

Evolution of Anti-HLA Antibody Tests

J Michael Cecka
UCLA Immunogenetics Center

Prediction of Hyperacute Rejection by the Crossmatch Test

Outcome	Pos	Neg	No Test	NoAb
Hyperacute	24	4	6	4
Fail <3 mo	0	4	6	32
Fail >3 mo	1	7	3	22
Surv <3 mo	2	1	2	6
Surv >3 mo	3	11	6	104
Total	30	27	23	168

PRA Determination

Add Patient's serum
to all wells

Add one donor's
cells to each well

Add complement

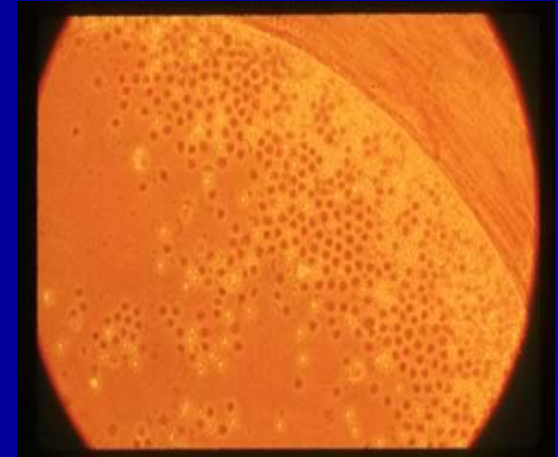
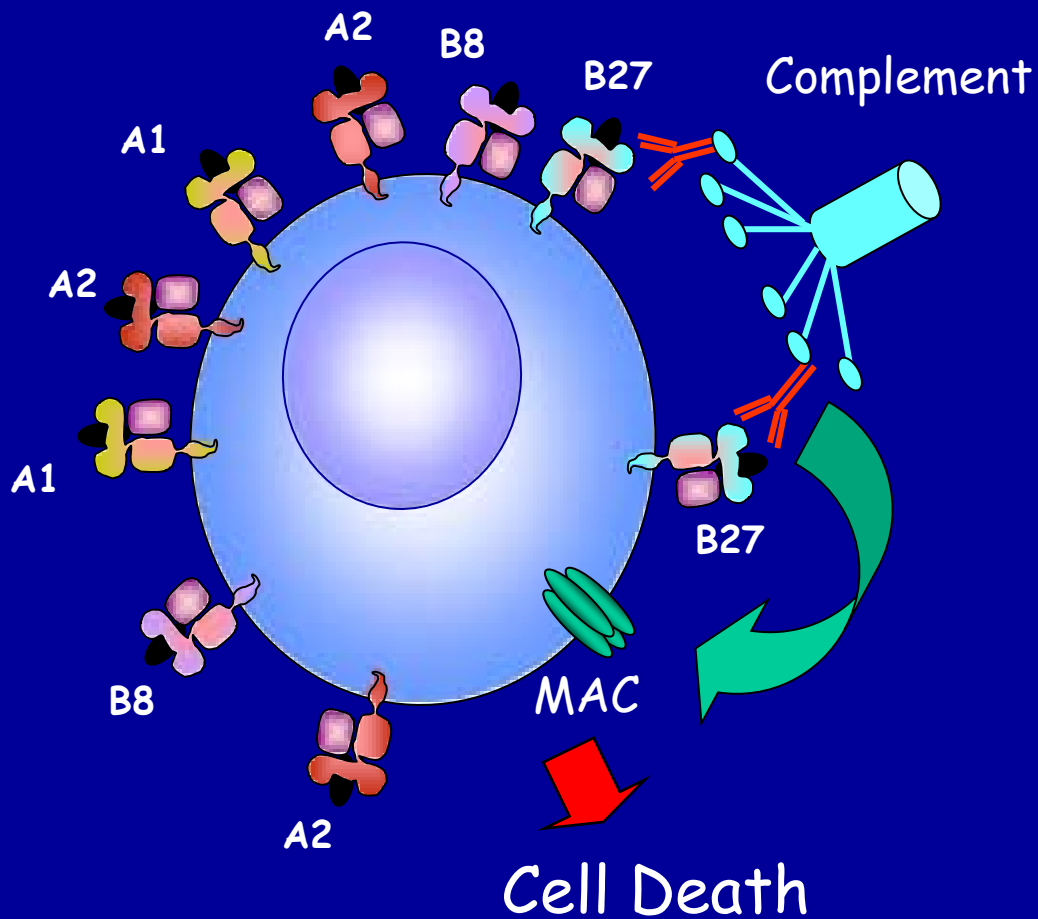
Add vital dye

