

*The Use of Flow Cytometry
for Transplant Compatibility
Analysis*

Thomas C. Shanahan, Ph.D., ABMLI
Upstate New York Transplant Laboratories
Upstate New York Transplant Services, Inc.

Transplant Compatibility

Terminology

Histocompatibility Analysis

The immunologic assays applicable to the prediction and prevention of antibody-mediated rejection

Alloantibody

Humoral mediator of rejection directed against foreign tissue antigens

Histocompatibility Antigens

Protein products of polymorphic genes that induce alloantibody formation; *Human Leukocyte (HLA) Antigens*

HLA Antigens

HLA Class I Antigens

HLA Class II Antigens

A locus

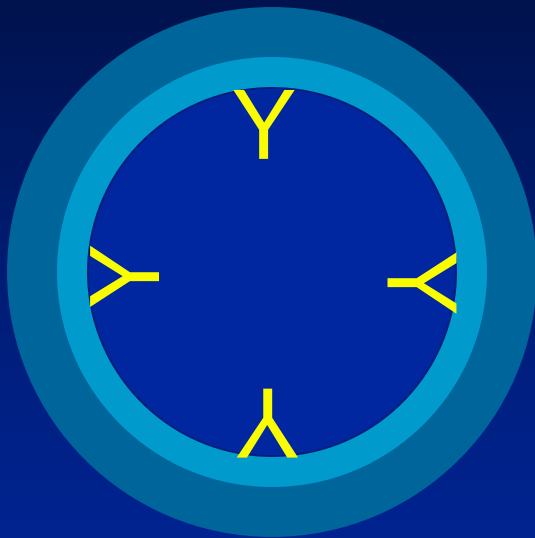
B locus

DR locus

A1	A30	B5	B35	B50	B63	DR1	DR10
A2	A31	B7	B37	B51	B64	DR2	DR11
A3	A32	B8	B38	B52	B65	DR3	DR12
A9	A33	B12	B39	B53	B67	DR4	DR13
A10	A34	B13	B40	B54	B70	DR5	DR14
A11	A36	B14	B41	B55	B71	DR6	DR15
A19	A43	B15	B42	B56	B72	DR7	DR16
A23	A66	B16	B44	B57	B73	DR8	DR17
A24	A68	B17	B45	B58	B75	DR9	DR18
A25	A69	B18	B46	B59	B76		
A26	A74	B21	B47	B60	B77		
A28	A80	B22	B48	B61	B78		
A29		B27	B49	B62	B81		

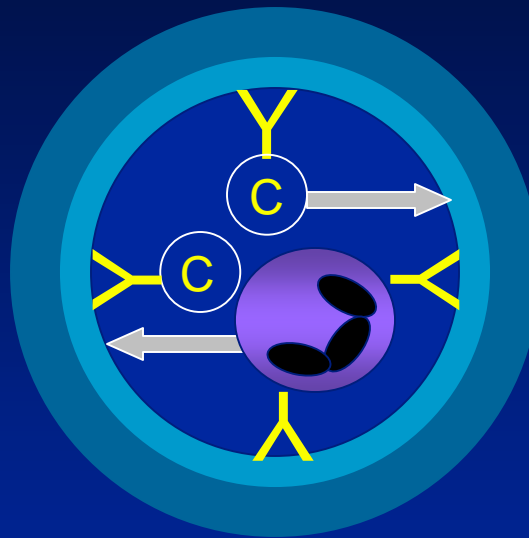
Alloantibody-Mediated Rejection

A



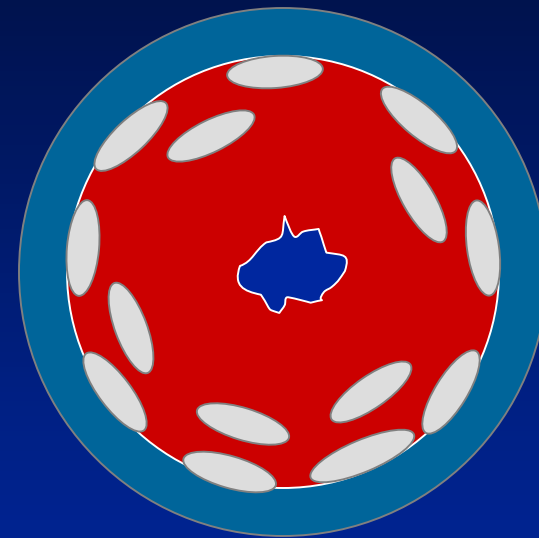
Antibody reacts with the endothelial lining of the blood vessel wall

