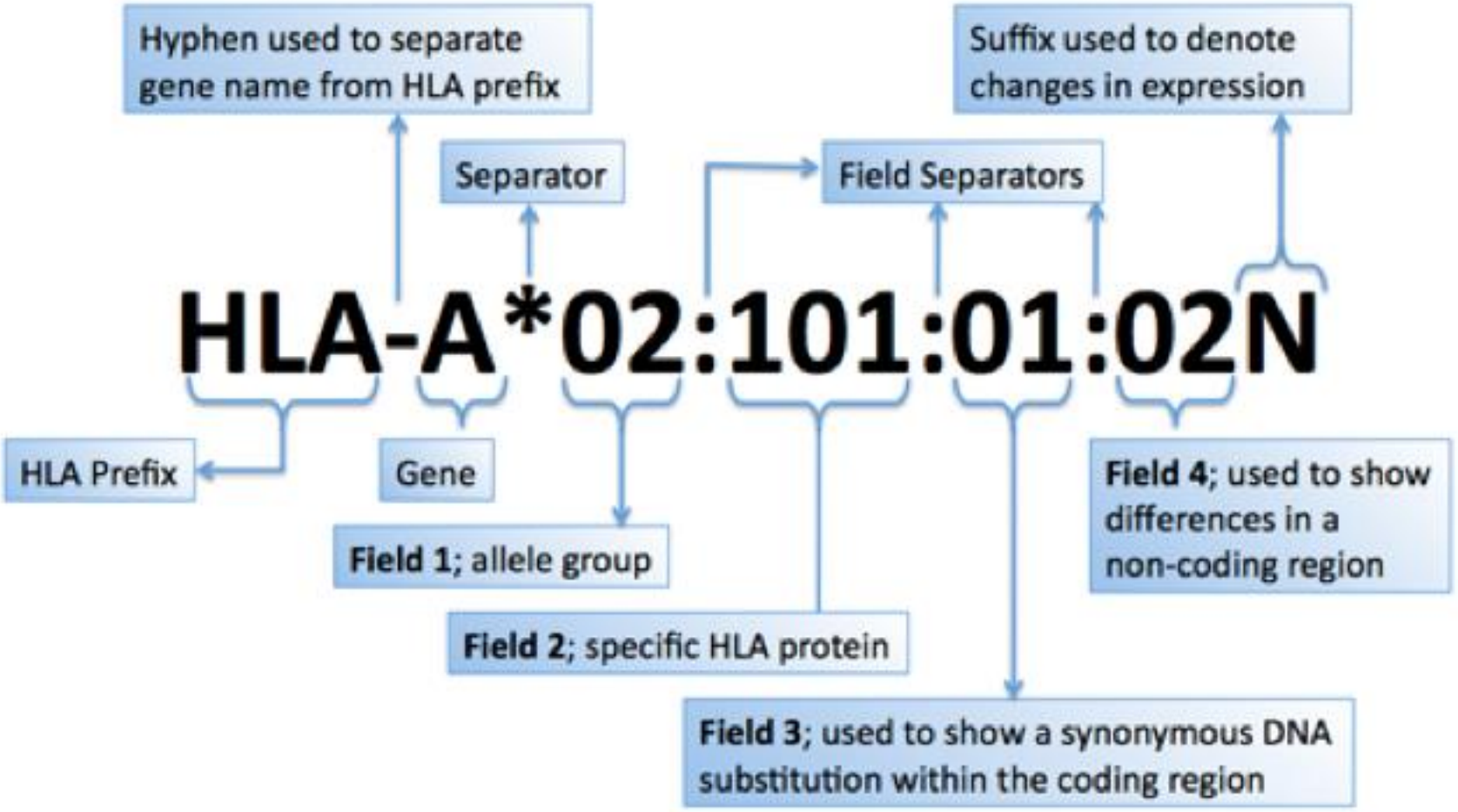
Expressed on all nucleated cells

HLA Polymorphism



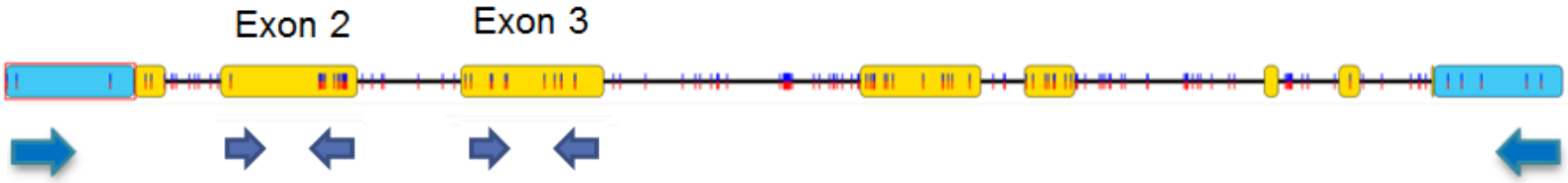
HLA-A	HLA-B	HLA-C
A*01:01:01:01	B*07:02:01	C*01:02:01
A*01:01:01:02N	B*07:02:02	C*01:02:02
A*01:01:01:03	B*07:02:03	C*01:02:03
A*01:01:02	B*07:02:04	C*01:02:04
A*01:01:03	B*07:02:05	C*01:02:05
A*01:01:04	B*07:02:06	C*01:02:06
A*01:01:05	B*07:02:07	C*01:02:07
A*01:01:06	B*07:02:08	C*01:02:08
A*01:01:07	B*07:02:09	C*01:02:09
A*01:01:08	B*07:02:10	C*01:02:10
A*01:01:09	B*07:02:11	C*01:02:11
A*01:01:10	B*07:02:12	C*01:02:12
A*01:01:11	B*07:02:13	C*01:02:13
A*01:01:12	B*07:02:14	C*01:02:14
A*01:01:13	B*07:02:15	C*01:02:15
A*01:01:14	B*07:02:16	C*01:02:16
A*01:01:15	B*07:02:17	C*01:02:17

Current nomenclature of HLA



HLA Typing Resolution

- HLA antigens can be defined under different resolutions
  - Useful for determination of allele-specific DSA
  - Useful for epitope analysis/matching



Resolution	Example	Cost
Low-Resolution (antigen)	B35	$150/locus
Intermediate Resolution	B*35:XTNJ XTNJ= 01/09/11/27/28	$150/locus
High-Resolution	B*35:01	$300/locus

Part II. Methods of Antibody Detection

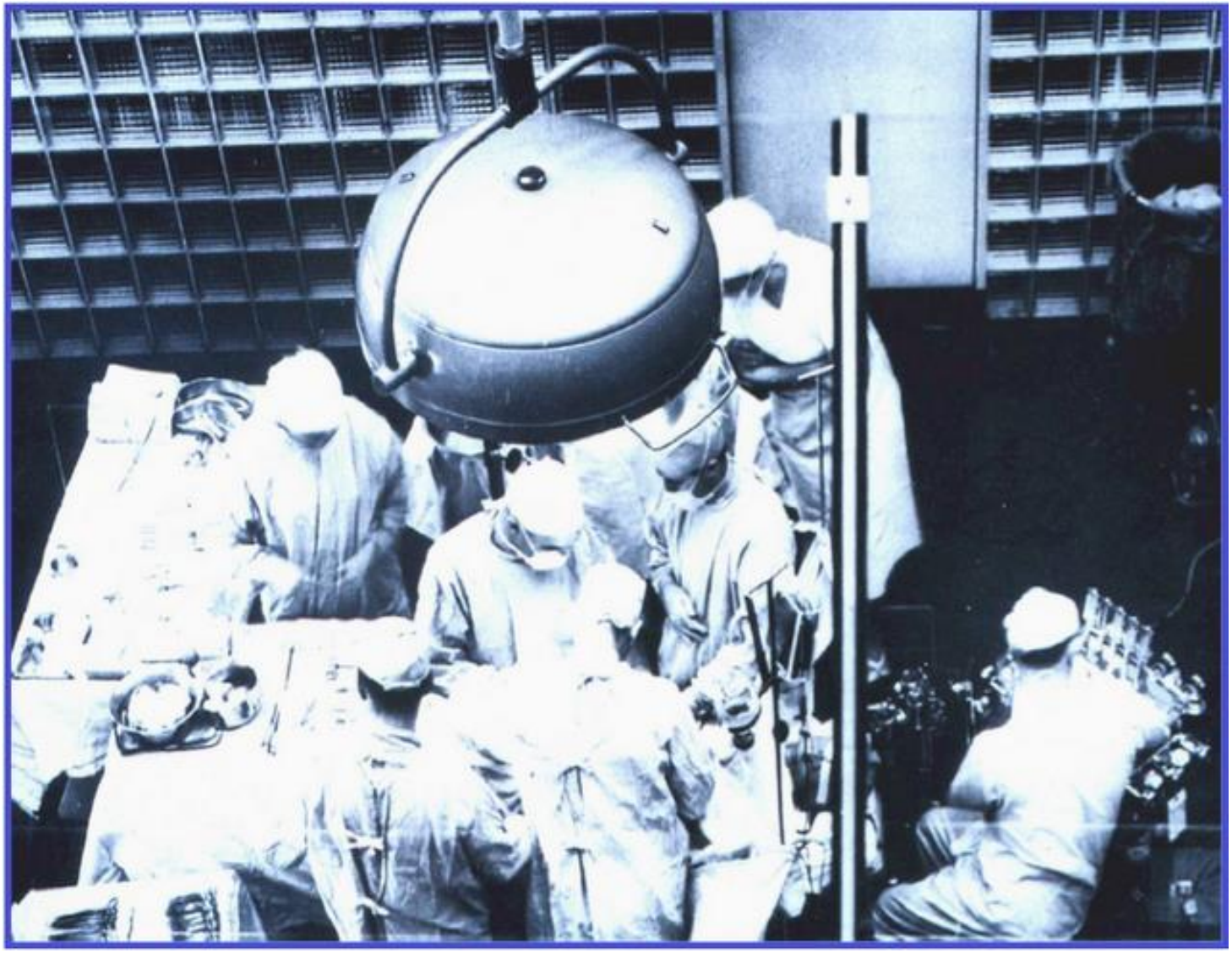
1. Complement-dependent cytotoxicity (CDC)
2. Flow cytometric crossmatch
3. Single-antigen bead (SAB) assay

“If I have seen further, it is by standing on the shoulders of giants”

