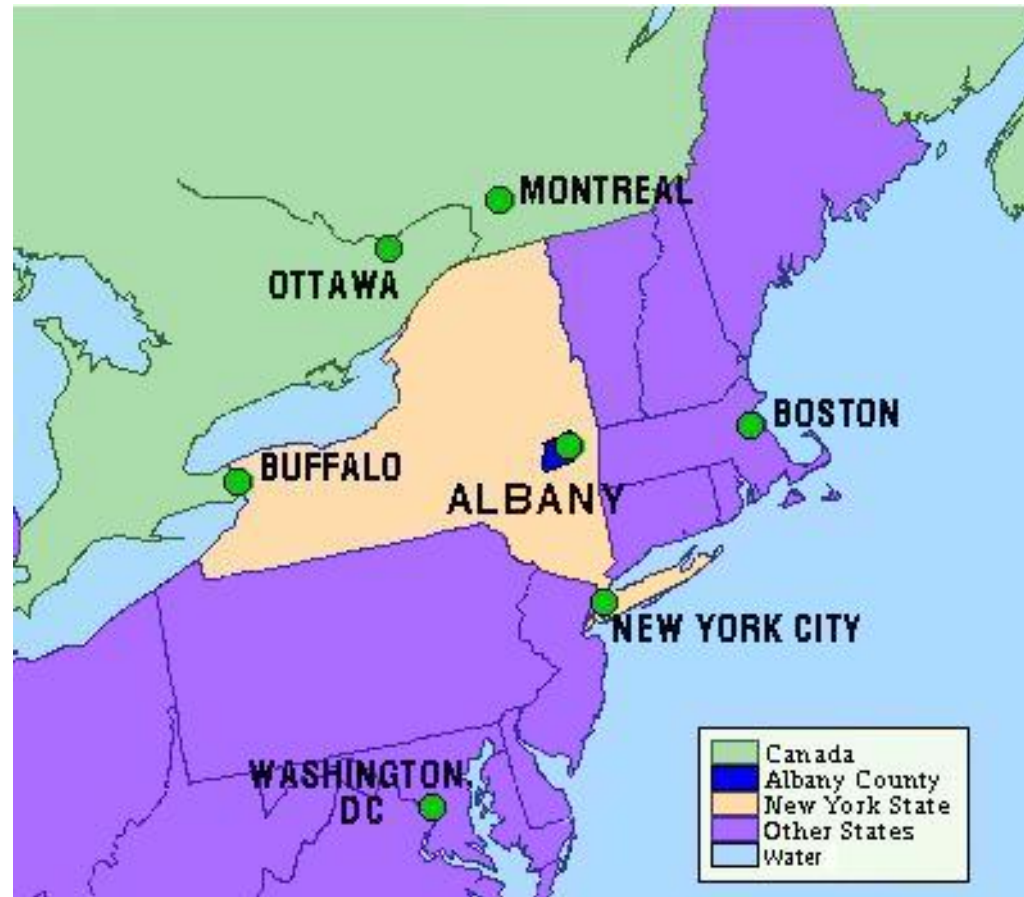


# **LUMINEX BASED ASSAYS**

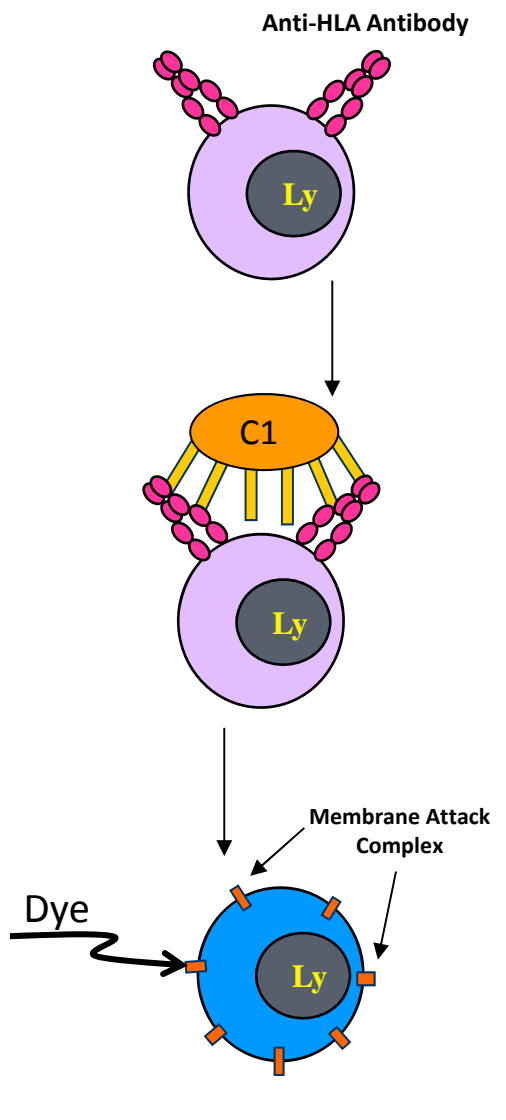
**Amy Hahn, PhD, D(ABHI)**