B



Complement fixation leads to neutrophil recruitment and endothelial destruction

C



Damaged endothelium causes platelet accumulation and thrombus formation

Approaches to Alloantibody Detection and Identification

Donor-Specific Antibodies (Crossmatch)

Allows for the detection of potentially harmful antibodies directed specifically against the donor's tissues.

Panel Reactive Antibodies (PRA)

Assesses the presence, scope, and specificity of HLA antibodies against *third-party* antigens

HLA Antibody Assessments

Complement Dependent Cytotoxicity

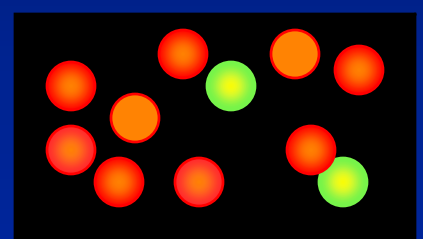
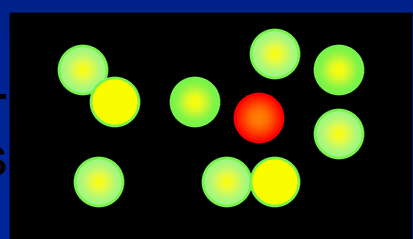
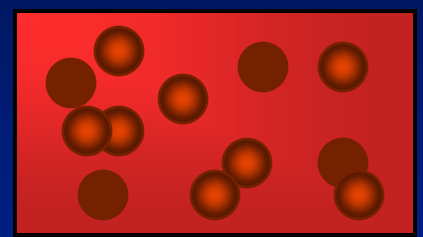
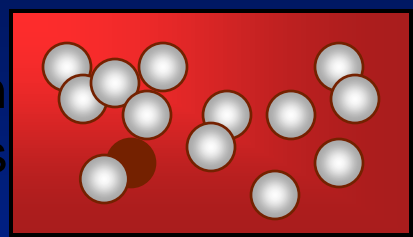
Donor Cells or Cell Panel
+
Patient's Serum
+
Complement

Exclusion dyes

Fluorochromes

Negative Reaction

Positive Reaction



Limitations of Cytotoxicity Assays

False Negatives

Due to:

- Low titer antibody
- Non-cytotoxic antibodies
- Class II antibodies

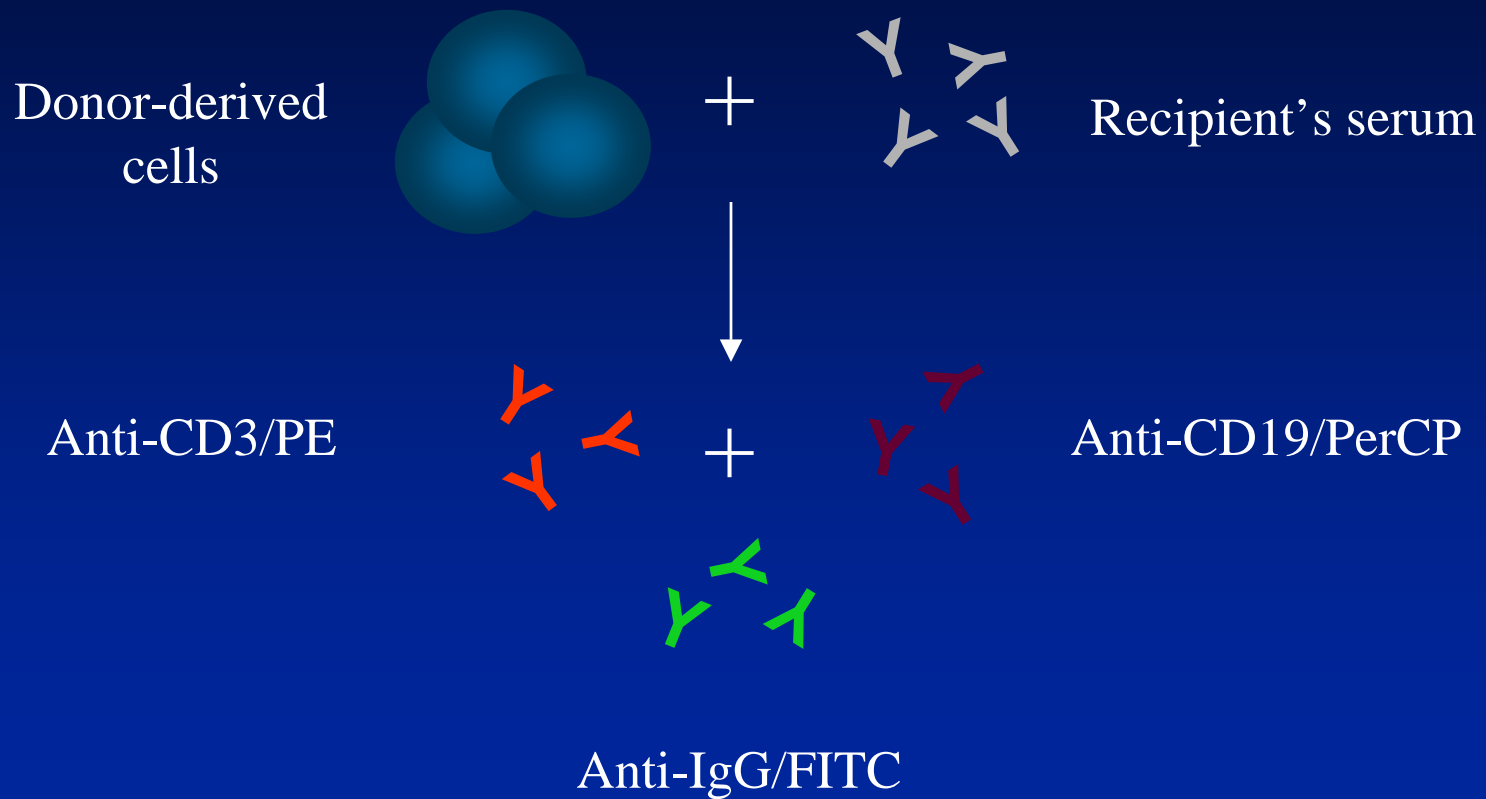
False Positives

Due to:

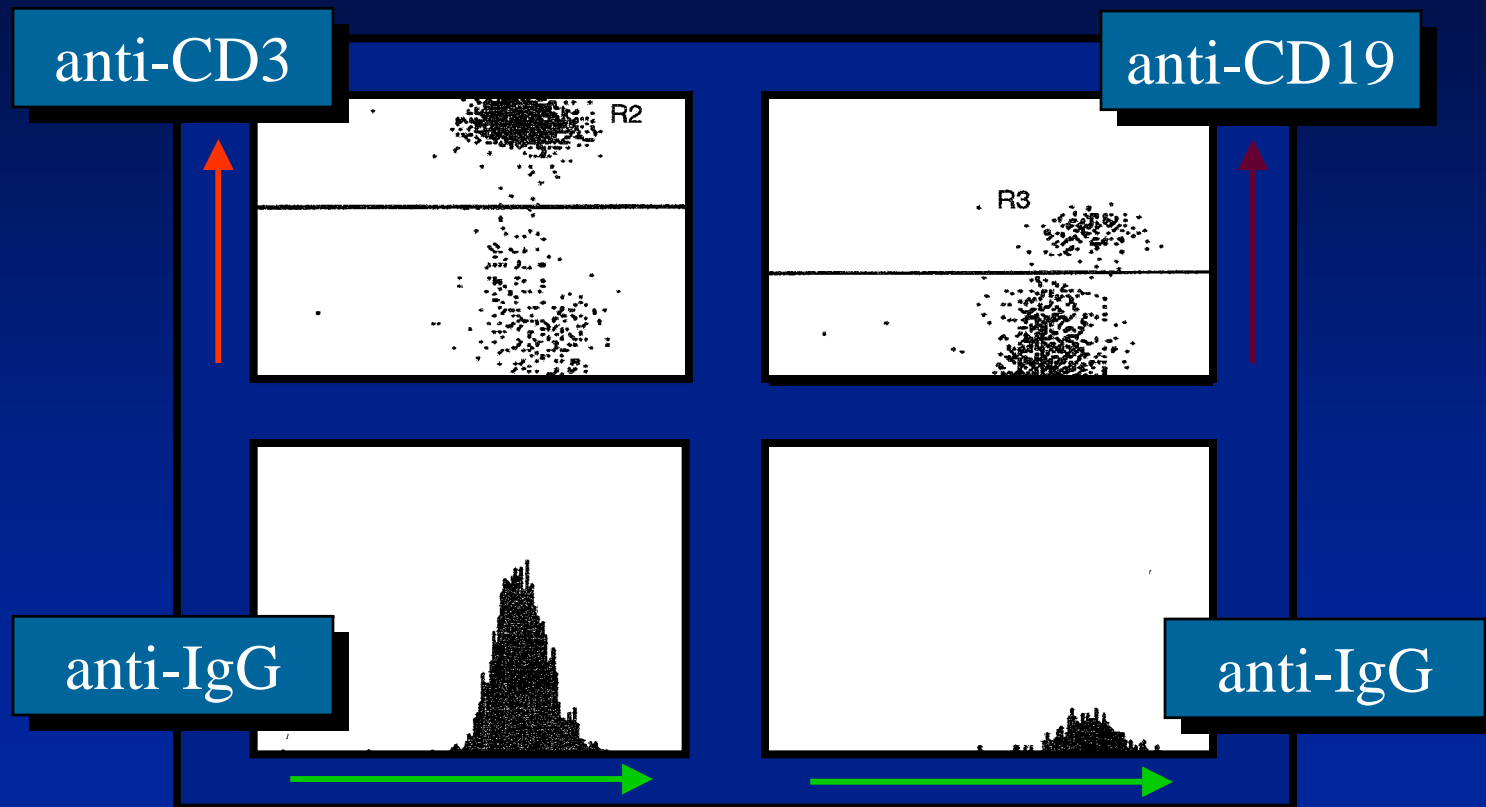
- Autoantibodies
- Irrelevant antibodies
- Immune complexes

Flow Cytometric Crossmatch

Sample Preparation



Flow Cytometric Crossmatch Data Analysis



Flow Cytometric Crossmatch

Interpretation

T cell reaction

B cell reaction

Specificity

negative

negative

none

positive

positive

HLA class I +/- II

negative

positive

HLA class II ?

positive

negative

low titer class I ?

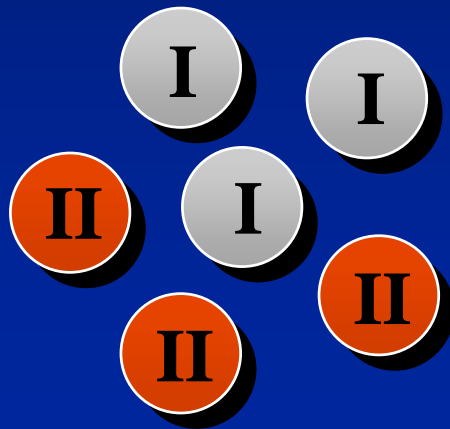
non-HLA antibody?