- Issac Newton, 1675

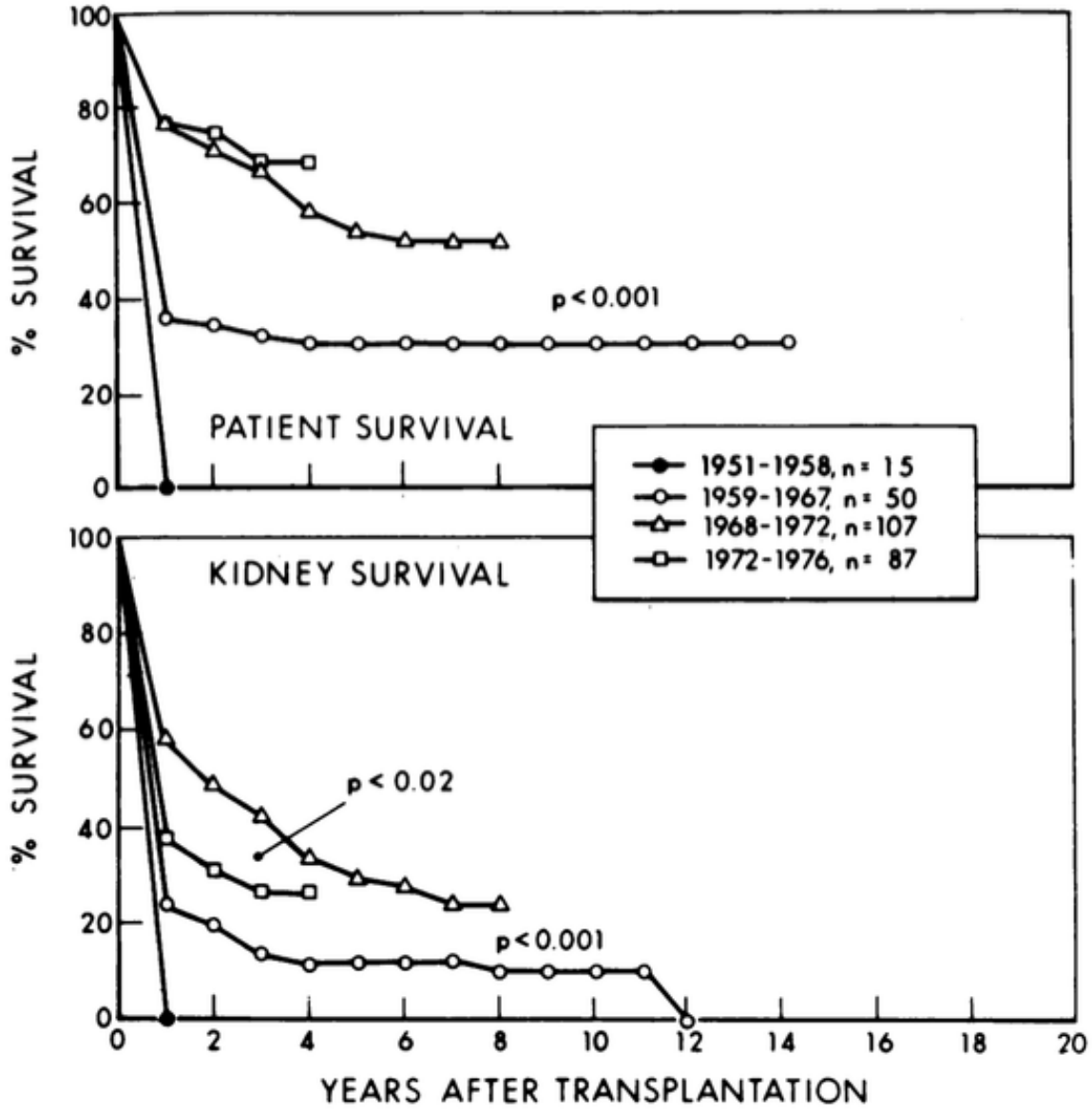
Famous Twins

First successful human kidney transplantation



- *December 23, 1954,* Brigham and Women's Hospital

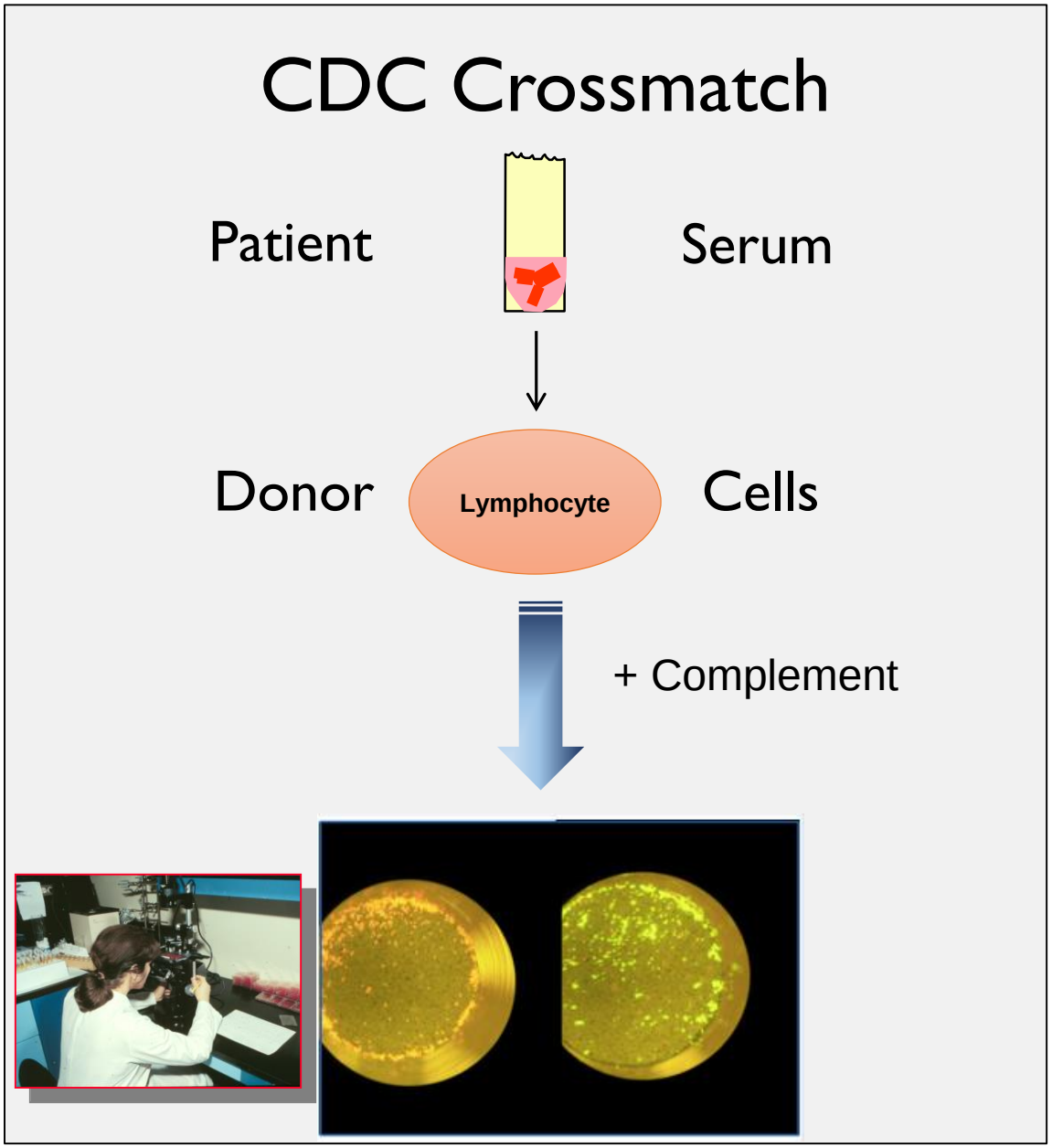
Stagnant outcomes: Imuran + steroids



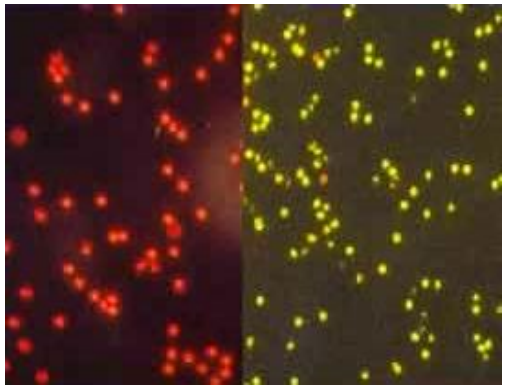
- Murray, Annals of Surgery, 1976

How to Overcome Hyperacute Rejection

HLA Antibody Detection: CDC crossmatch (cell-based)



Paul Terasaki
UCLA Medical School, CA, USA
b. 1929



Dead cells **Live cells**

ORIGINAL ARTICLE ARCHIVE

Significance of the Positive Crossmatch Test in Kidney Transplantation

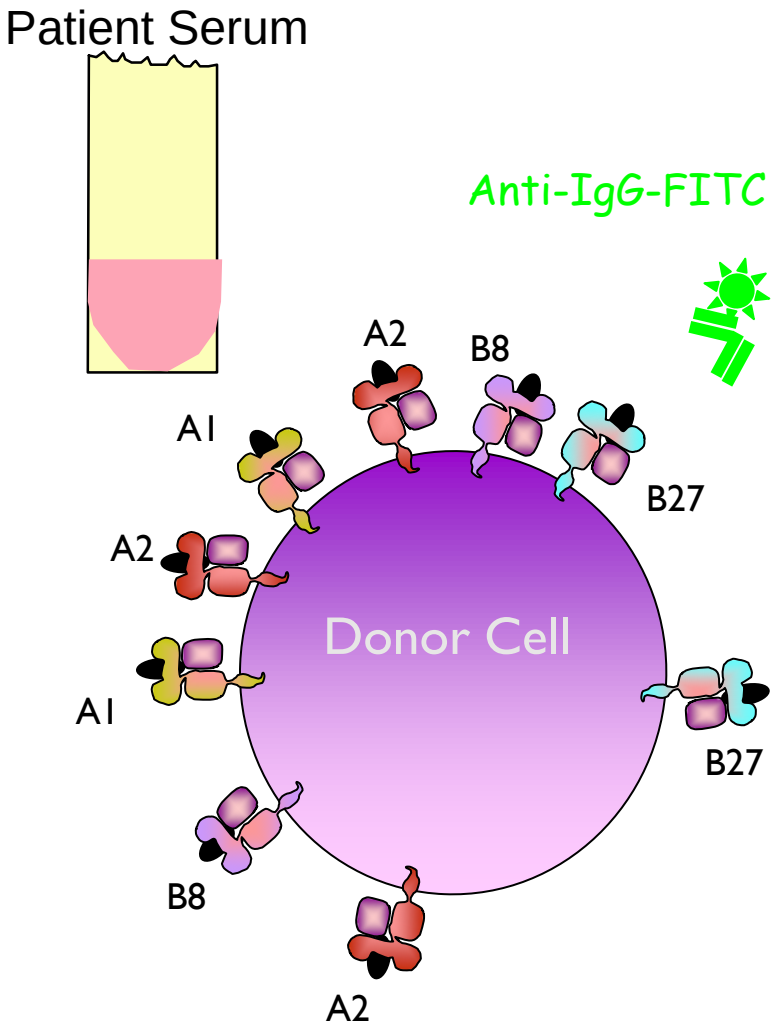
Ramon Patel, M.R.C.P., and Paul I. Terasaki, Ph.D.

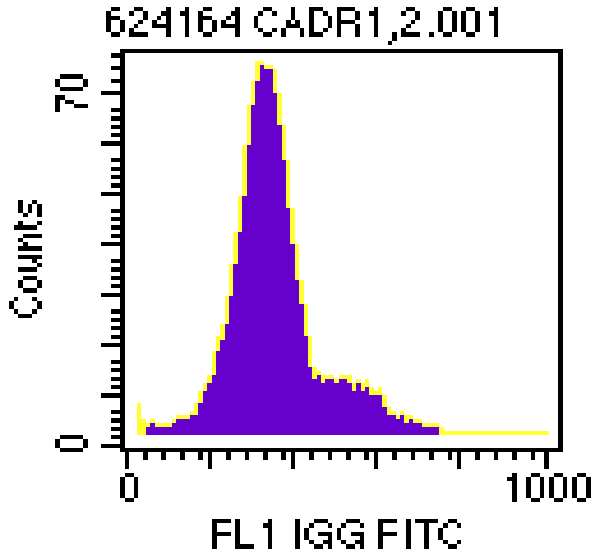
N Engl J Med 1969; 280:735-739 | April 3, 1969 | DOI: 10.1056/NEJM196904032801401

	CDC Positive	CDC Negative
No rejection	6	187
Rejection	24 (80%) *	8 (4%)

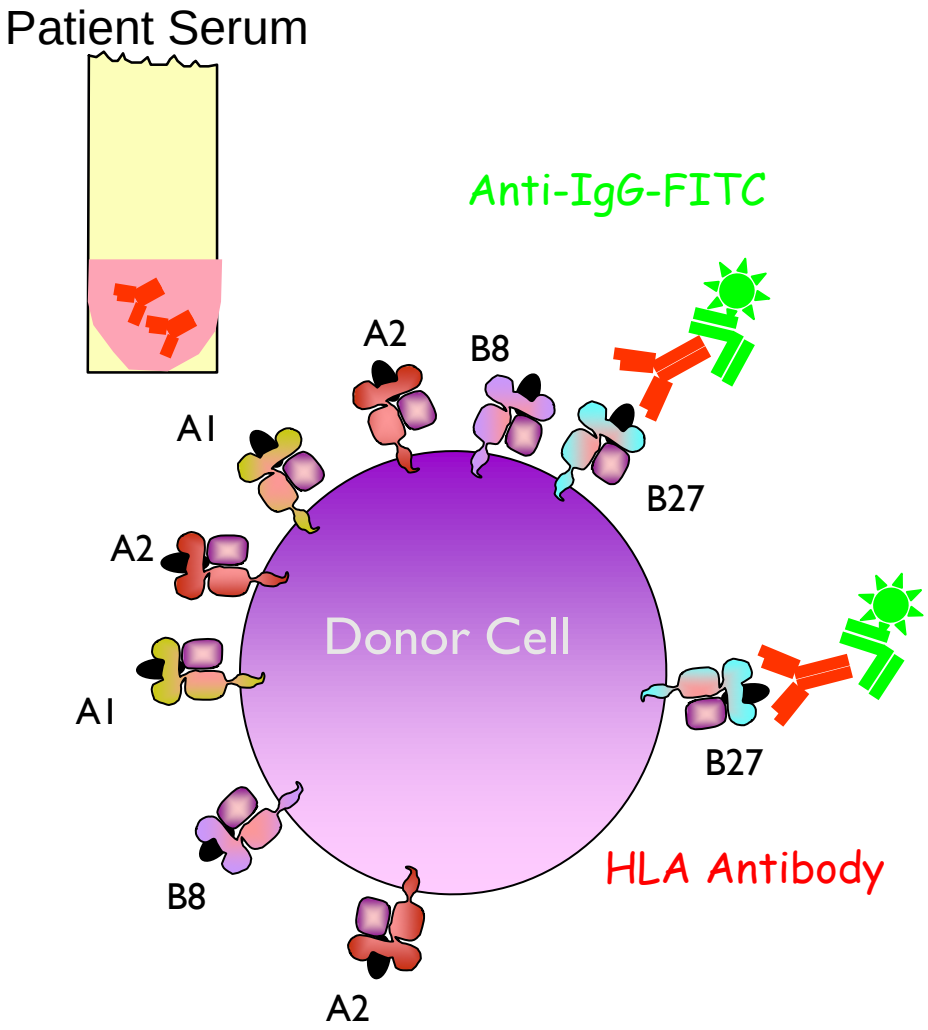
* $p < 0.001$

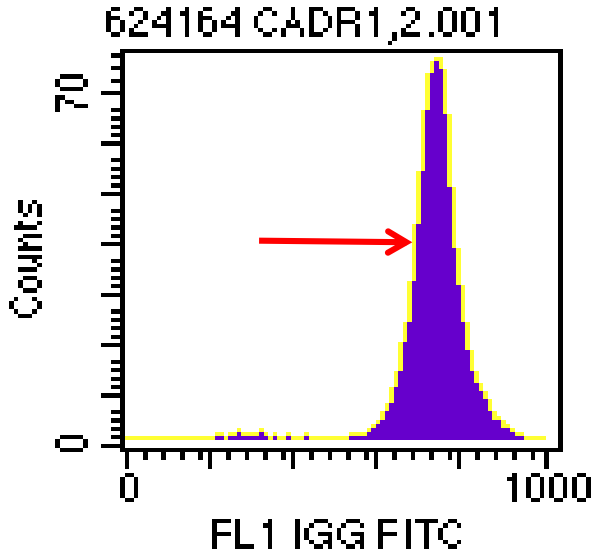
HLA Antibody Detection: Flow crossmatch (cell-based)





HLA Antibody Detection: Flow crossmatch (cell-based)





Interpretation of flow crossmatches

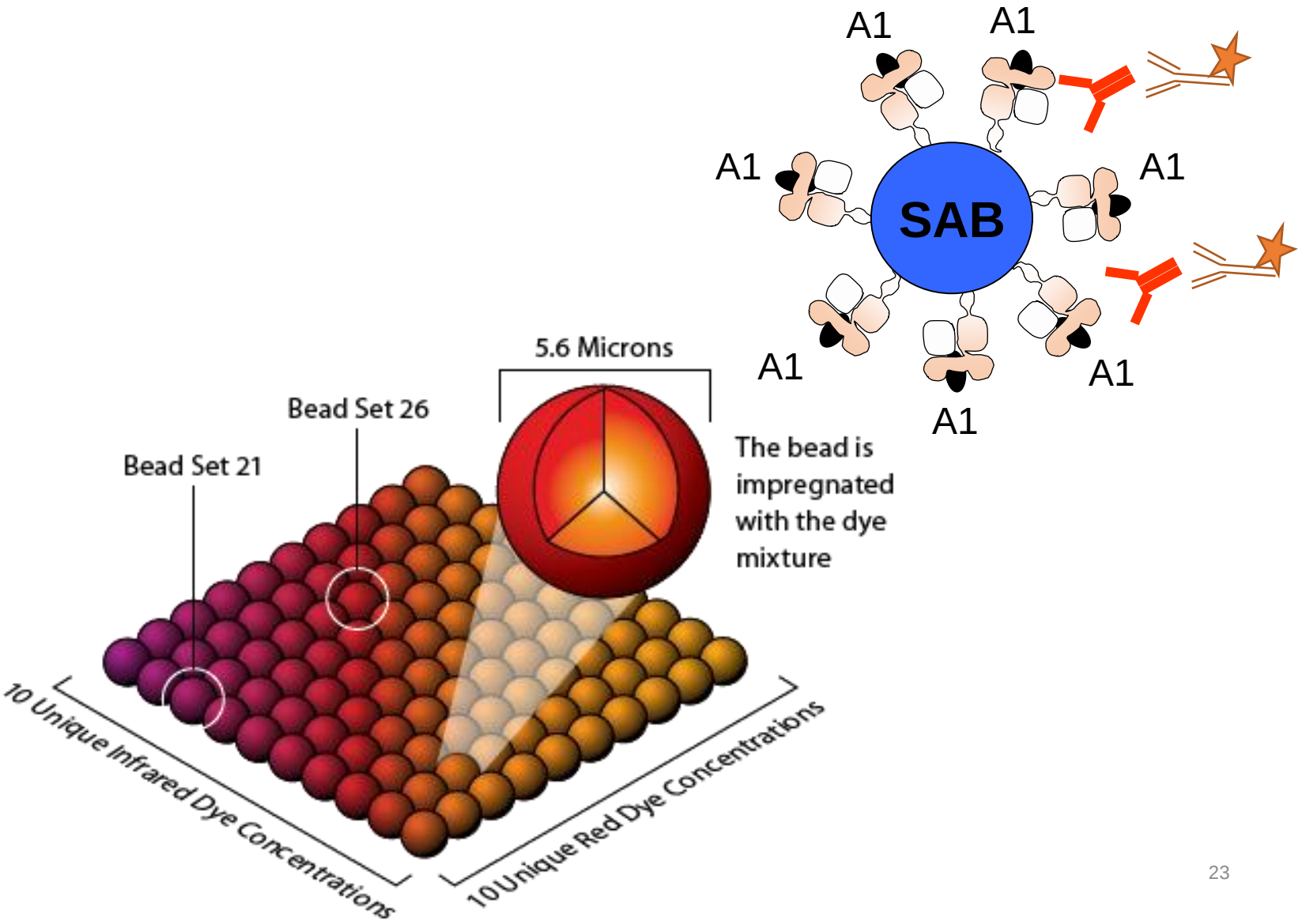
Table 3 Interpretation of Crossmatch result

Class I T-Cell XM	B-Cell XM	Class I & II	Interpretation
–ve	–ve		No DSAb to HLA class I or II OR DSAb titre too low to cause positive reaction OR (DSAb that is not complement-fixing – relevance unclear)
+ve	+ve		DSAb/s to HLA class I OR Multiple DSAbs to HLA class I +/– II
–ve	+ve		DSAb/s to HLA class II OR Low level DSAb/s to HLA class I
+ve	–ve		Technical error (possibly related to B-cell viability). The test should be repeated