**Albany Medical College**

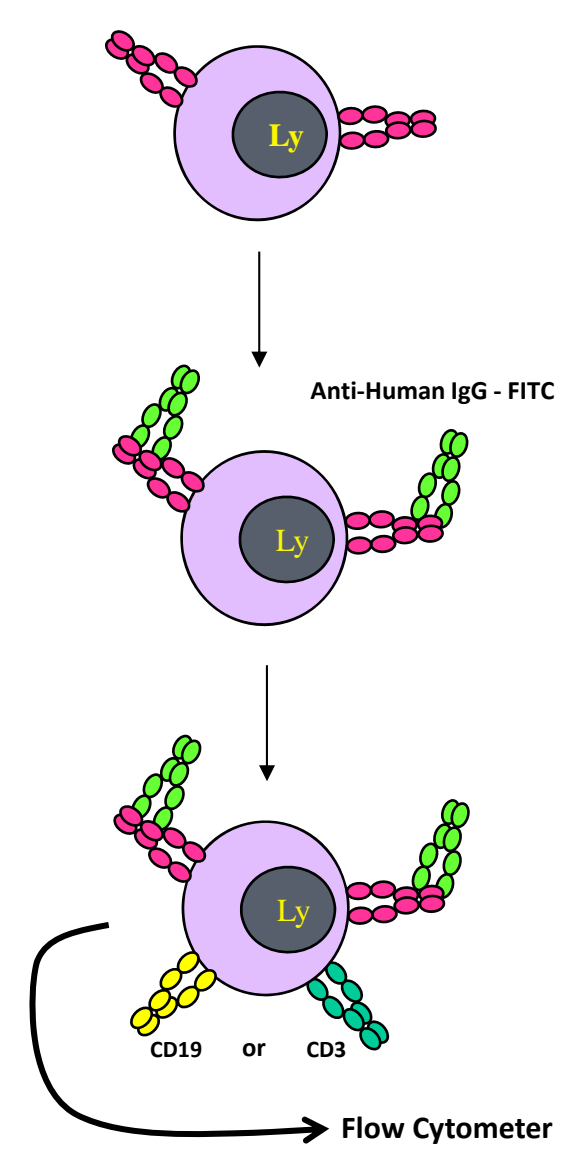
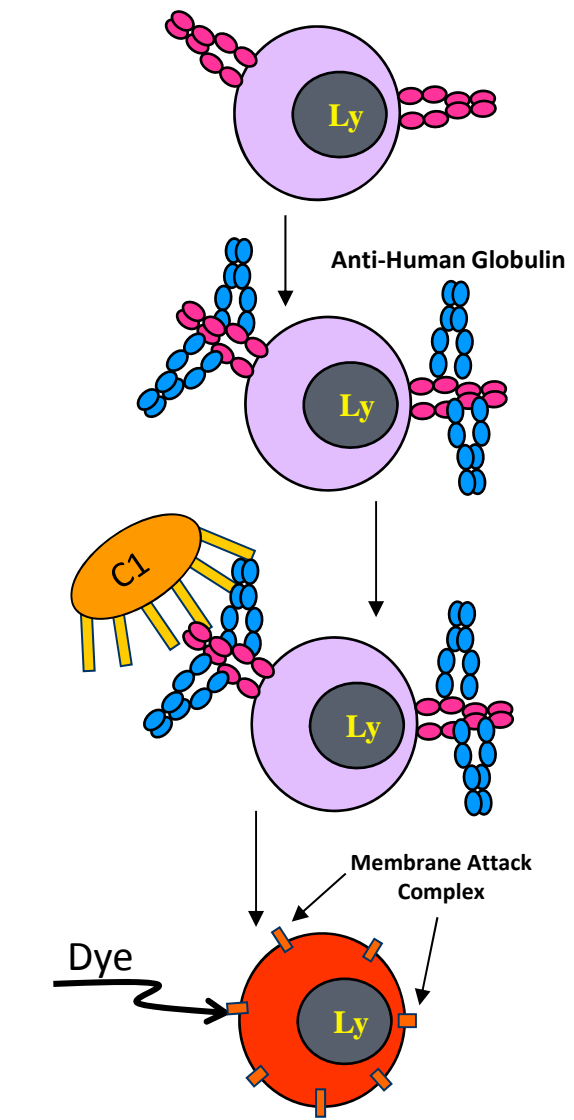
**Albany, NY**