HLA Antibody Identification

Qualitative Fluorimetry

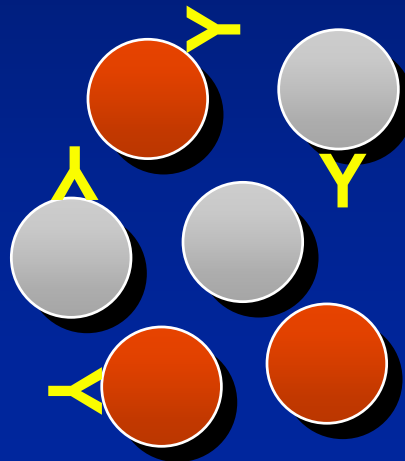
1

HLA-Coated
Latex Particles
(broad specificity)



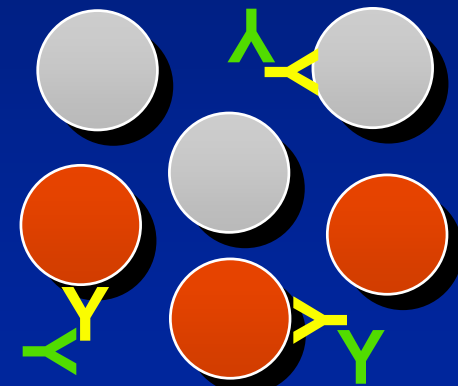
2

Patient
Serum



3

Goat Anti-
Human IgG
(with fluorochrome)

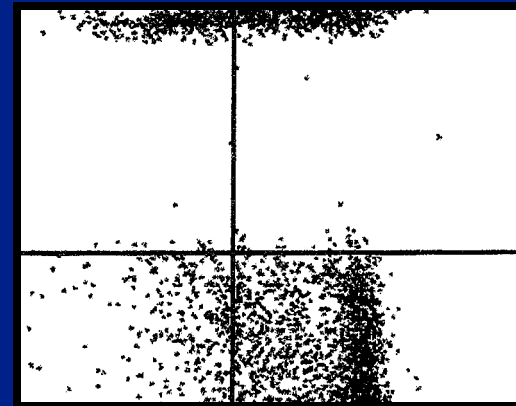
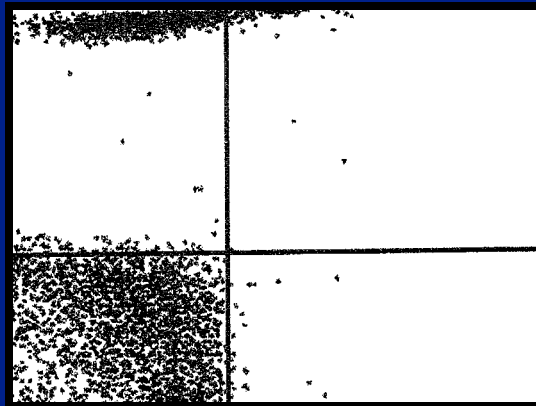