Estimate viability

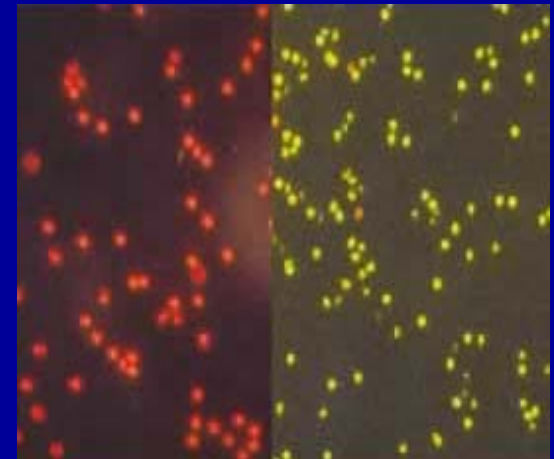
24% PRA

Complement Dependent Cytotoxicity (CDC) Crossmatching

Eosin



Fluorescein Diacetate +
Ethidium Bromide



Dead cells

Live cells⁴

Identifying HLA Antibodies

	A	B	Cw	
●	1	2 35 63	4 7	
●	1	11 37 62	6	
●	1	26 35 62	4	Anti-A1
●	1	26 8 57	2 6	
●	1	26 38 73	12 18	
●	1	32 42 44	6 17	Anti-B8
●	1	33 8 62	7 10	
●	1	34 35 57	6 16	
●	1	66 50 58	4 7	
●	2	11 13 55	1 7	
●	2	24 7 77	8 15	
●	2	26 39 46	1 7	

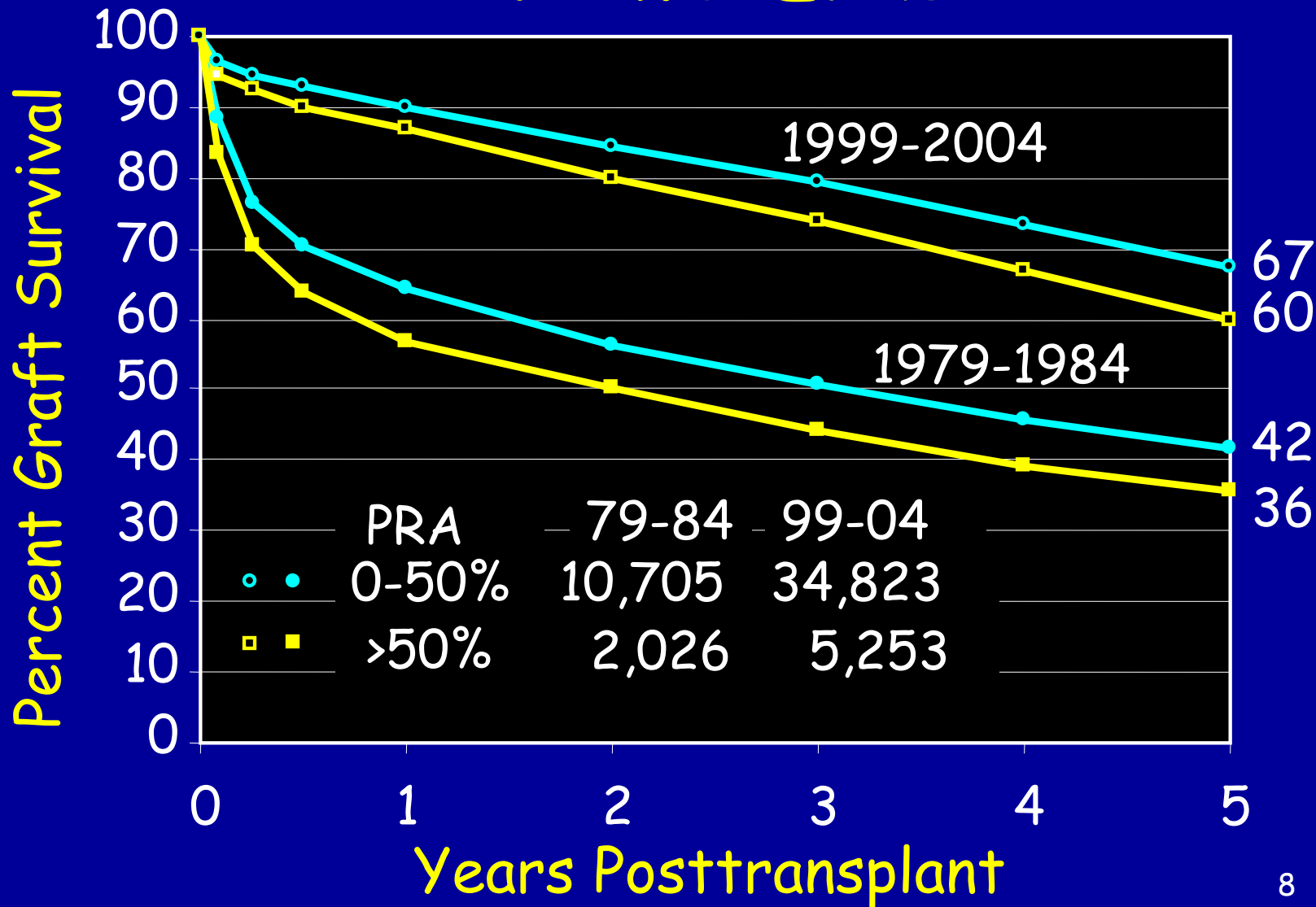
Consequences of Preformed anti-donor HLA Antibodies

- Hyperacute rejection
- Delayed graft function
- Accelerated acute rejection
- Chronic rejection
- Prolonged waiting times
- No transplantation