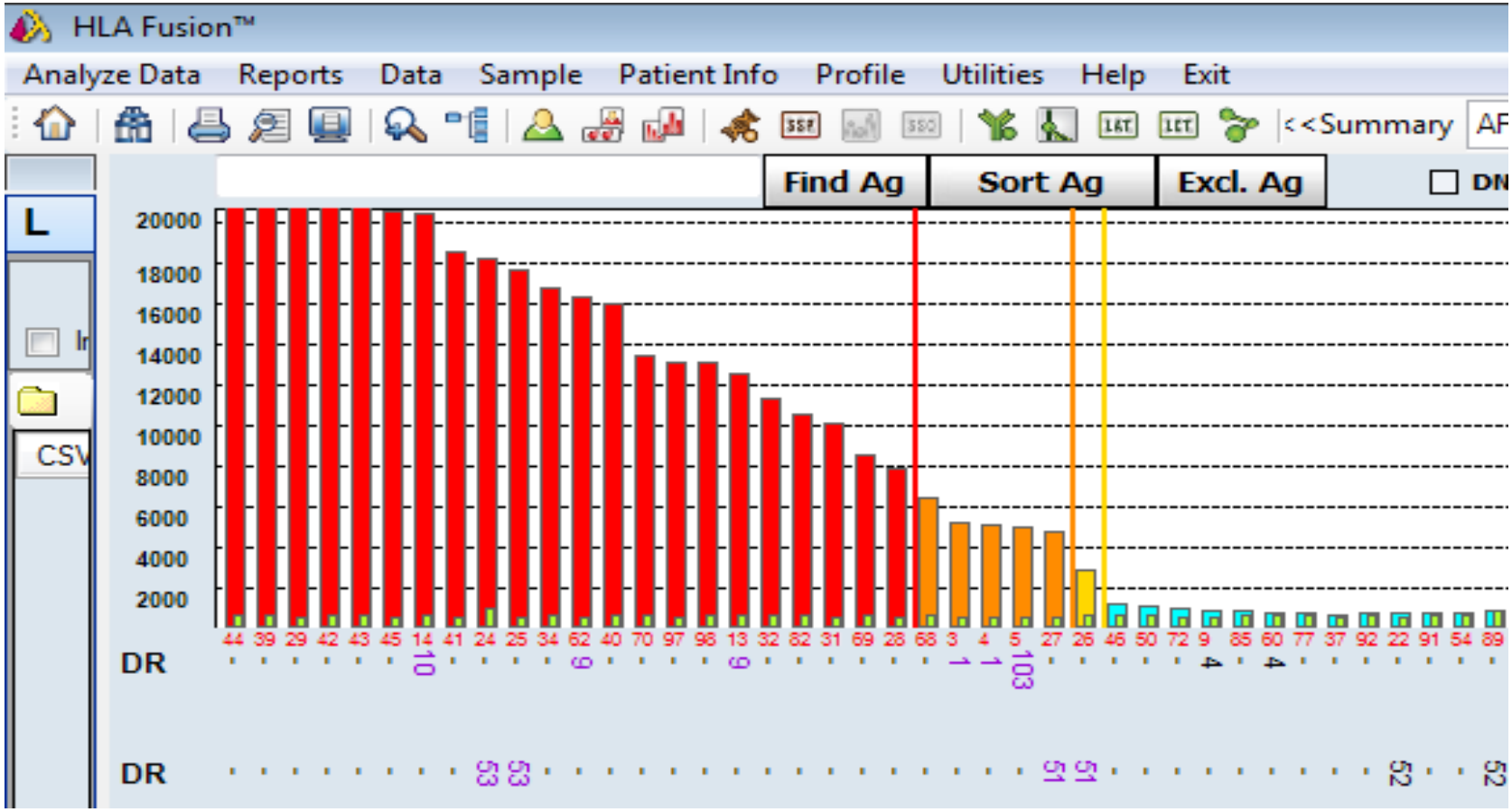
+ve, positive; –ve, negative, DSAb: donor-specific anti-HLA antibody; HLA, human leucocyte antigen, XM: crossmatch.

Mulley, Nephrology 16 (2011) 125–133

HLA Antibody Detection: single-antigen beads (solid phase)



HLA Antibody Detection: single-antigen beads (solid phase)



Question: What does it mean when a patient is highly sensitized? What is cPRA?

cPRA (calculated panel of reactive antibody)

- cPRA = estimates the percentage of donors in a given population who carry unacceptable antigens for a recipient

CPRA CALCULATOR

UNACCEPTABLE ANTIGENS

Check the antigens that are unacceptable.

Check all A unacceptable antigens:

☐ 1 ☑ 2 ☐ 3 ☐ 9 ☐ 10 ☐ 11 ☐ 19 ☐ 23 ☑ 24 ☐ 25

☐ 26 ☐ 28 ☐ 29 ☐ 30 ☐ 31 ☐ 32 ☐ 33 ☐ 34 ☐ 36 ☐ 43

☐ 66 ☐ 68 ☐ 69 ☐ 74 ☐ 80 ☐ 203 ☐ 210 ☐ 2403 ☐ 6601 ☐ 6602

Check all B unacceptable antigens:

☐ 5 ☑ 7 ☐ 8 ☐ 12 ☐ 13 ☐ 14 ☐ 15 ☐ 16 ☐ 17 ☐ 18

☐ 21 ☐ 22 ☐ 27 ☐ 35 ☐ 37 ☐ 38 ☐ 39 ☐ 40 ☐ 41 ☐ 42

☐ 44 ☐ 45 ☐ 46 ☐ 47 ☐ 48 ☐ 49 ☐ 50 ☐ 51 ☐ 52 ☐ 53

☐ 54 ☐ 55 ☐ 56 ☐ 57 ☐ 58 ☐ 59 ☐ 60 ☐ 61 ☐ 62 ☐ 63

☐ 64 ☐ 65 ☐ 67 ☐ 70 ☐ 71 ☐ 72 ☐ 73 ☐ 75 ☐ 76 ☐ 77

☐ 78 ☐ 81 ☐ 82 ☐ 703 ☐ 804 ☐ 1304 ☐ 2708 ☐ 3901 ☐ 3902 ☐ 3905

☐ 4005 ☐ 5102 ☐ 5103 ☐ 7801 ☐ 8201

Check BW unacceptable antigen:

○ 4 ○ 6 ○ N/A

Check all C unacceptable antigens:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

☐ 12 ☐ 13 ☐ 14 ☐ 15 ☐ 16 ☐ 17 ☐ 18

Check all DR unacceptable antigens:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

☐ 11 ☐ 12 ☐ 13 ☐ 14 ☐ 15 ☐ 16 ☐ 17 ☐ 18 ☐ 103 ☐ 1403

☐ 1404

Check DR51/52/53 unacceptable antigens:

☐ 51 ☐ 52 ☐ 53

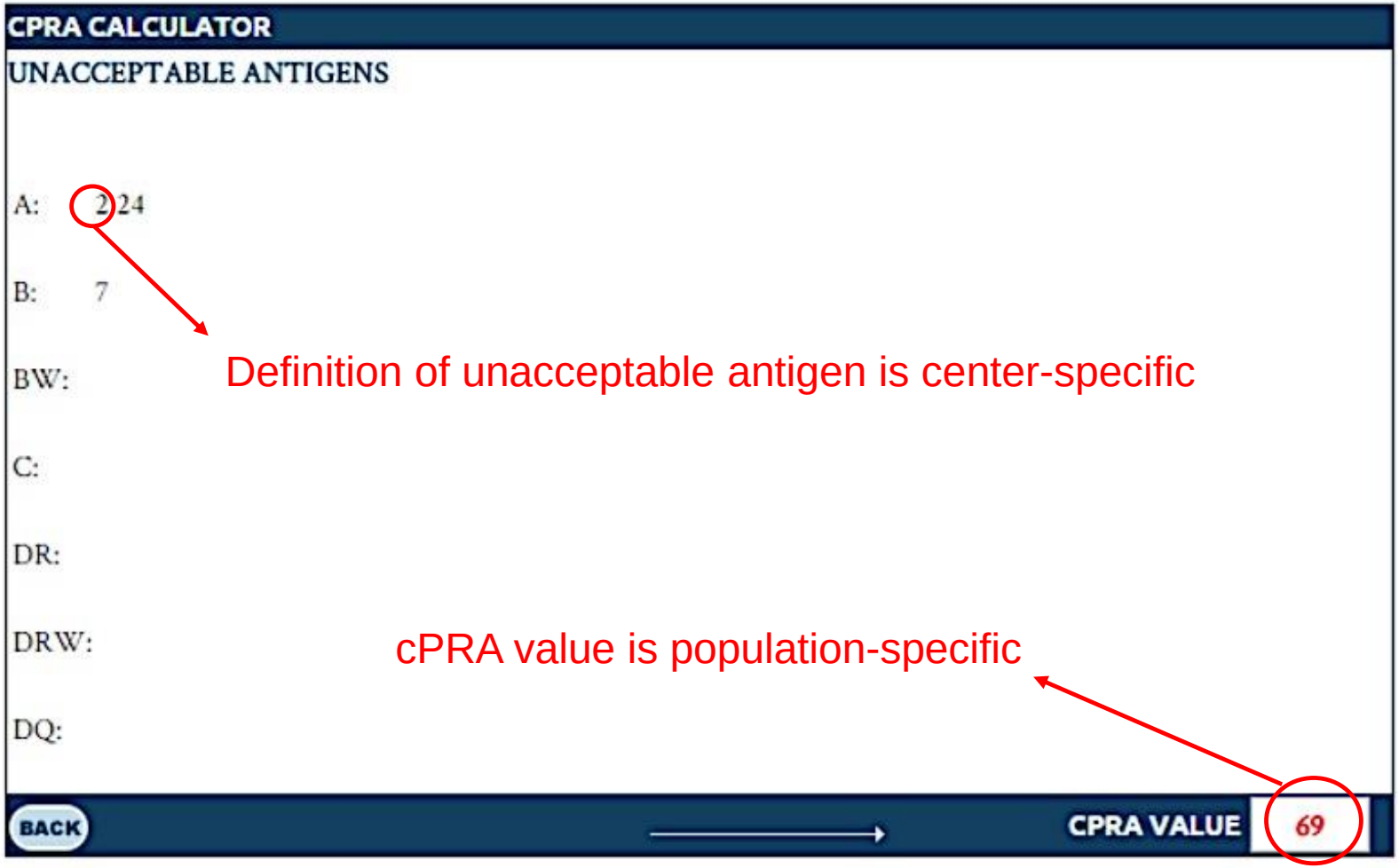
Check all DQ unacceptable antigens:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9

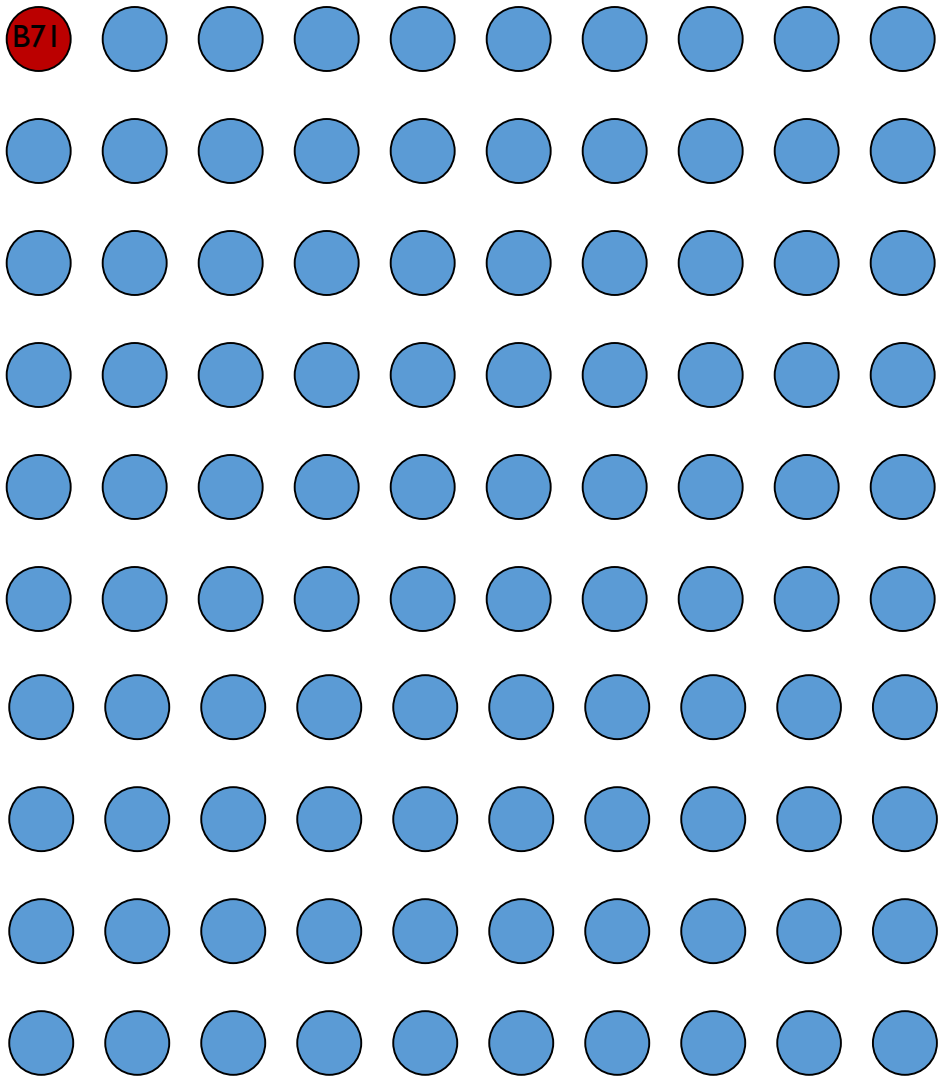
Reset Calculate

cPRA calculator

cPRA calculator



Antigen frequency matters



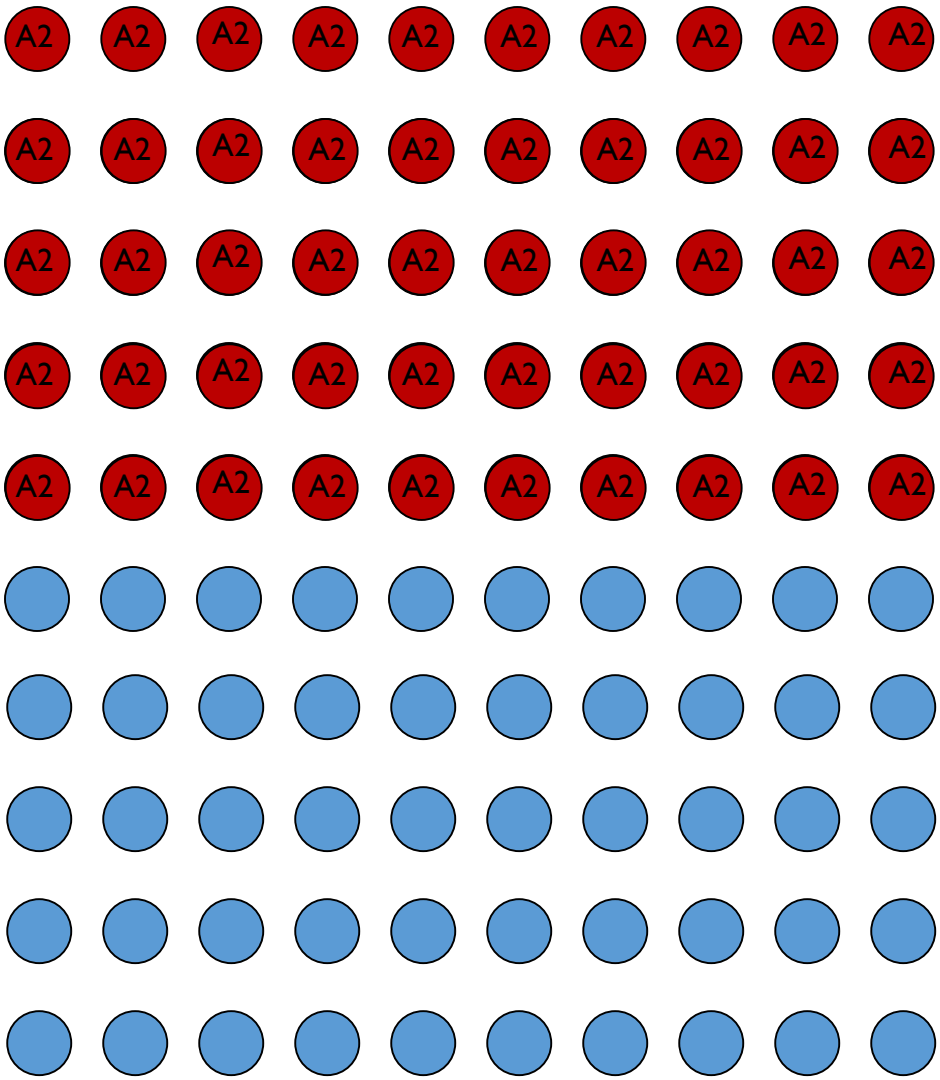
Patient 1:

Antibody: B71

cPRA = 1/100

= 1%

Antigen frequency matters



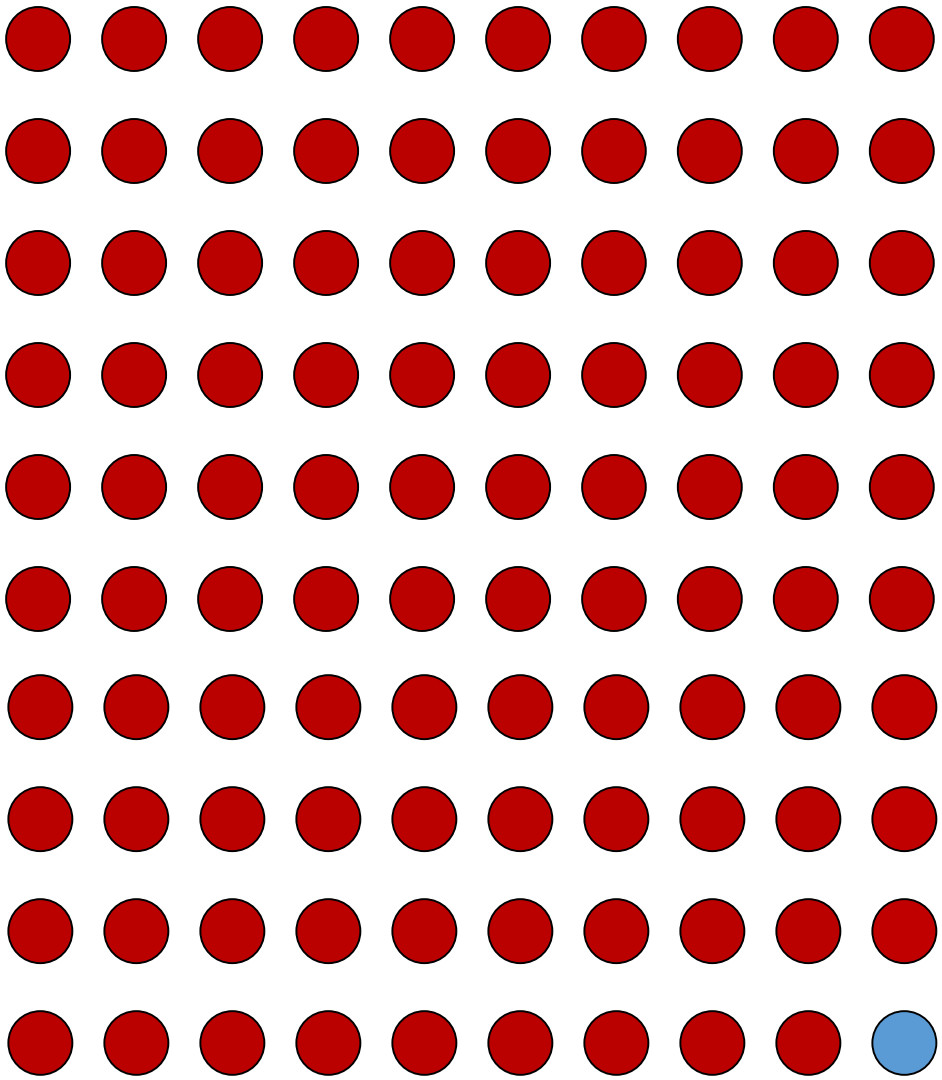
Patient 2:

Antibody: A2

cPRA = 50/100

= 50%

Antigen frequency matters



Patient 3:

cPRA = 99/100

= 99%

How can someone make so many antibodies?

- Mrs. MC

Cumulative Class I Antibody Specificities

A:24 80
B:35 37 46 49 50 51 52 53 56 62 71 72 75 77 78
Cw:6 7 9 10

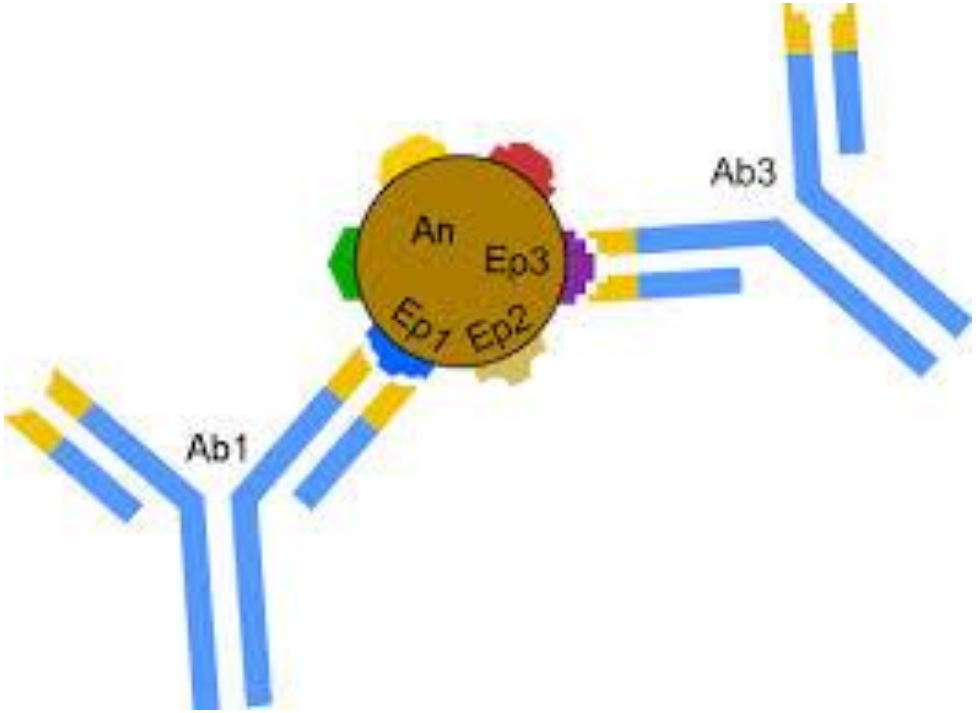
Cumulative Class II Antibody Specificities

DR:1 4 8 9 10 11 12 13 14 15 16 17 18 0103
DRw:51 52
DQ:5
DP:3 6 11 13

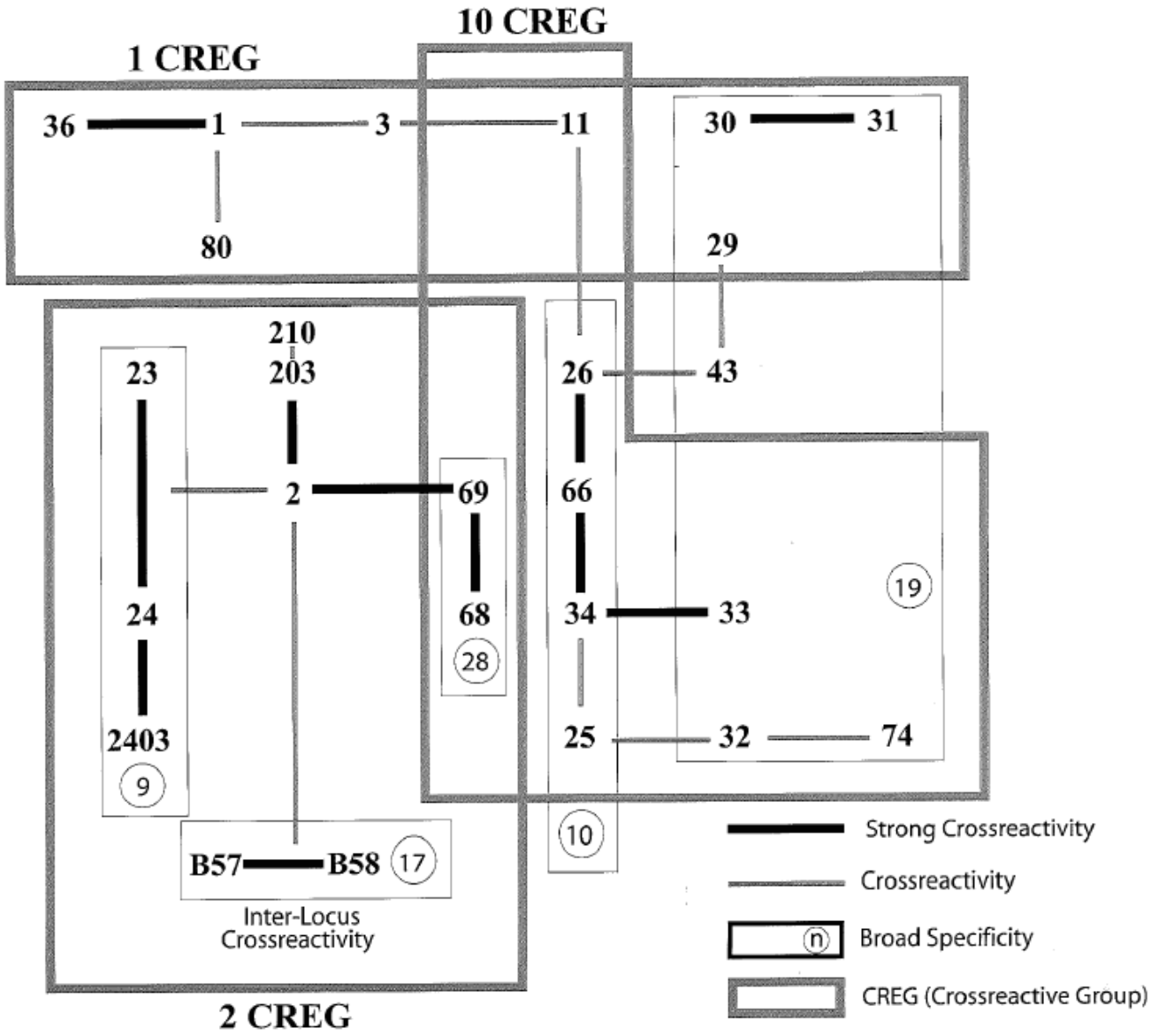
Cumulative Combined Calculated PRA

cPRA: 100% Last Date Calculated: 02-Feb-2015

Antibodies recognize epitopes



Antibodies form in cross-reactive group species (CREG)



Example of epitope spreading

HLA Antibody Investigation

Recipient Sera	Test	% Pos
02-May-2014	Luminex Class I IgG PRA	16
02-May-2014	Luminex Class II IgG PRA	0
02-May-2014	Luminex Class I Single Antigen	

Case Comments

HLA antibody update for sample dated: 2 MAY 2014

No new Class I or Class II specificities detected.

Clinical Comments

Reported Date	Reported Comment

Cumulative Class I Antibody Specificities

A:2 23

HLA Antibody Investigation

Recipient Sera	Test	% Pos
03-Aug-2015	Luminex Class I IgG Ab Screen	
03-Aug-2015	Luminex Class II IgG Ab Screen	

Case Comments

ABO blood groupings performed by VGH Blood Transfusion Services.

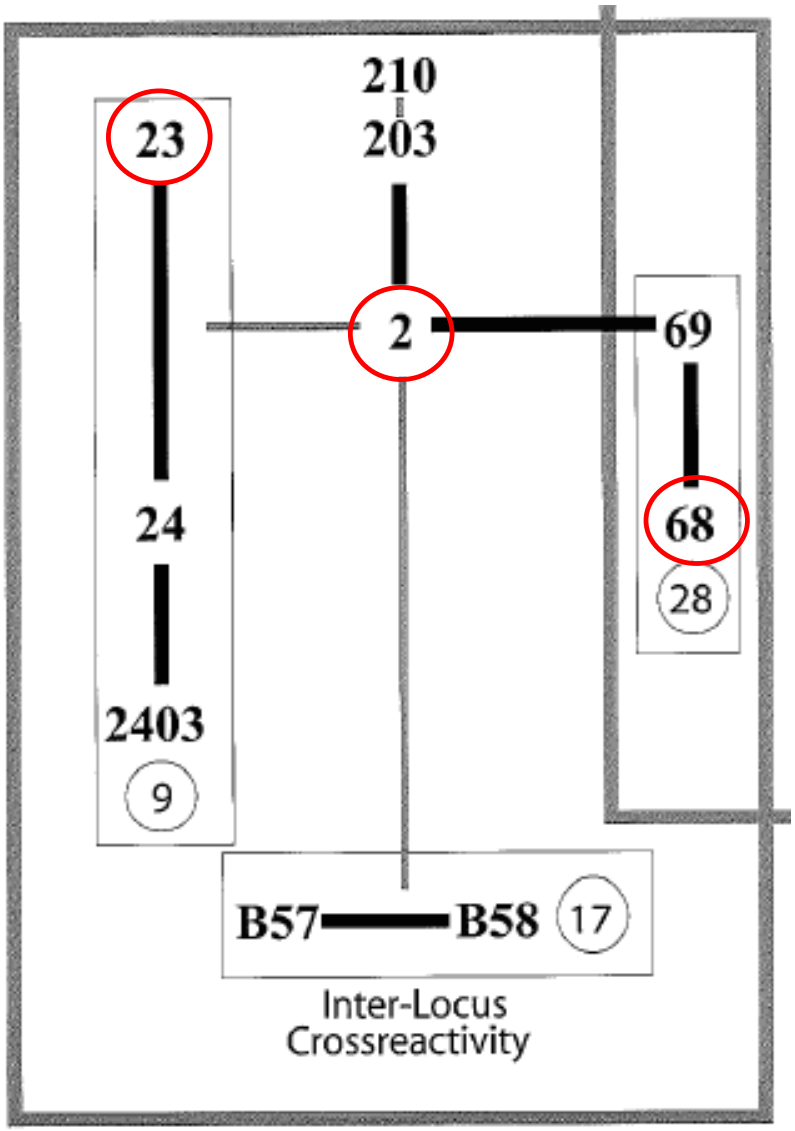
Transplanted: 15 SEP 2015

Clinical Comments

Reported Date	Reported Comment

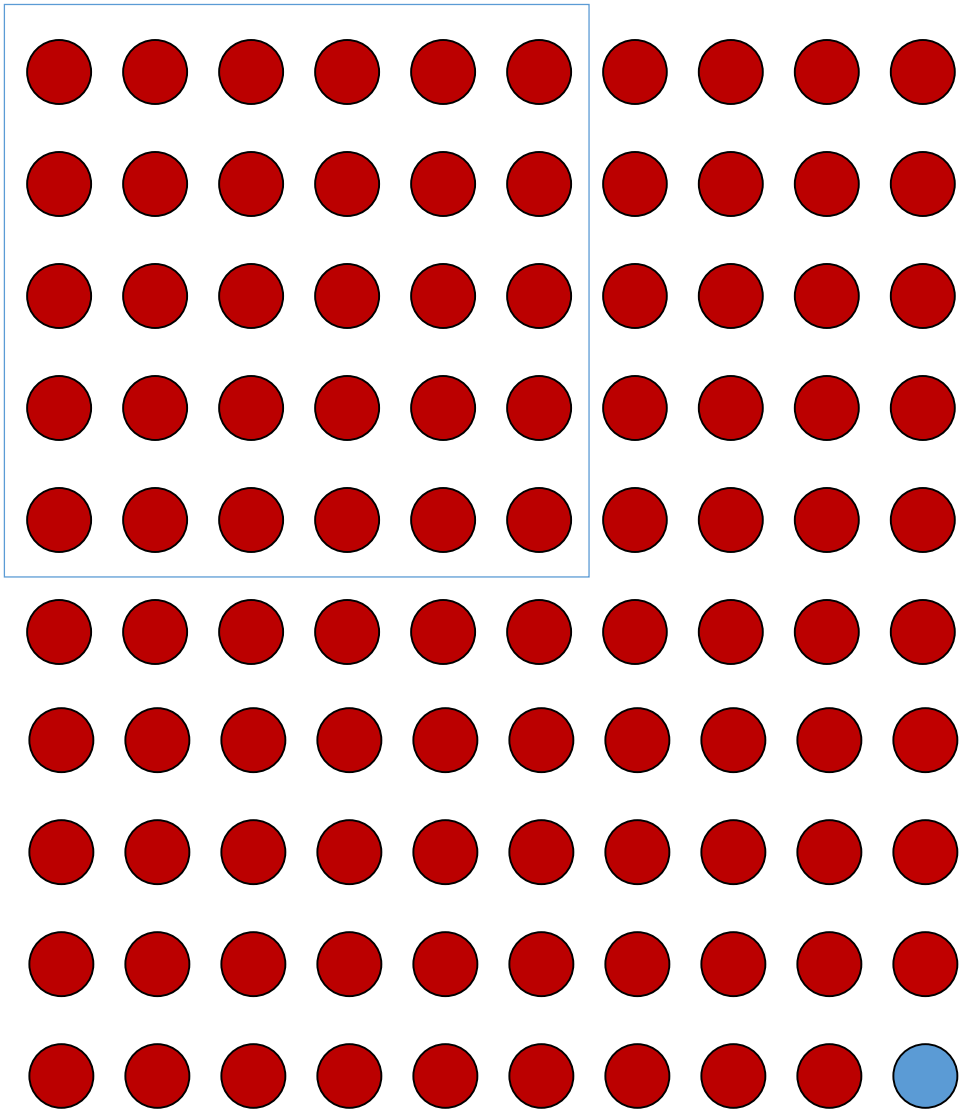
Cumulative Class I Antibody Specificities