Fluorimetric Analysis

HLA Antibody Differentiation

Class II

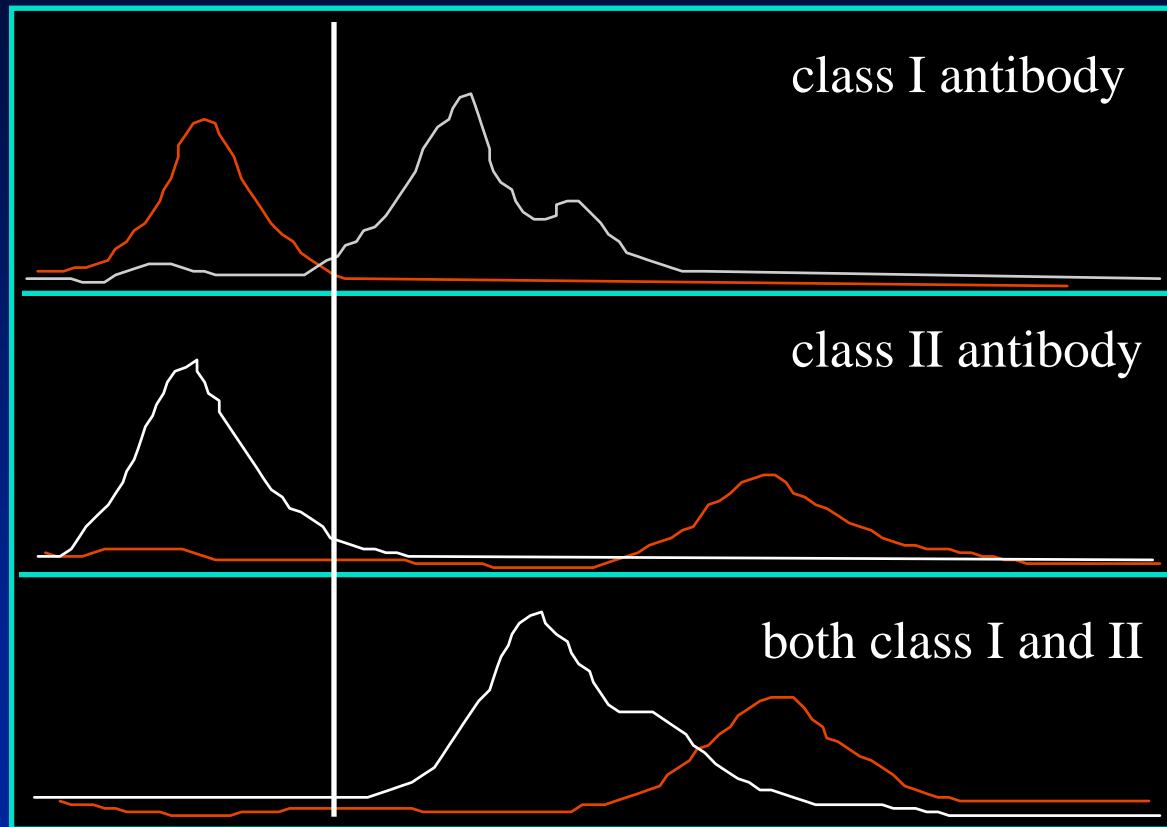
Class I



Negative

Positive

Fluorimetric Analysis



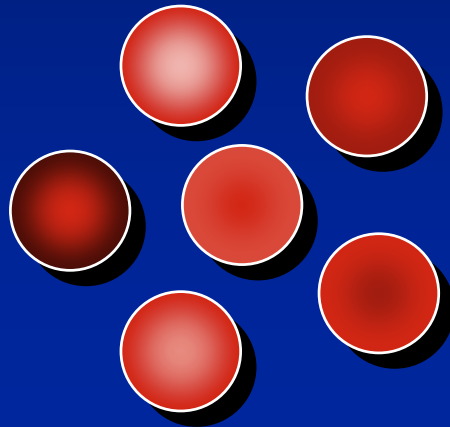
Relative Green Fluorescence

HLA Antibody Identification

Quantitative Fluorimetry

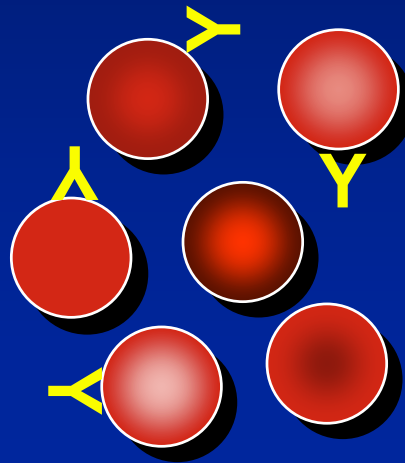
1

HLA-Coated
Latex Particles
(unique specificity)