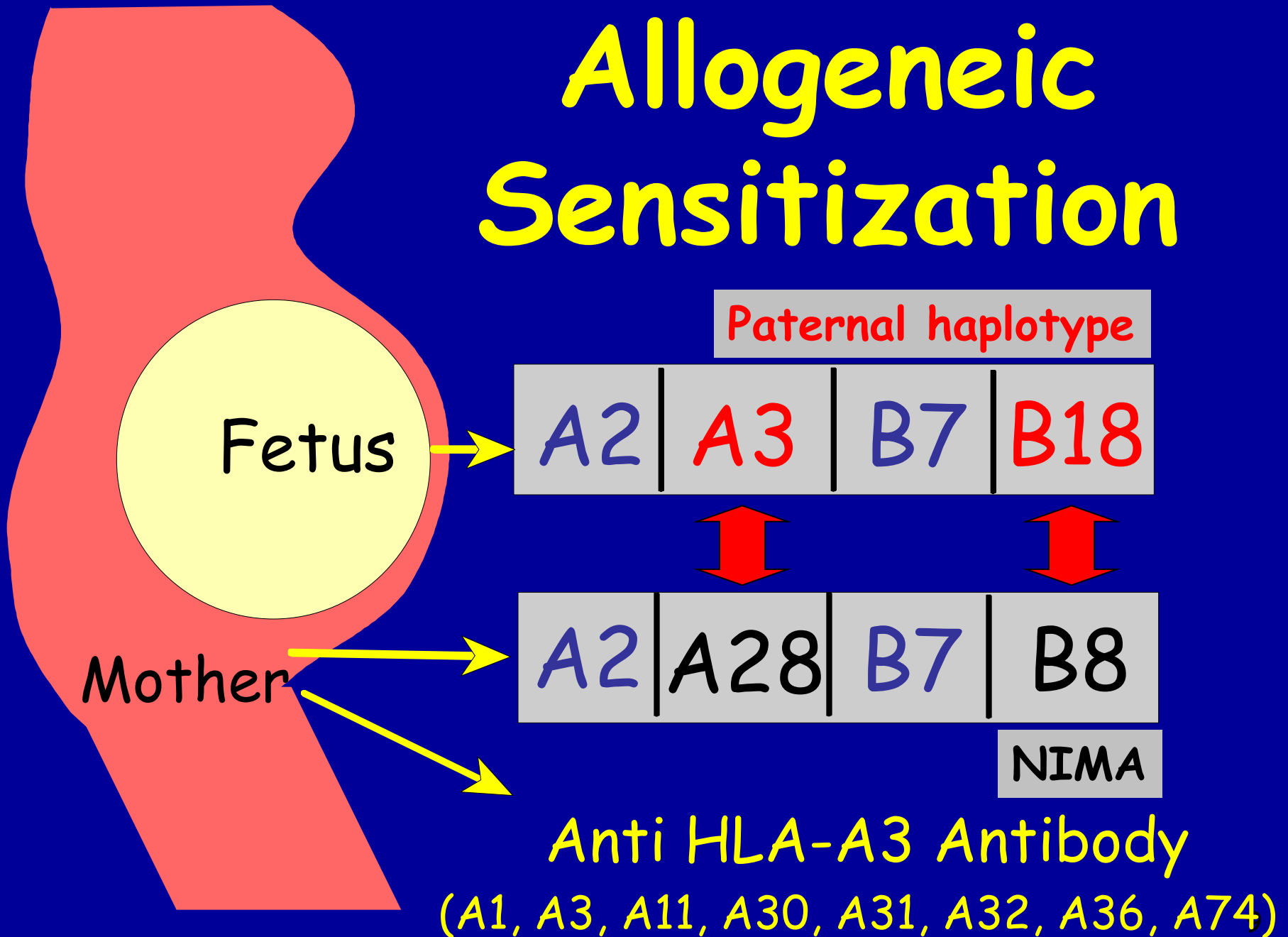
The Problem

- Avoid donor HLA-specific antibodies
 - Crossmatch tests
 - Complement-dependent cytotoxicity
 - Anti-human globulin (AHG) enhancement
 - Flow cytometry
 - Virtual
 - Panel-reactive antibody (PRA)
 - Complement-dependent cytotoxicity
 - Anti-human globulin (AHG) enhancement
 - Flow cytometry
 - Solid-phase tests