A:2 23 68



2 CREG

Solution for highly sensitized patients

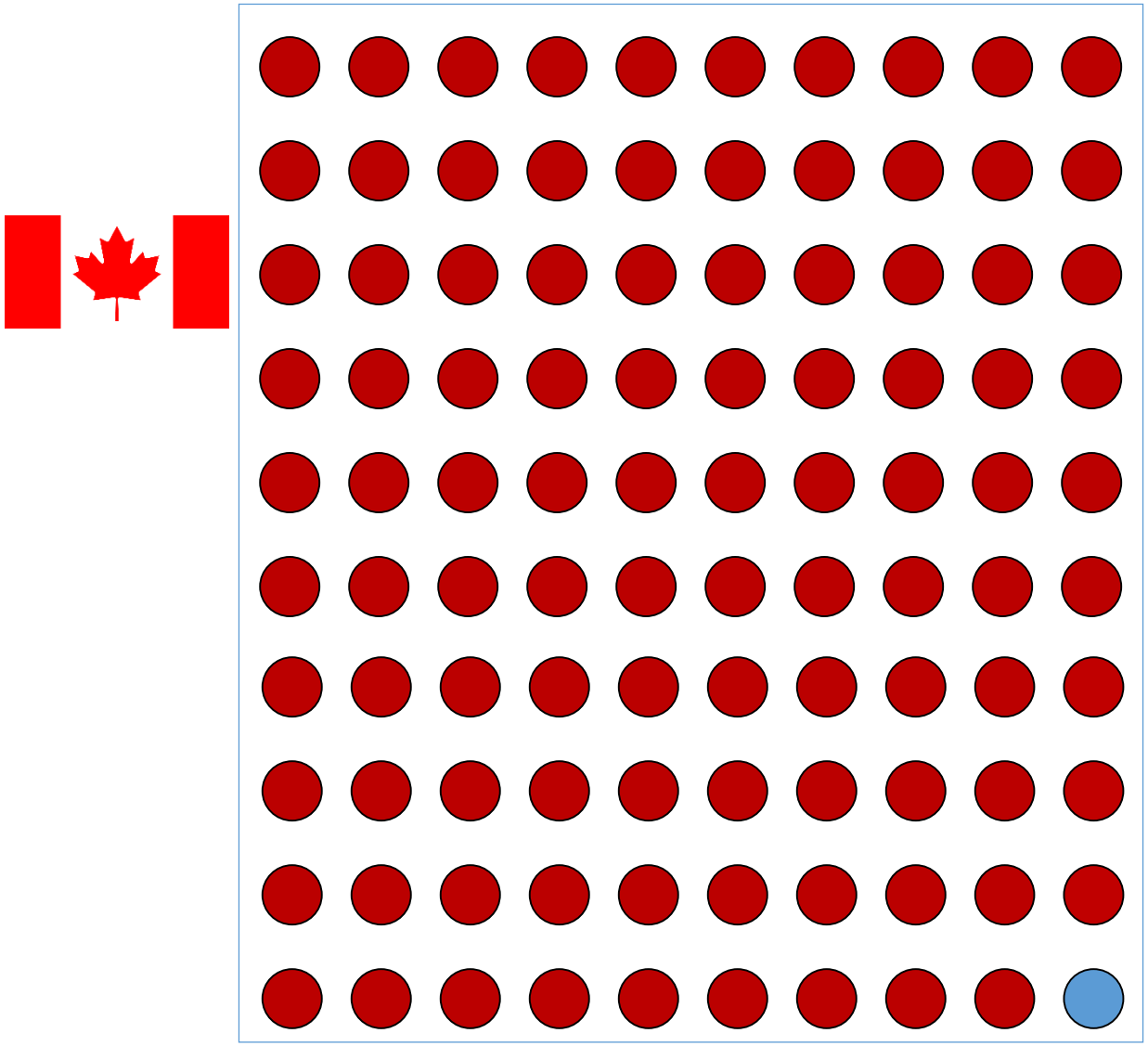


Patient 3:

cPRA = 99/100

= 99%

Solution for highly sensitized patients



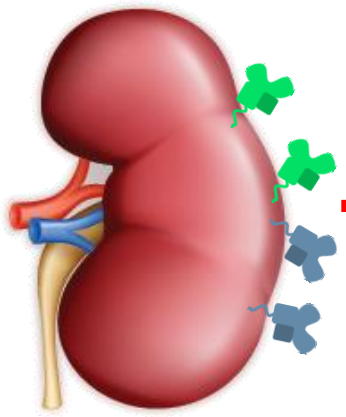
Patient 3:

cPRA = 99/100

= 99%

A Solution for highly sensitized patients (HSP program)

Virtual crossmatch

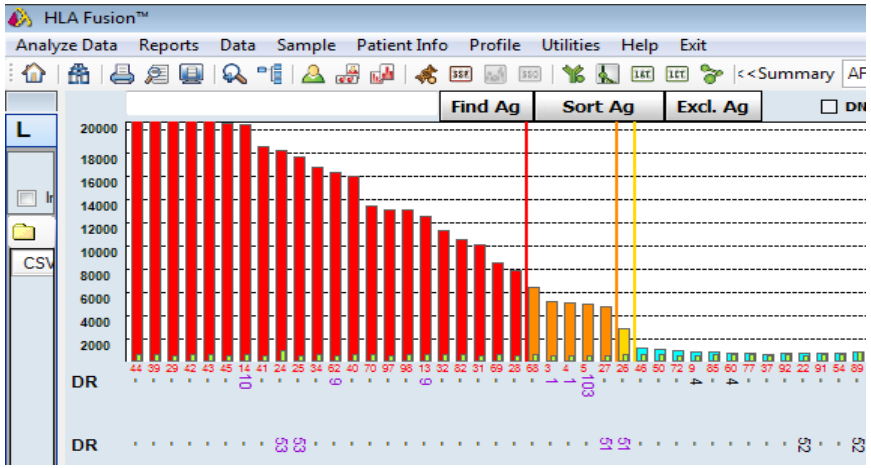


Compatible
?

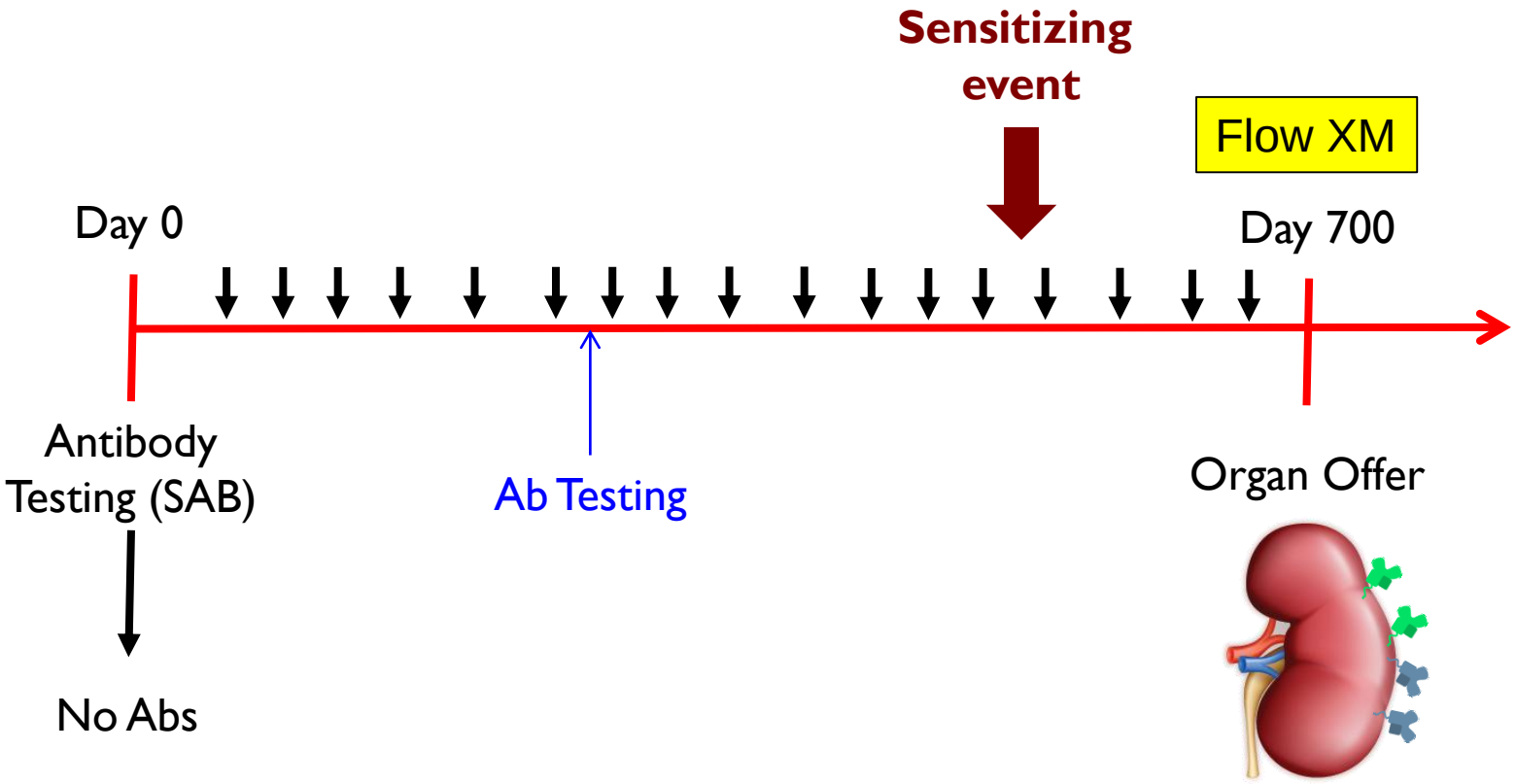
Donor: Vancouver

Recipient: Toronto

Donor Typing		
A	2	26
B	58	27
C	7	1
DR	17	1
DQ	2	5



Redundant assays to prevent AMR



Limitations of virtual crossmatch:

- Assume donor typing is correct
- Assume recipient antibody profile is correct
- Assume no new sensitizing events

Clinical Case:

- Recipient FE: 60 y.o. Female, cPRA = 99%
- Deceased donor offer from Ontario
- DCD Donor: 68 Male, terminal Cr 70