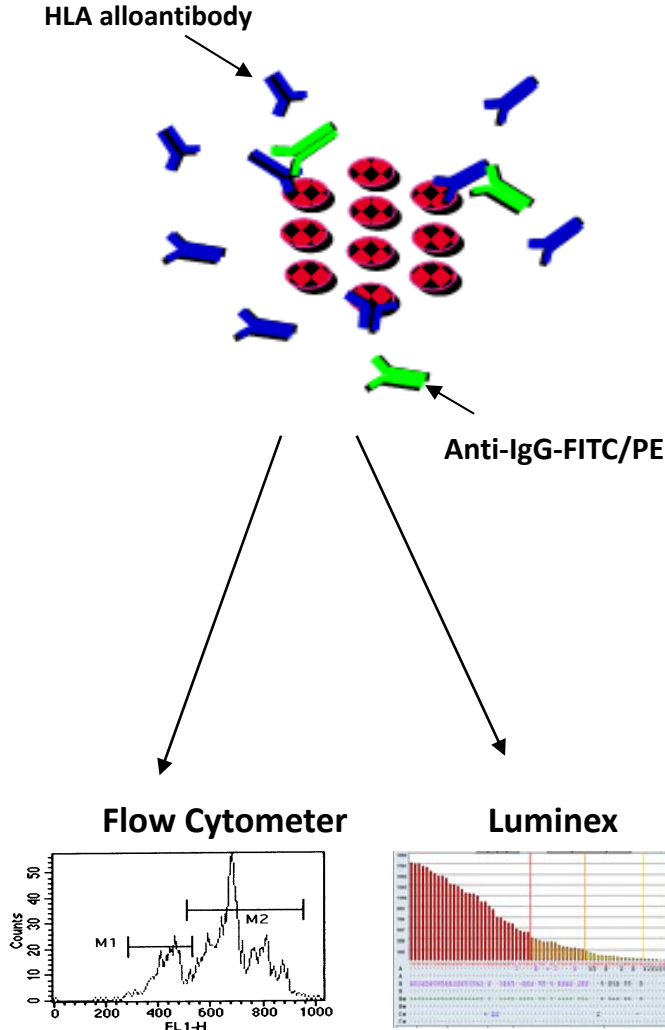
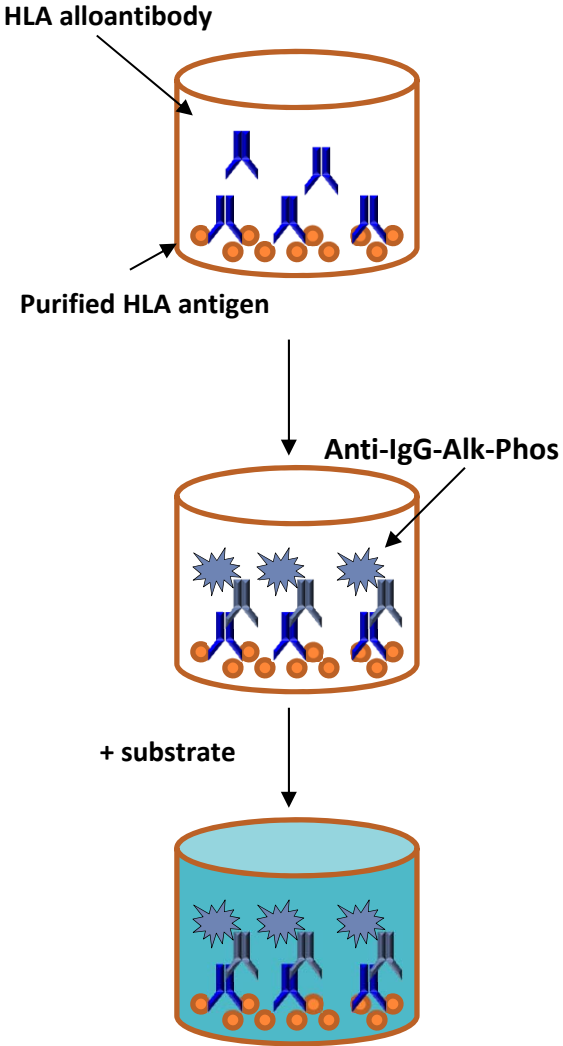
# Evolution of HLA Antibody Detection - Cellular