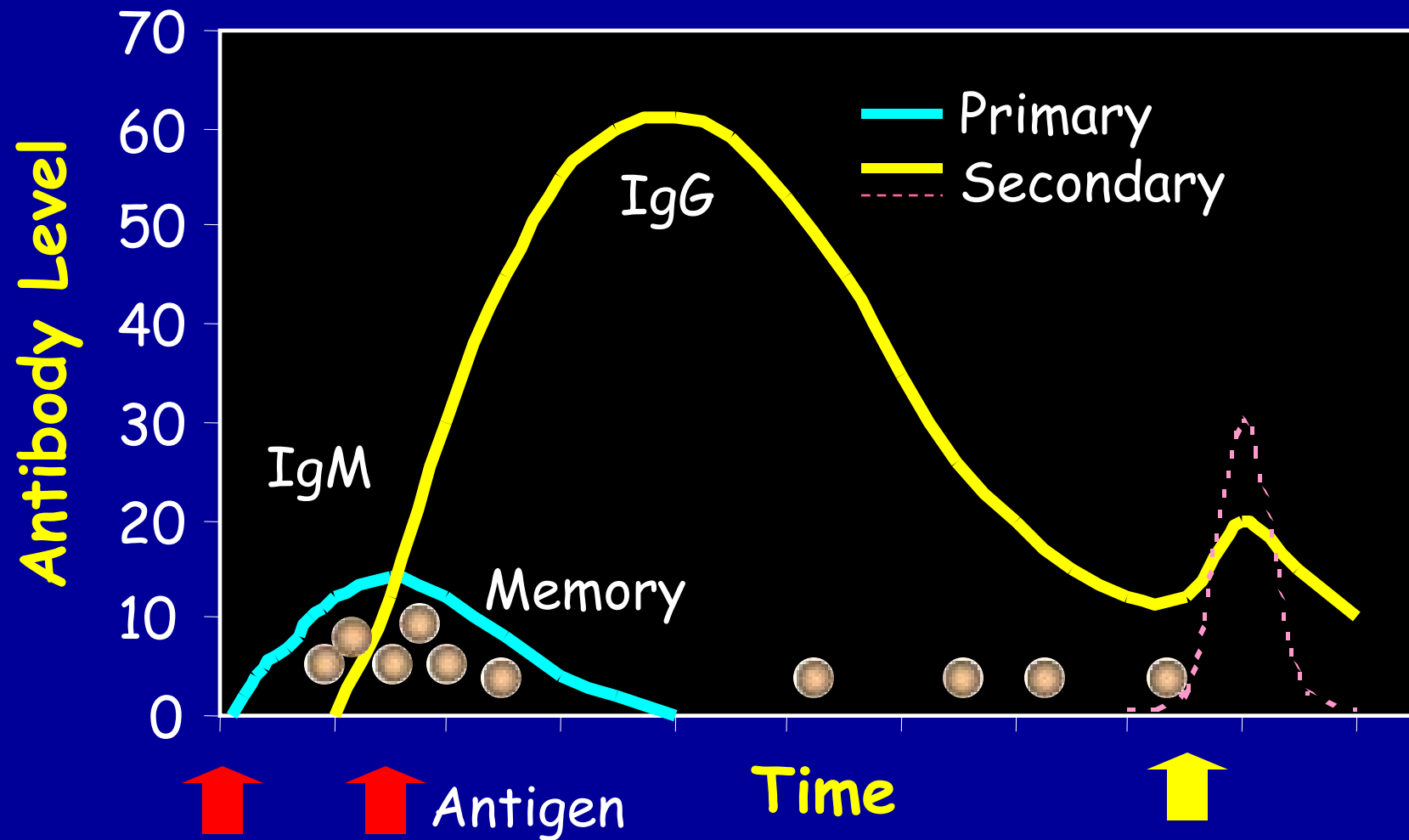
Effect of Sensitization in Two Eras



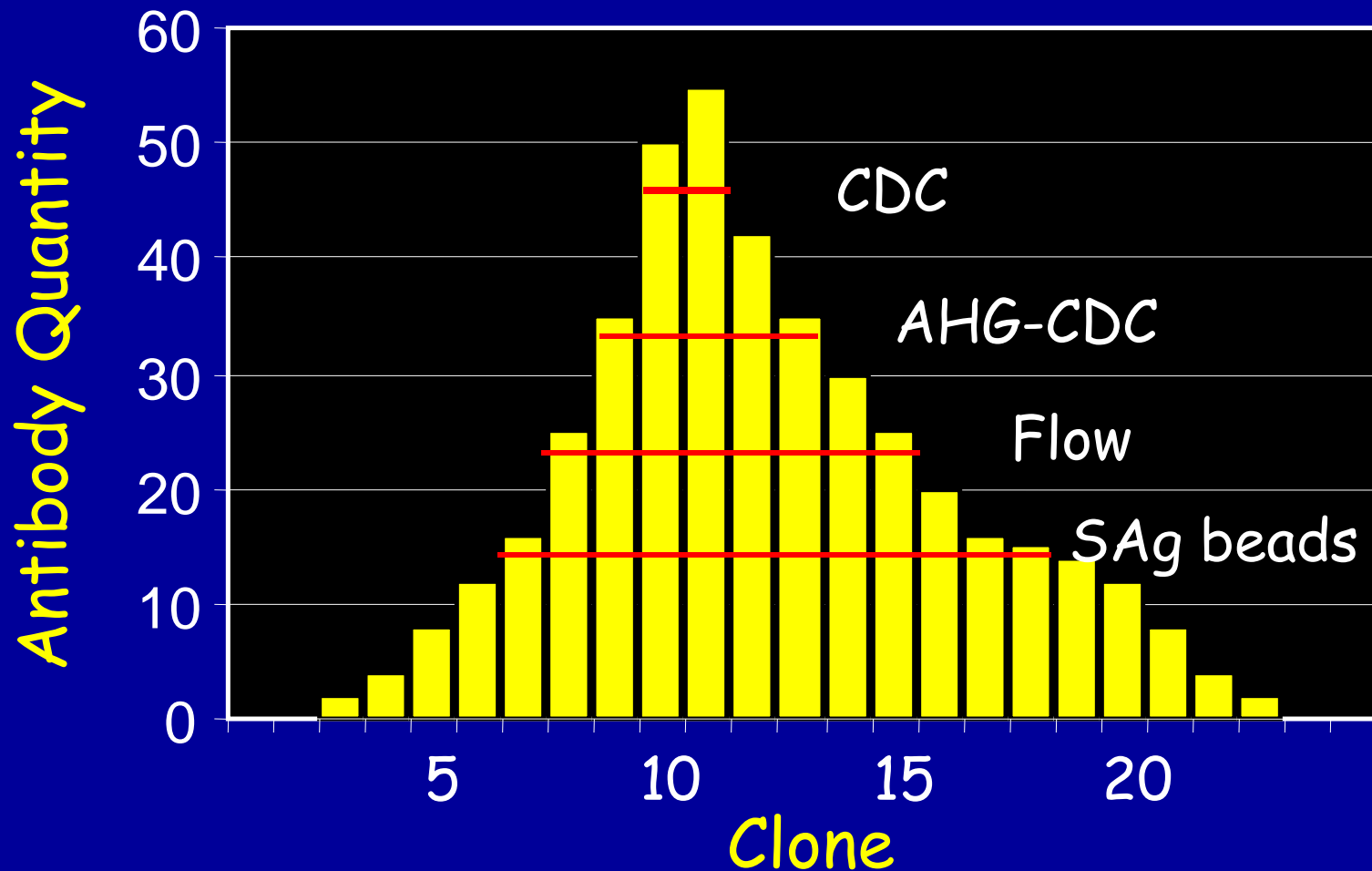