Cumulative Class I Antibody Specificities

A:3 25 26 29 31 33 34 66 68 69

B:48 55 57 58 61

Cumulative Class II Antibody Specificities

DR:1 4 9 10 11 14 15 0103

DRw:51 53

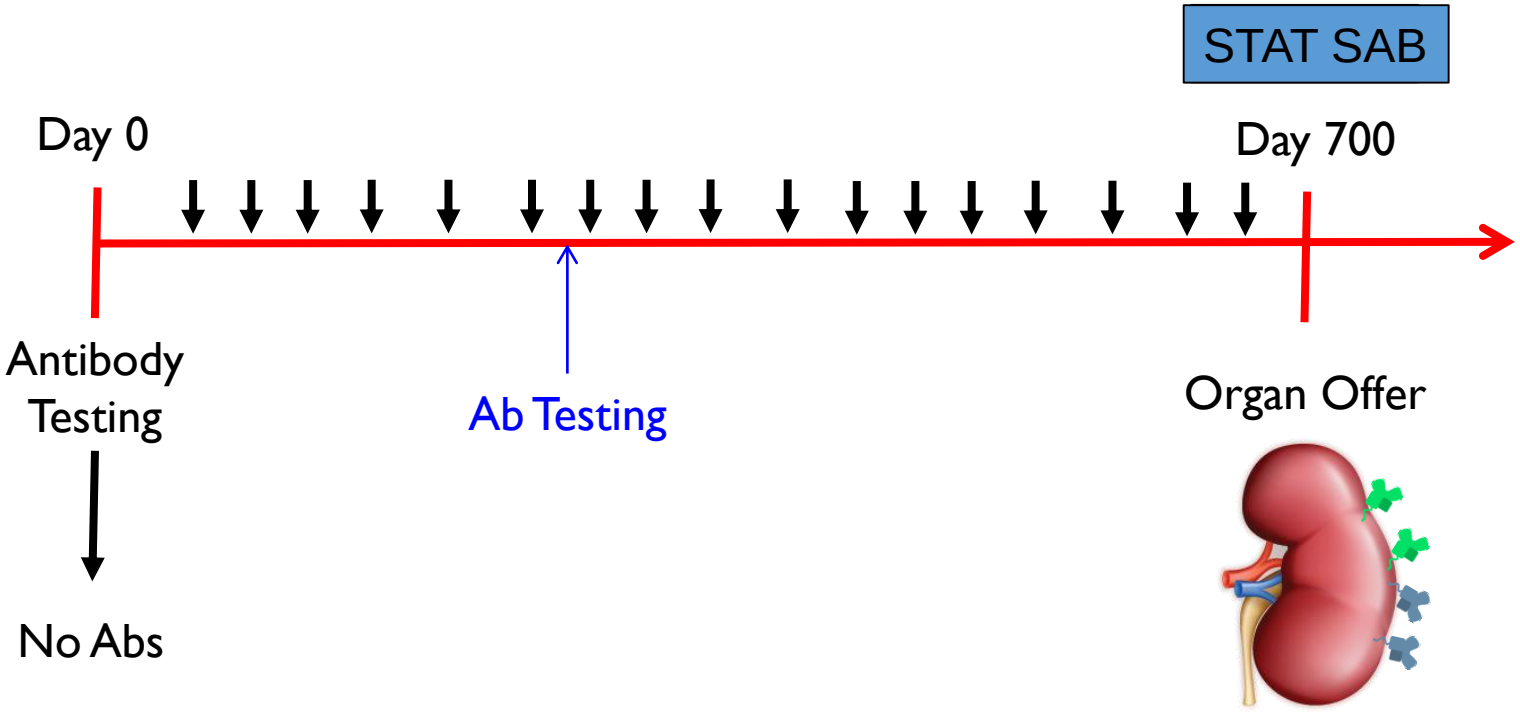
DQ:2 5

DP:1 20

Cumulative Combined Calculated PRA

cPRA: 99% Last Date Calculated: 28-Jun-2016

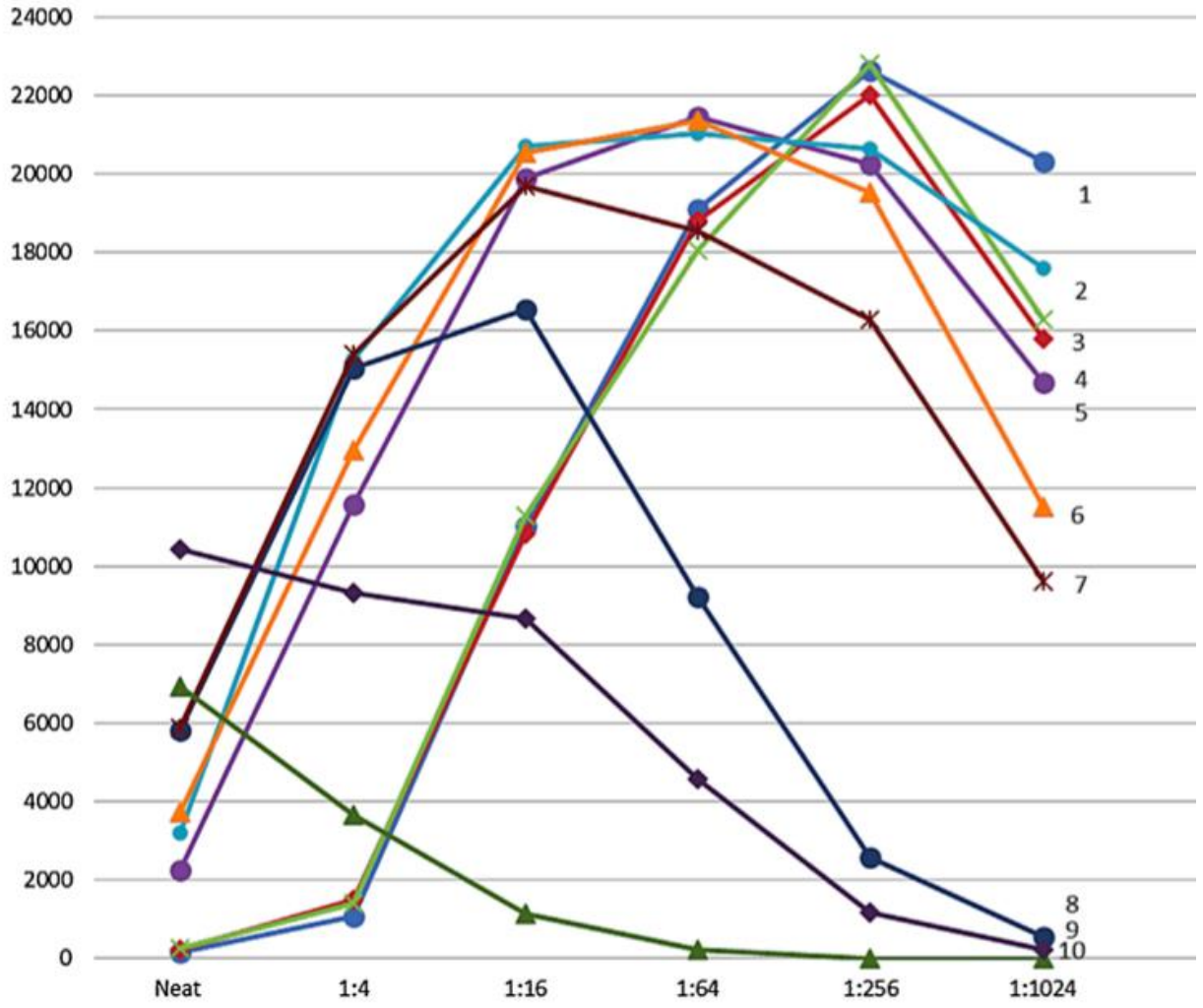
Redundant assays to prevent AMR



Limitations of virtual crossmatch:

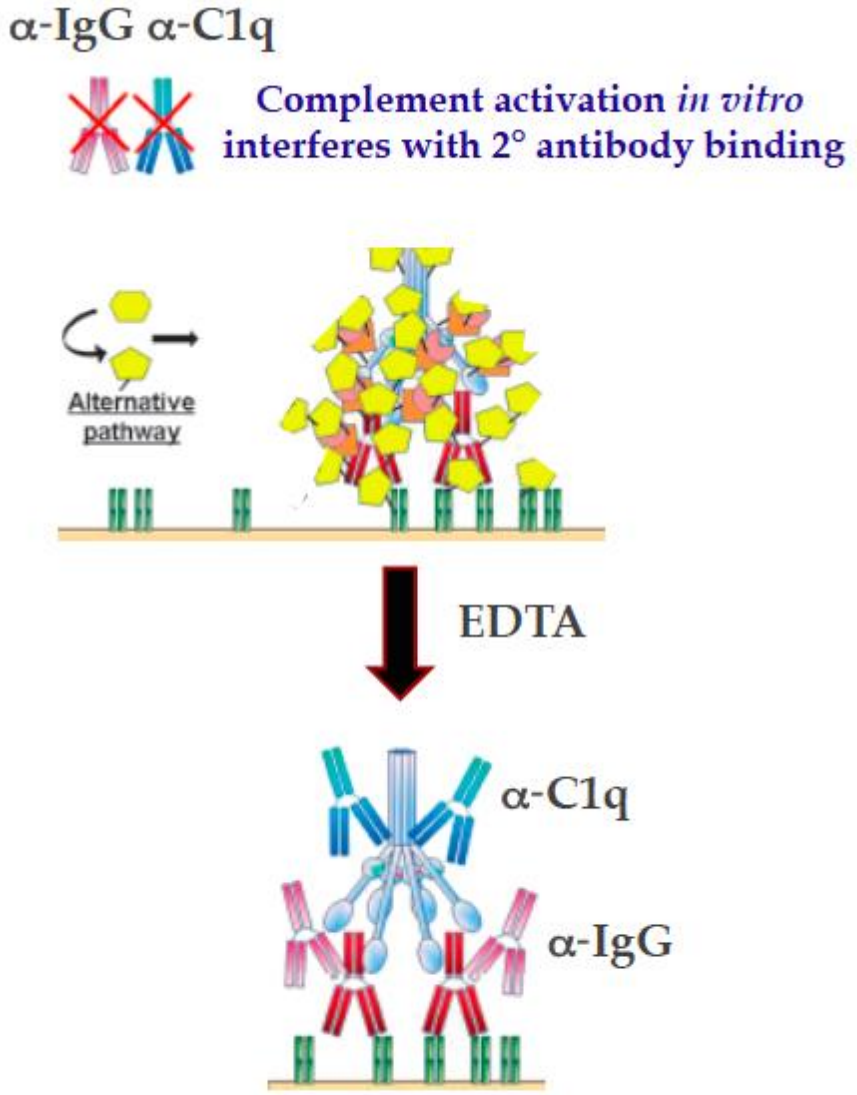
- Assume donor typing is correct
- Assume recipient antibody profile is correct
- Assume no new sensitizing events

Additional pitfalls: prozone effect



Tambur, AJT, 2015

Additional pitfalls: epitope pattern on beads



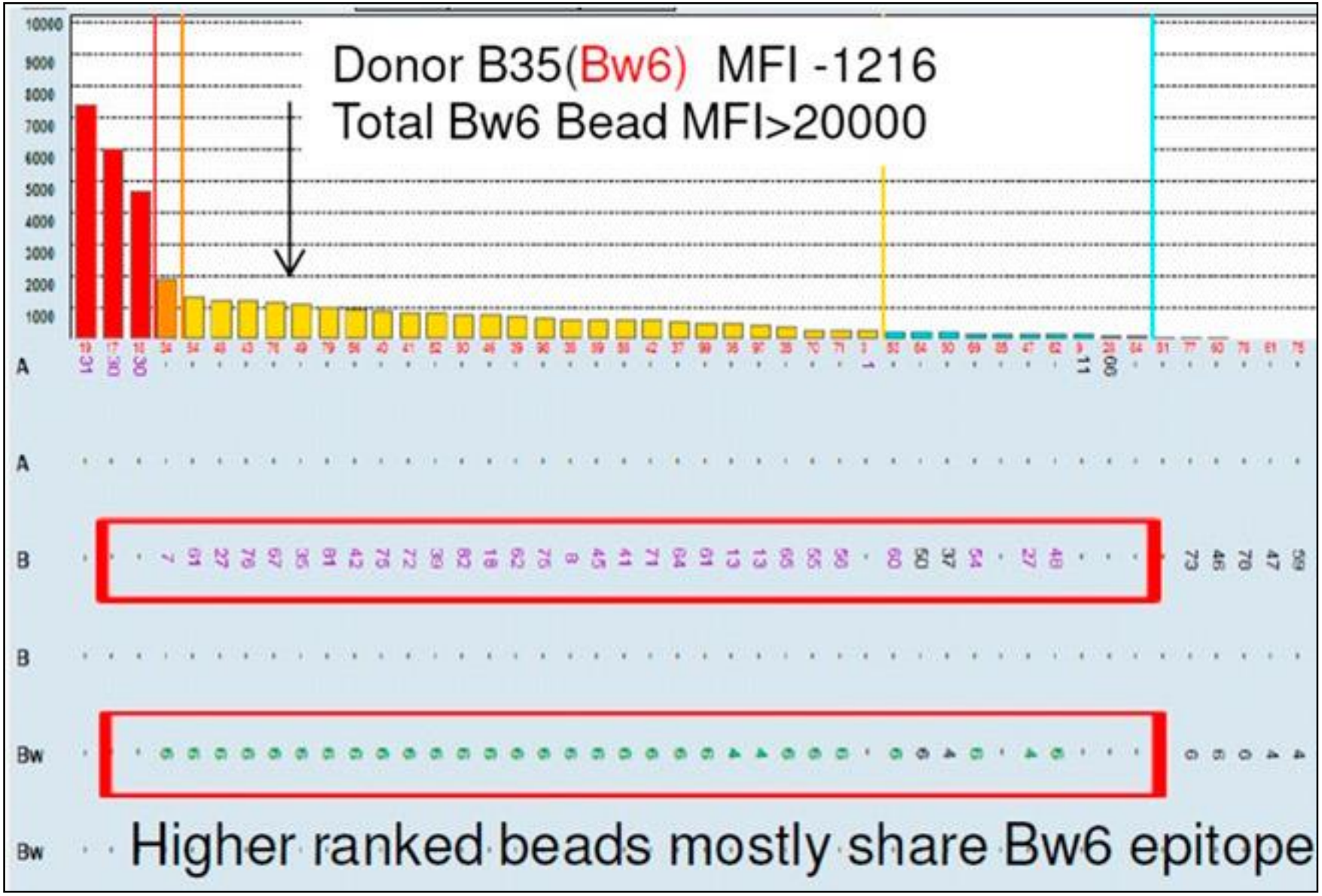
Visentin et al, Transplantation (2014) 98:625-631

- Slide courtesy Peter Nickerson

How to eliminate complement interference:

1. Serum dilution
2. EDTA
3. DTT
4. Heat

Additional pitfalls: epitope pattern on beads

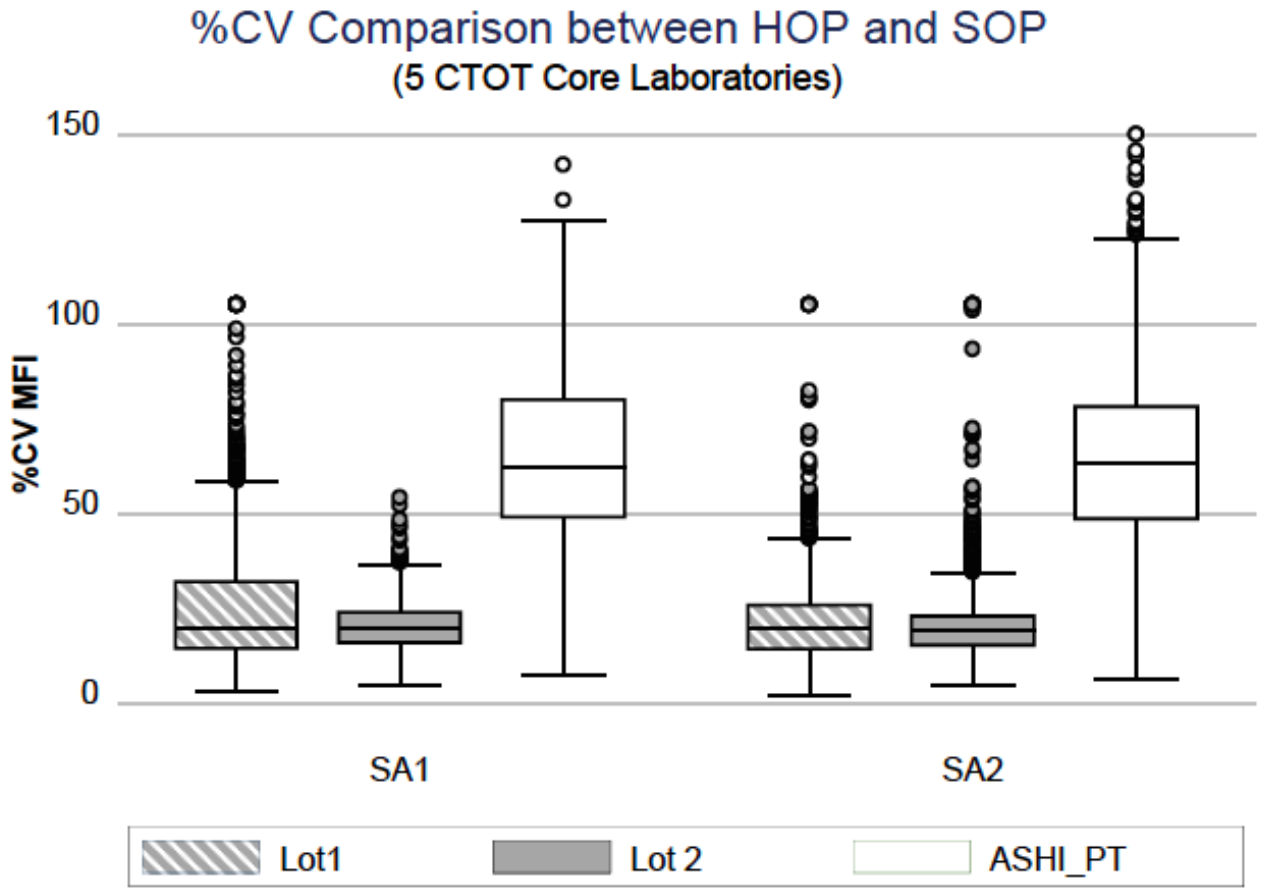


Konvalinka, JASN, 2015

SAB is a semi-quantitative assay

Not Licensed as a Quantitative Assay

- Slide courtesy Peter Nickerson



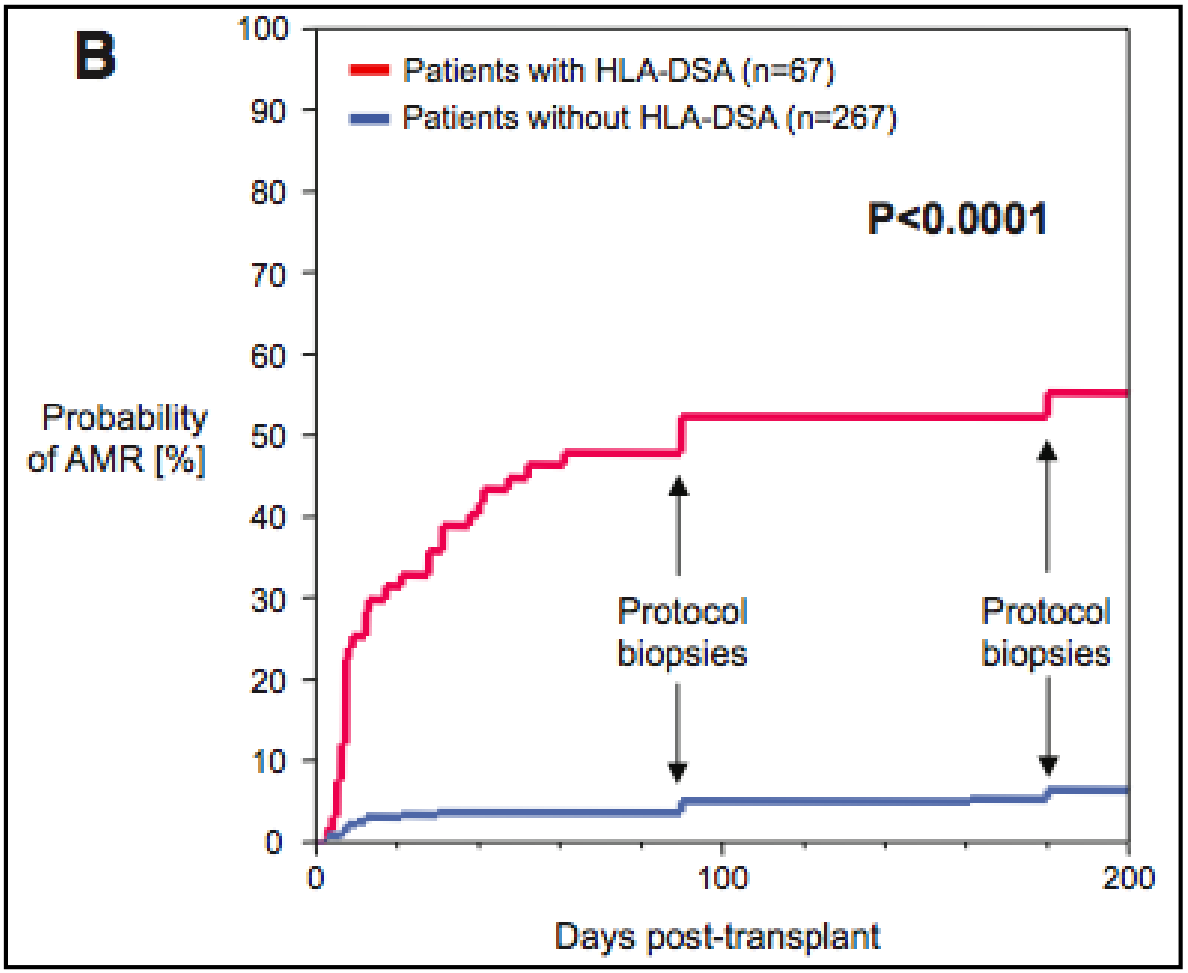
Quantitative Assay FDA requires CV < 20%