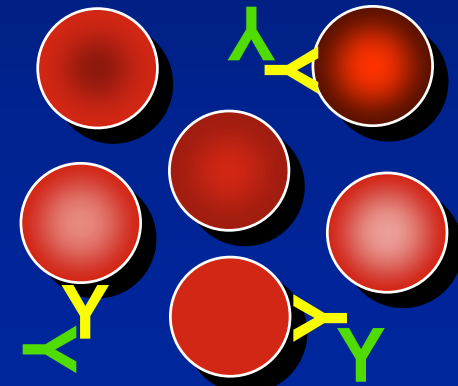
2

Patient
Serum



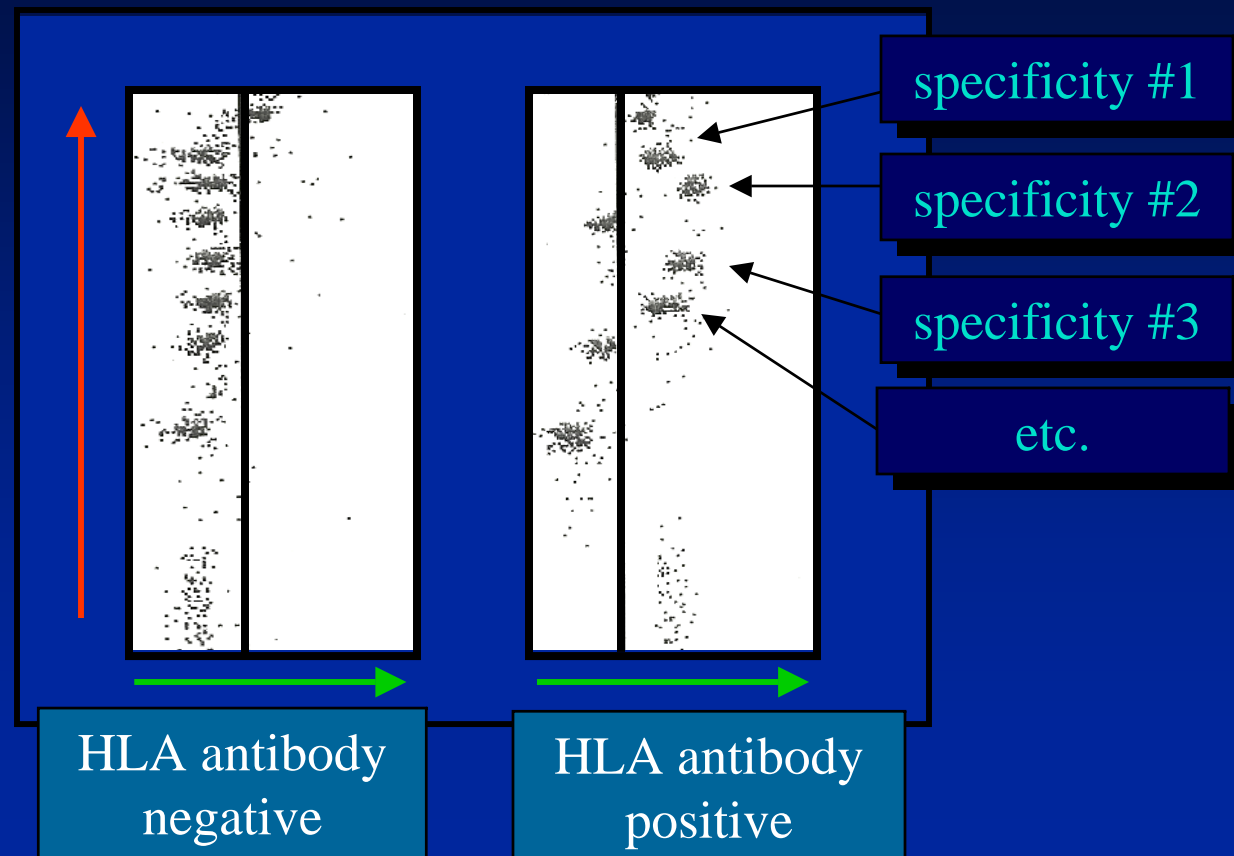
3

Goat Anti-
Human IgG
(with fluorochrome)



Fluorimetric Analysis

HLA Antibody Identification



Flow Cytometric Enhancement of Sensitivity & Specificity

- Detection of low titer HLA antibodies
- Detection of non-cytotoxic HLA antibodies
- Detection of B cell specific class II antibodies
- Identification of IgM class autoantibodies
- Elimination of reactions attributed to immune complexes
- Distinction of clinically insignificant IgG class antibodies

Flow Cytometry and Transplant Compatibility Analysis

Summary

- Sensitivity prevents unfavorable outcomes
- Specificity avoids unfair exclusions
- Antibody detection must be correlated with allo-antibody differentiation and identification for proper interpretation
- Advancements in flow cytometry may reduce the extent of compatibility analysis required for successful transplantation.