Allogeneic Sensitization



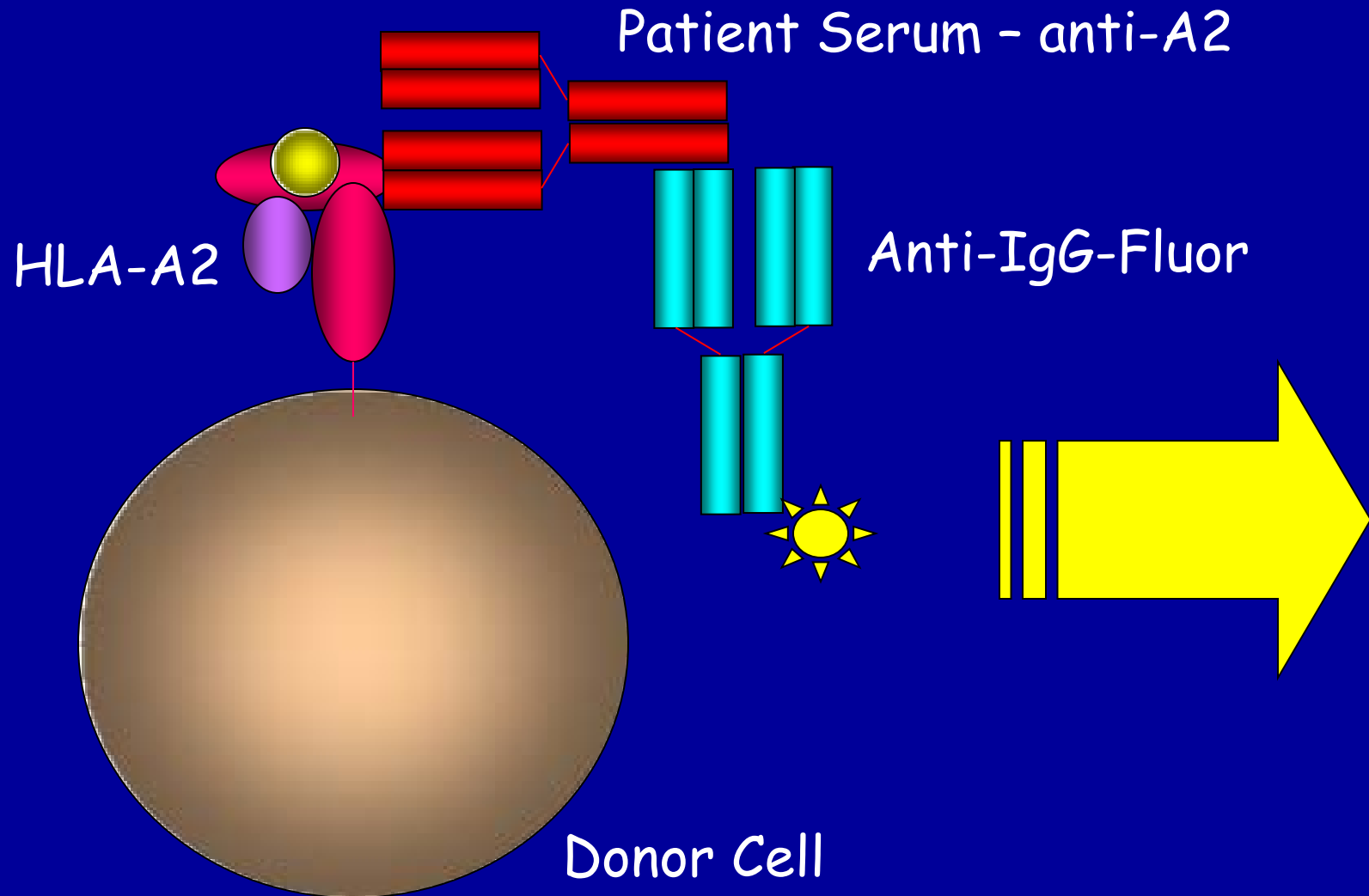
Kinetics of Primary and Secondary Humoral Immune Responses