Reed et al., AJT (2013) 13:1859-1870

Part III. DSA Risk Stratification

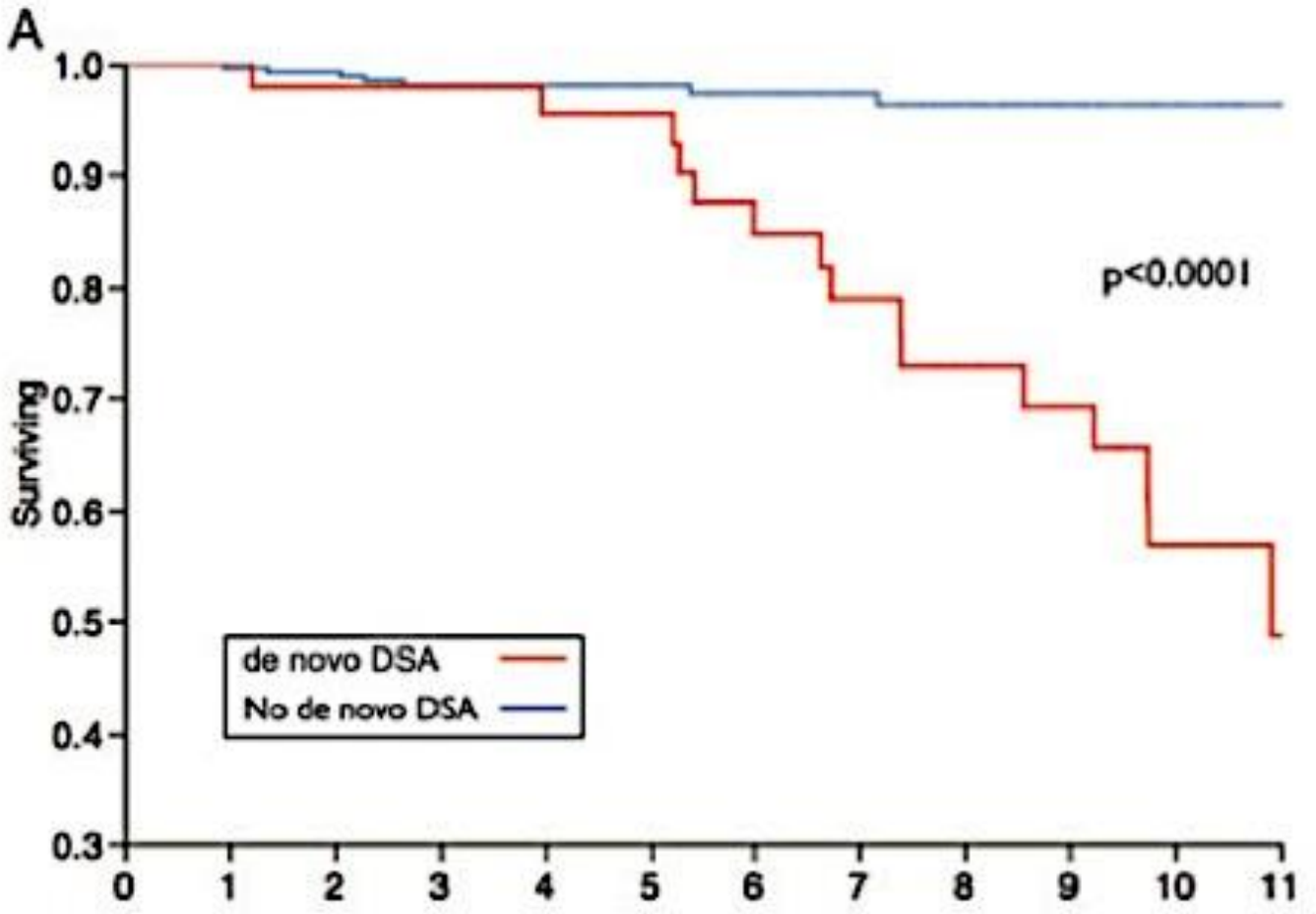
FACULTY OF MEDICINE

DSA increase the risk of AMR



Amico et al, Transplantation • Volume 87, 2009

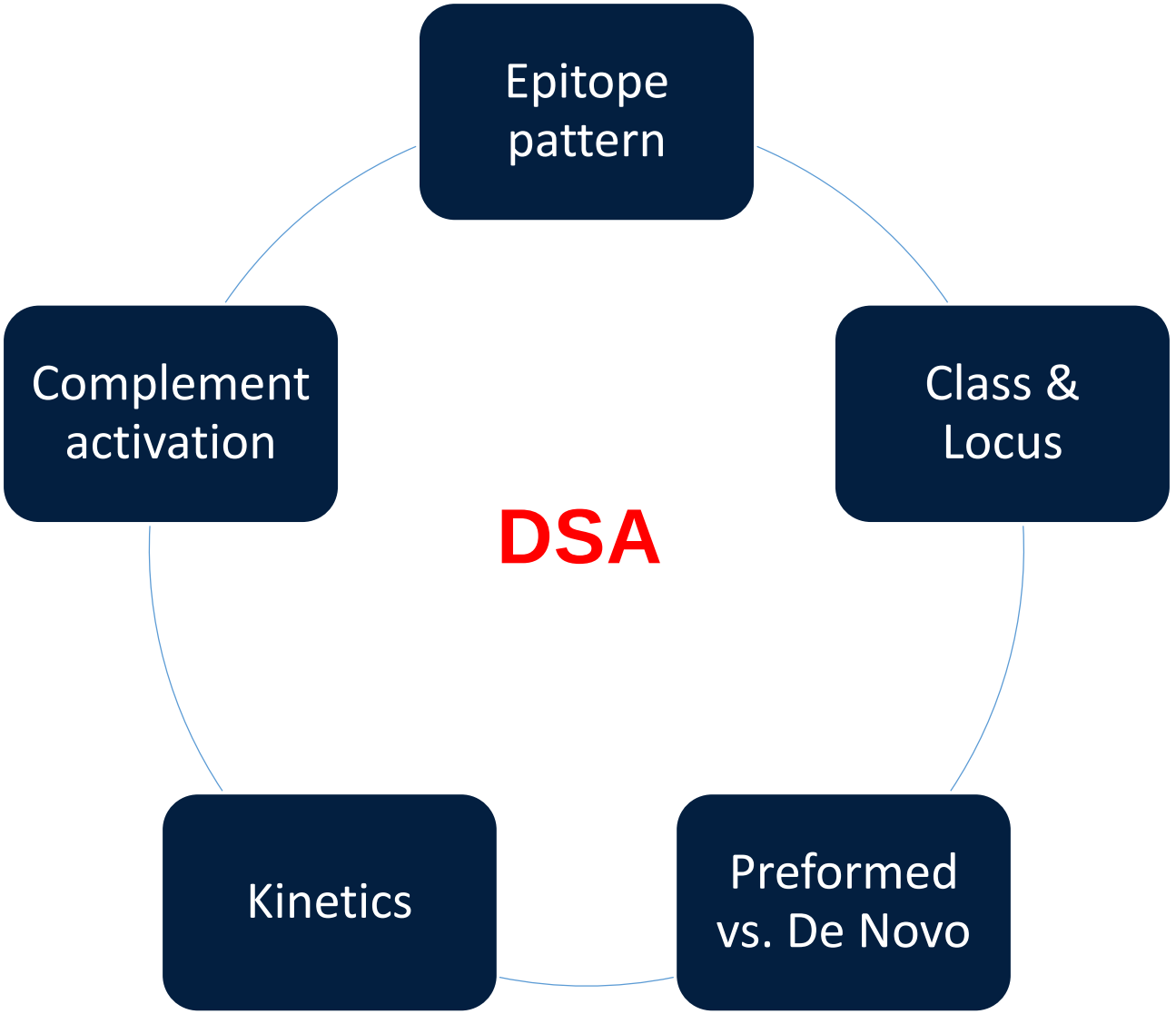
De Novo DSA associate with inferior long-term outcome



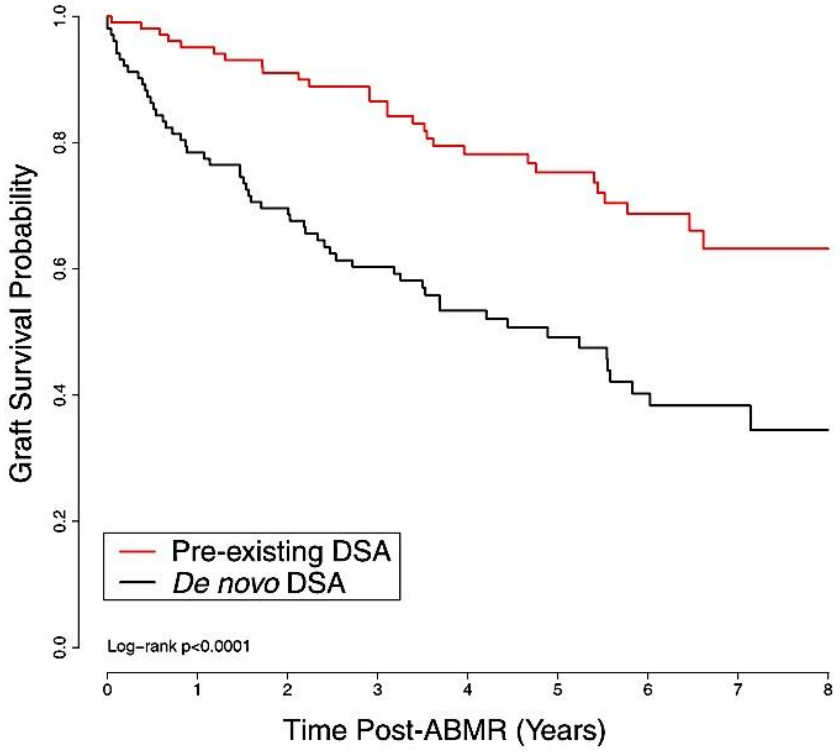
- 10-year graft survival is significantly reduced (44% vs 93%)

Wiebe, AJT 2012

Determinants of the pathogenicity of DSA



DSA consideration: inferior graft survival with de novo DSA



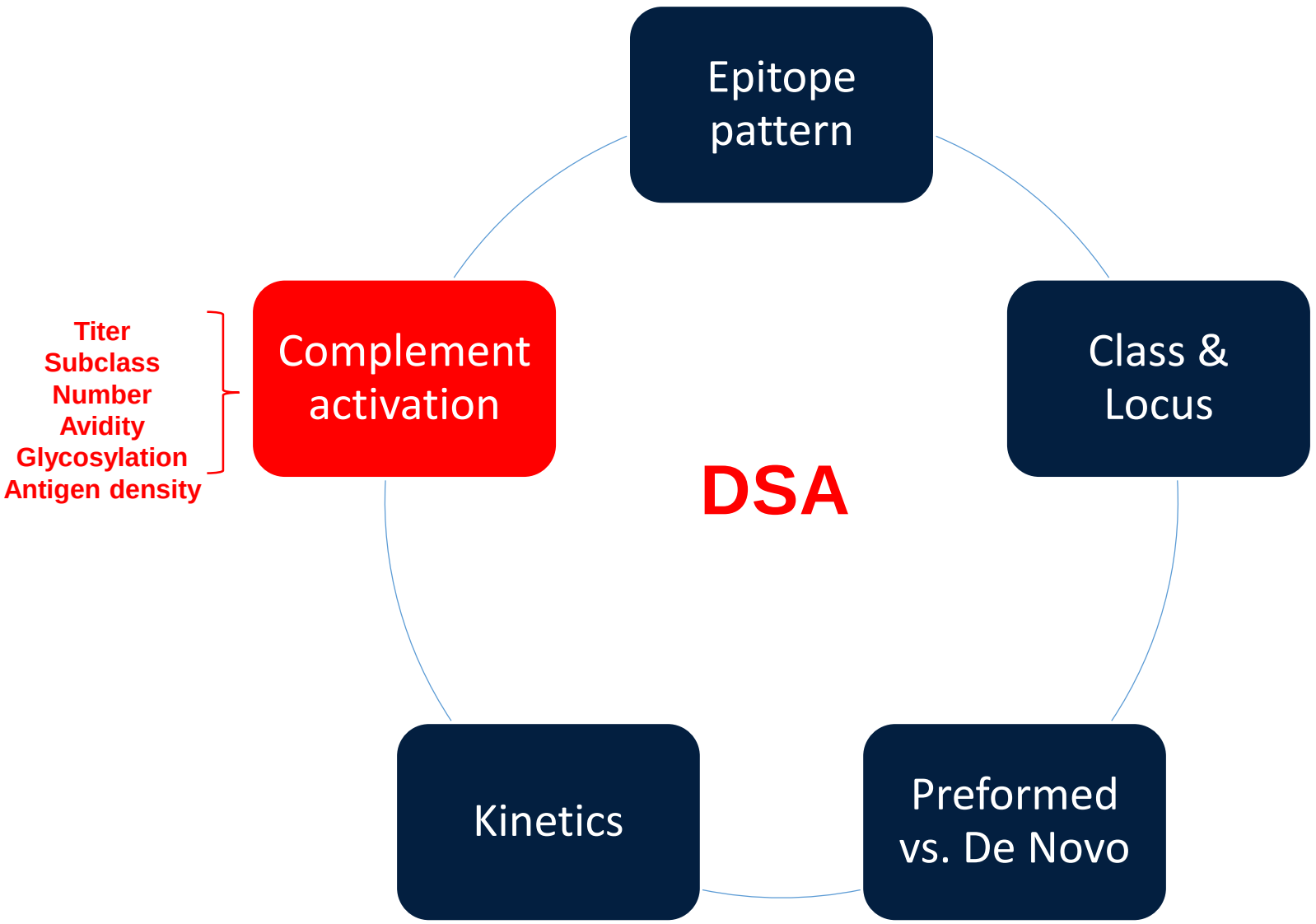
N at Risk									
Pre-existing DSA	103	95	87	74	61	49	32	17	11
De novo DSA	102	80	70	56	43	31	22	10	4