Dynamics and Detection of the Antibody Response

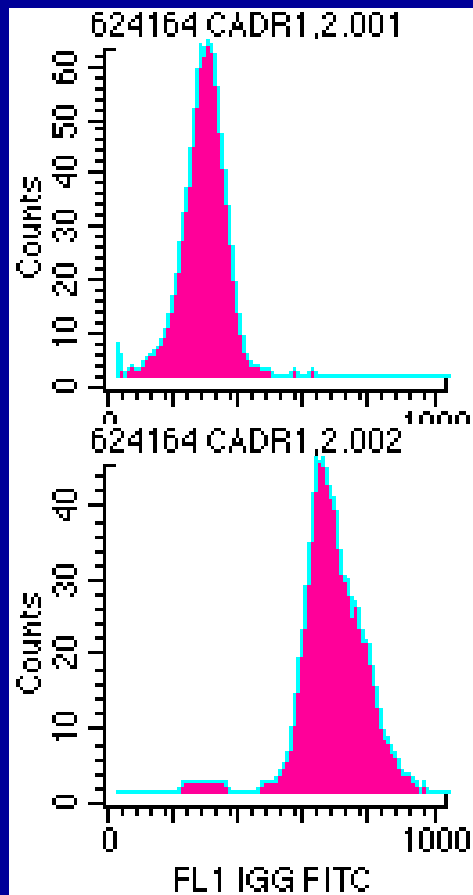


The Flow Cytometry Crossmatch

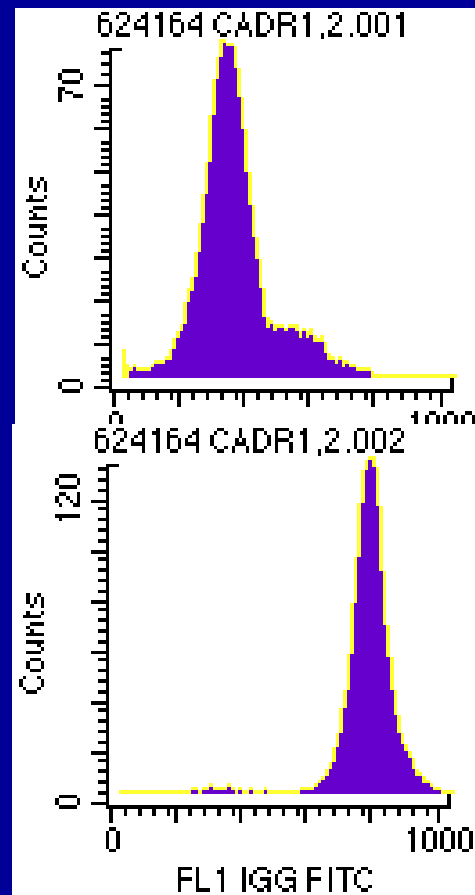


Flow Cytometer

T-CELL



B-CELL



NEG CONTROL

T-MC = 265

B-MC = 318

PATIENT

T-MC = 638

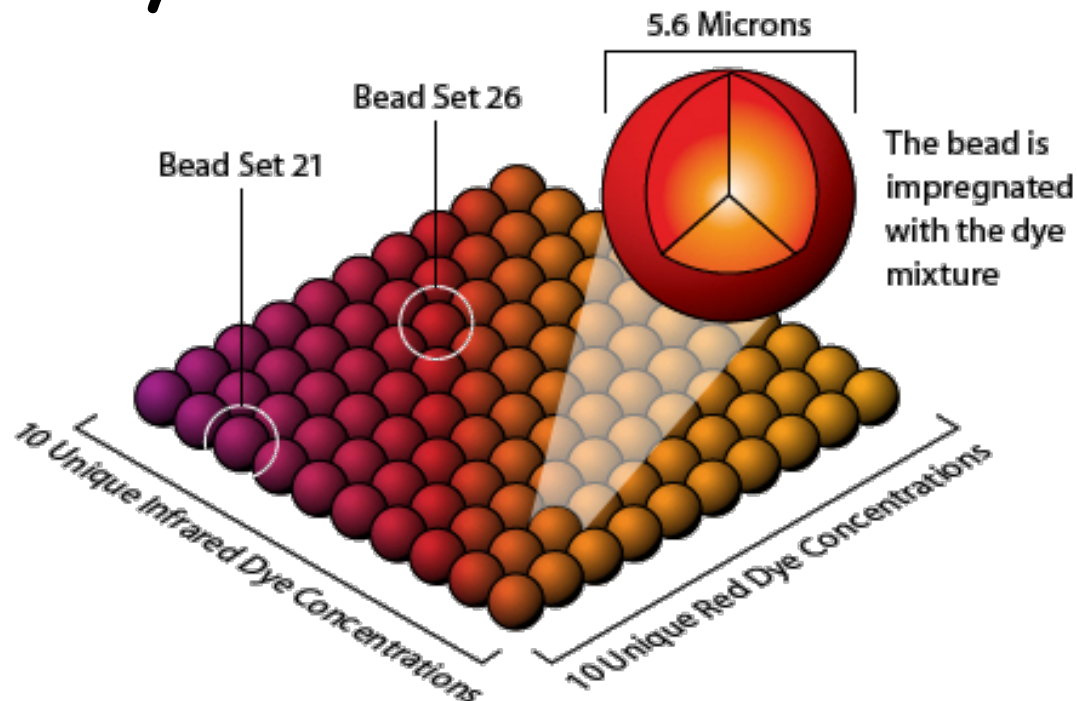
MCS = 373

B-MC = 743

MCS = 425

Luminex Technology

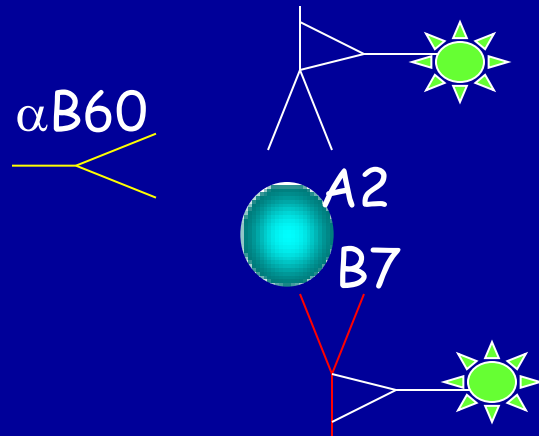
Unique bead sets are color coded using different ratios of two fluorescent dyes, beads are distinguished based on FL intensity



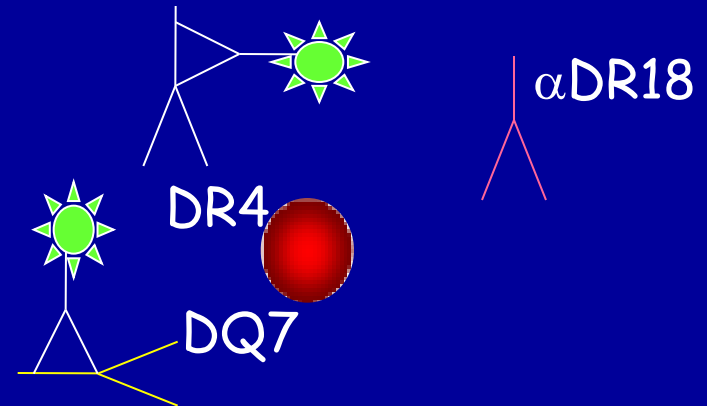
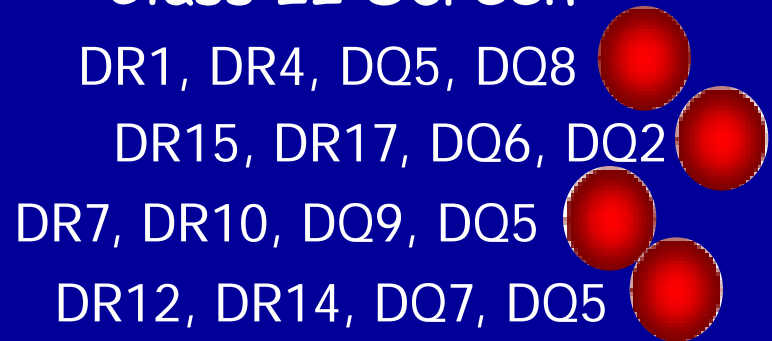
Screen and ID

(HLA Phenotype beads)