Aubert, JASN 2017

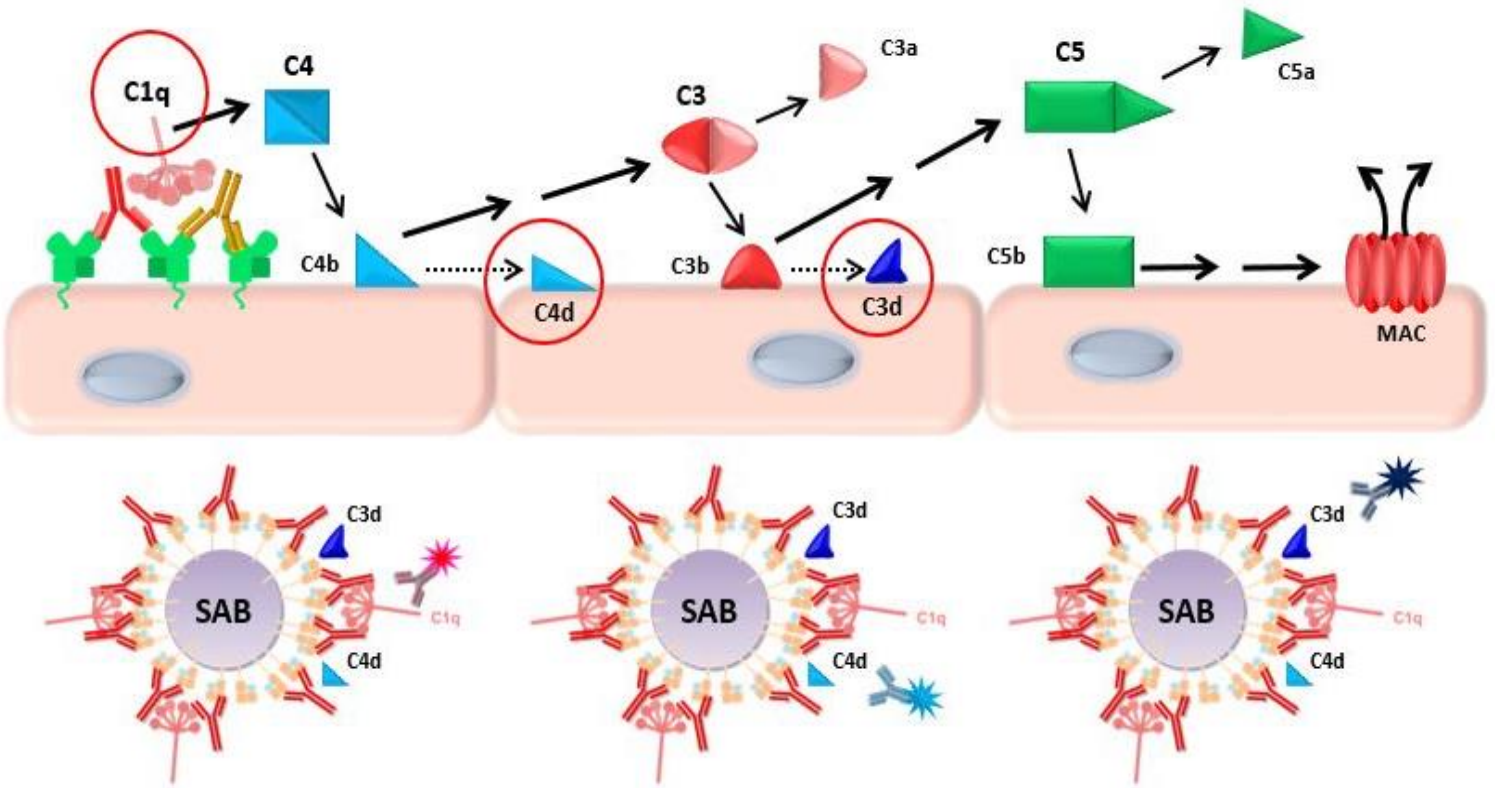
DSA consideration: de novo DSA are mainly class II

	Willicombe Transplantation 2012	DeVos Kidney Int 2012	Musat AJT 2011	Smith AJT 2011	Palmer Transplantation 2002
Population	Renal	Renal	Liver	Heart	Lung
De Novo DSA	18.2% (92/502)	18% (62/347)	63% (27/43)	33% (57/173)	10% (9/90)
DQ DSA	**54.3%** (50/92)	**53%** (33/62)	**81%** (22/27)	**72%** (41/57)	**56%** (5/9)

Determinants of the pathogenicity of DSA



In vitro functional assessment of complement activation

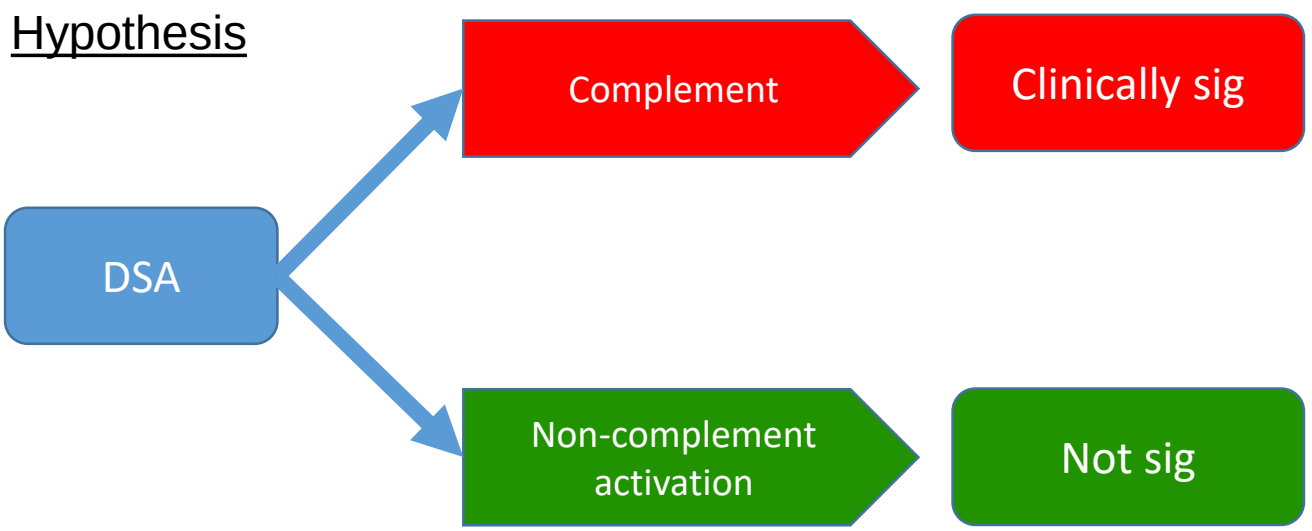


	C1q Assay	C4d Assay	C3d Assay
Complement source	Purified exogenous C1q	Normal human serum	Normal human serum
Reagent	Anti-C1q antibody	Anti-C4d antibody	Anti-C3d antibody

Figure 1. Schematic of the classical complement pathway. Different components in the pathway (C1q, C4d, C3d) are targeted in complement dependent assays to evaluate the complement-activating potential of HLA antibodies.

Lan J, et al. Transplantation 2017 (In Print)

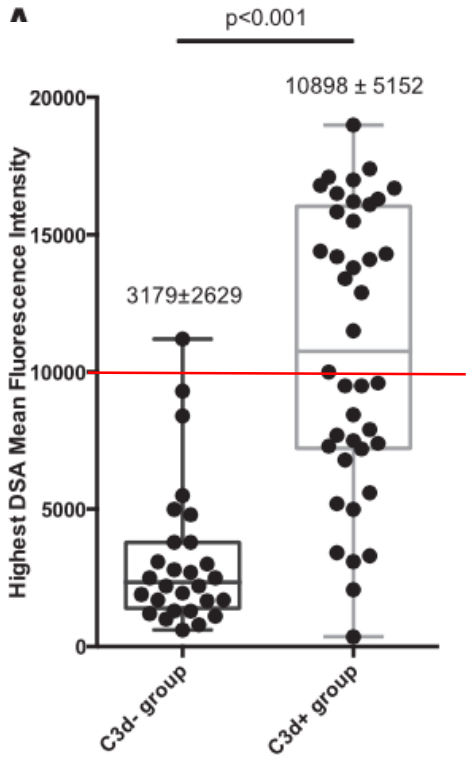
Hypothesis: using complement activation to risk-stratify DSA



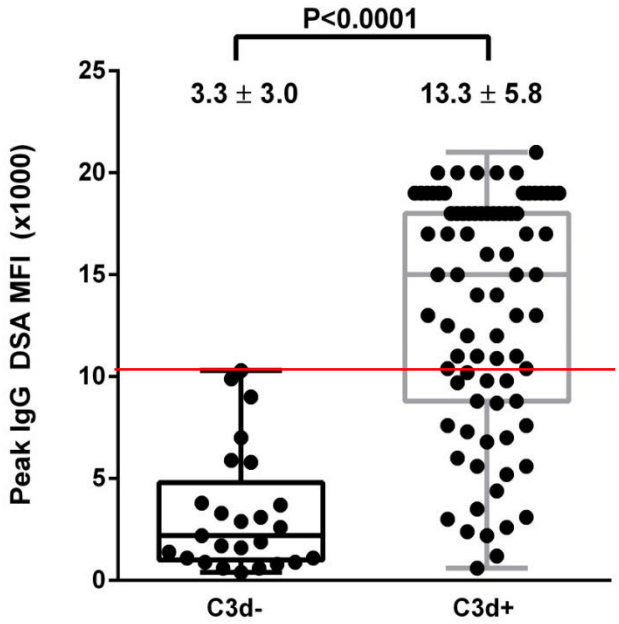
Complement binding assessment assays

C1q predictive of outcomes	C1q not predictive of outcomes
Yabu et al, Transplantation 2011	Crespo et al, Transpl Immunol 2013 (Pre-Tx)
Loupy et al, NEJM, 2013	Otten et al, AJT, 2012 (Pre-Tx)
Freitas et al, Transplantation, 2013	Ginevri et al, AJT, 2012
Fichtner et al, Pediatr Nephrol 2016	Wiebe et al, AJT, 2016 (not sig in multivariate)
Bamoulid et al, Transplantation, 2016	
Sicard et al, JASN, 2014 (C3d)	
Comoli et al, AJT, 2016 (C3d)	
Guidicelli et al, JASN, 2016 (C1q)	
Lefaucheur et al, JASN, 2016 (IgG subclass)	
Viglietti et al, JASN, 2016 (IgG3, C1q)	

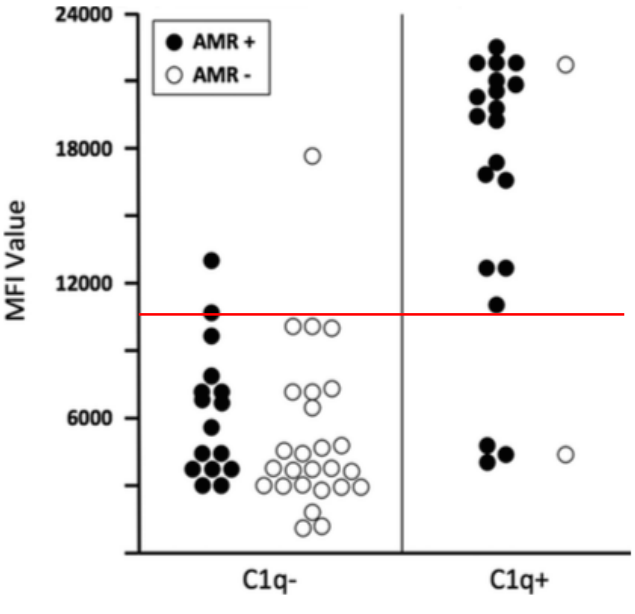
Antibody concentration and complement activation



Sicard et al, JASN, 2014



Lan et al, submitted



Yell, Transplantation, 2015

Manipulation of antibody concentration affects C1q positivity

Effects of normalization of C1q + DSA MFI values to levels comparable C1q – on Luminex-C1q activity

	N	MFI	Luminex-C1q
C1q + DSA	12	18,233 + 4268	+
C1q + DSA-diluted	12	6784 + 3386	–
C1q – DSA	22	5864 + 2686	–

In vitro C1q positivity is related to antibody concentration

Effects of serum concentration on C1q-binding activity of C1q – DSA

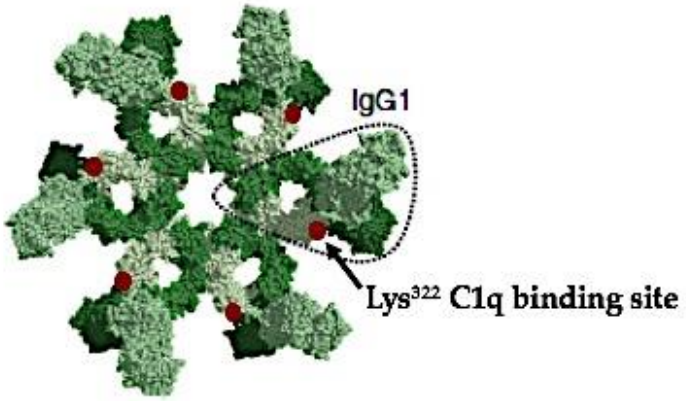
	Neat		Concentrated	
Sample	MFI	Luminex-C1q	MFI	Luminex-C1q
1	5489	Neg	12,243	Pos
2	4924	Neg	10,125	Pos
3	6985	Neg	13,112	Pos
4	5573	Neg	11,832	Pos
5	6323	Neg	7125	Neg
6	3794	Neg	5793	Neg

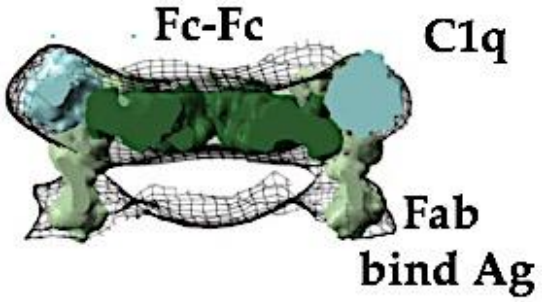
Yell, Transplantation, 2015

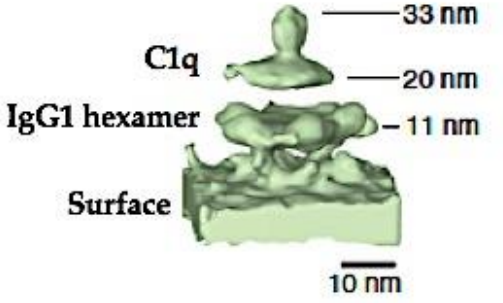
Assembly of IgG hexamers on cell surface is required for complement activation

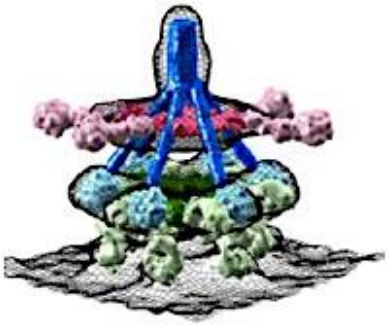
Slide Courtesy of Peter Nickerson

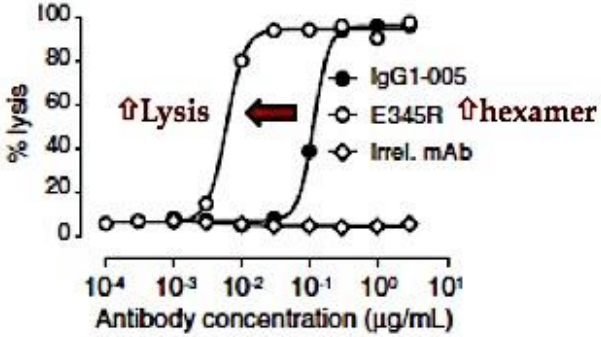
Diebolder, Science, 2014











Prevalence of isolated weak/non-complement binding IgG subclasses

Prevalence of isolated weak/non-c' activating HLA DSA is rare

	Patient cohort	Prevalence of isolated IgG2/IgG4 Ab
Lefaucheur, JASN, 2016	n=125	4%
Schaub, Transplantation, 2014	n=73	5%
Arnold, Transpl Int, 2013	n=274	1%
Lowe, Human Immunol, 2013	n=51	1%

Complement dependent assays

- Antibody concentration is the dominant determinant of complement activation
- Isolated non-complement-activating antibodies (IgG2, IgG4) are rare in the clinical setting (1-5%)
- Non-complement-activating DSAs can also be pathogenic, although the pattern of injury may be less severe than c’-activating

THANK YOU

Questions?

James.Lan@vch.ca