Class I Screen

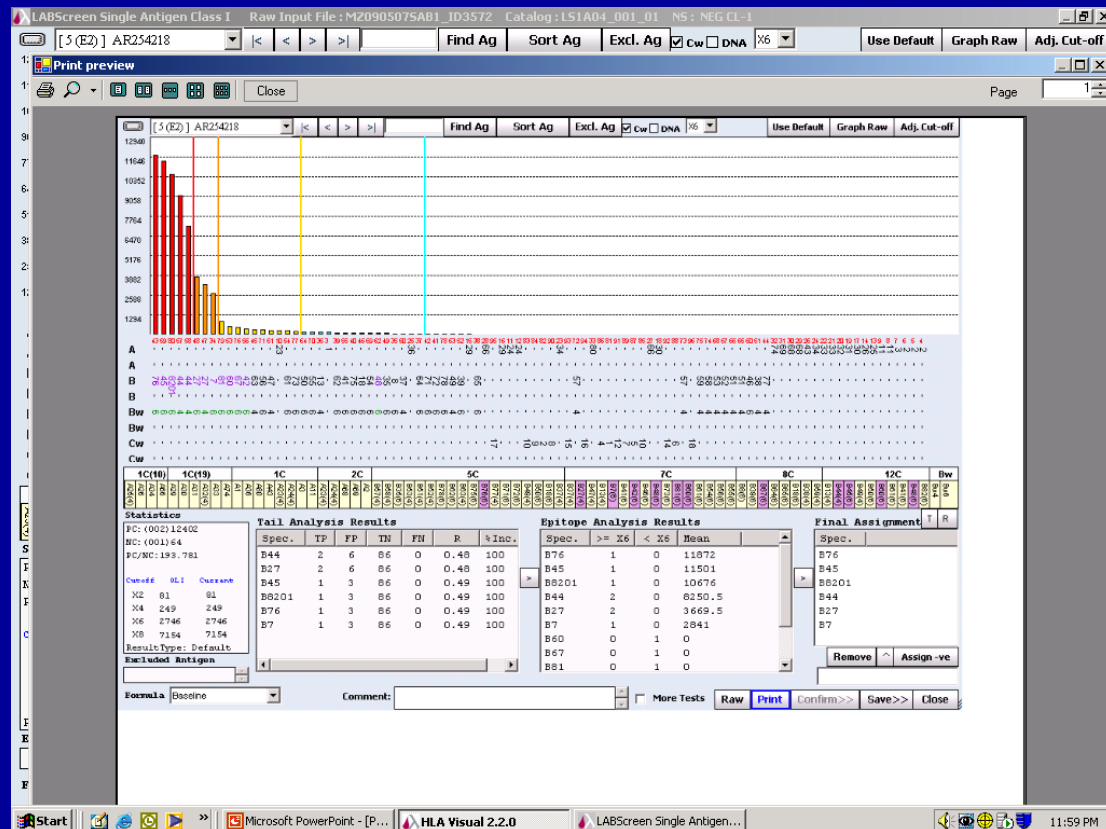
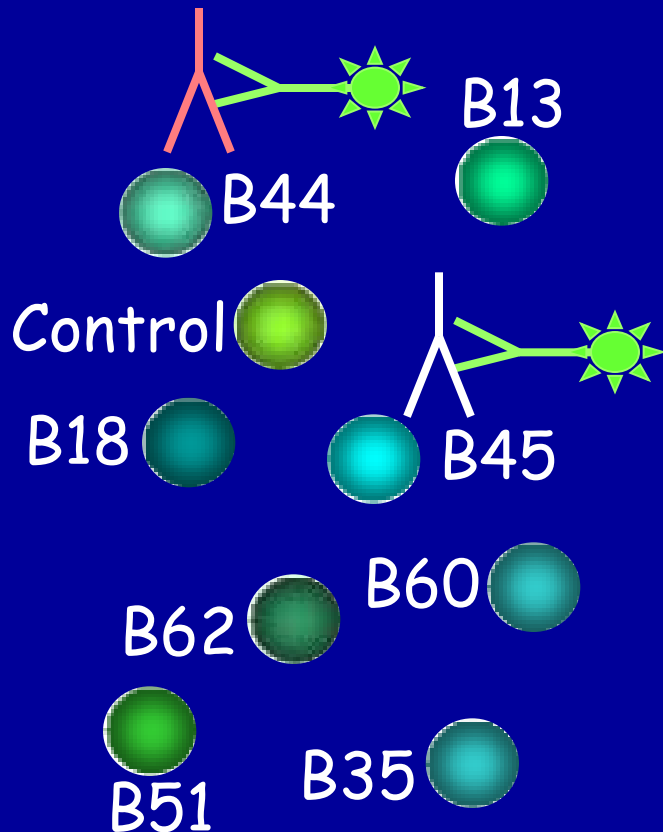


Class II Screen



Precise Identification of Antibodies

Single Antigen Beads



Unacceptable HLA Antigens

Patient anti-A2 + anti-DR4 + anti-DQ5
 50% cPRA 60% cPRA 70% cPRA

Potential Donors

A				B				DR				DQ			
1	68	8	13	4	15	2	5	3	24	18	39	1	4	4	4
2	24	7	18	1	10	5	5	24	43	27	45	1	8	4	8
2	29	13	51	8	14	4	8	11	33	51	64	15	18	5	7
23	26	49	62	1	17	2	5	2	25	39	65	9	17	4	9
2	68	39	71	15	16	5	6	2	23	44	45	13	18	7	8
1	36	7	44	9	17	4	9	1	2	8	62	4	17	4	7
69	74	55	60	4	7	7	8	2	34	57	61	11	14	2	4

Summary

- HLA antibody tests have evolved
 - CDC < AHG CDC < Flow cytometry ~ ELISA ~ SP screen < SP ID < Single antigen
 - Precision has improved for broadly sensitized patients
 - Specificity of antibodies can be determined
- Effects of antibody on transplant outcomes are clear and widely accepted
 - Some controversies remain about importance of weak donor HLA-specific antibodies
 - Immune memory cannot be quantified