Gebel and Bray



# Evolution of HLA Antibody Detection – Solid Phase



# ANTIBODY IDENTIFICATION ISSUES

- cell vs. solid phase
- level of resolution
  - pooled cells vs. single cell vs. single antigen
- limitations of method
  - missing antigens in panel
  - crossreactivity
  - sensitivity
  - linkage disequilibrium
  - ability to sort out high PRA sera
  - cryptic epitopes exposed on denatured antigens
- confounding variables
  - “sticky” sera
  - interfering factors
  - autoantibody
  - antibody reduction therapies
- clinical relevance
  - relationship to crossmatch results
  - relationship to graft survival

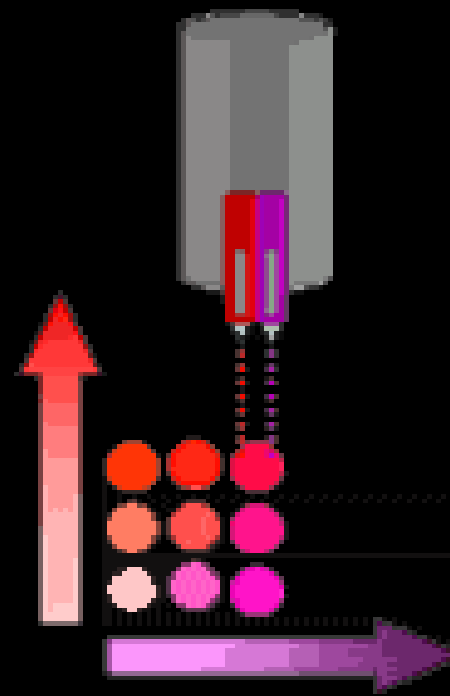


# MICROSPHERE (BEAD) ARRAY TECHNOLOGY

- “marriage” of flow cytometry and probe hybridization methods
- detected by single application flow cytometer
  - Luminex
- bead particles colored using 2 dyes
  - gradient of colors
  - coloration becomes an integral component of each bead
- molecules covalently linked to beads
  - HLA proteins (for antibody screening / identification)
  - oligonucleotides ( for molecular typing)

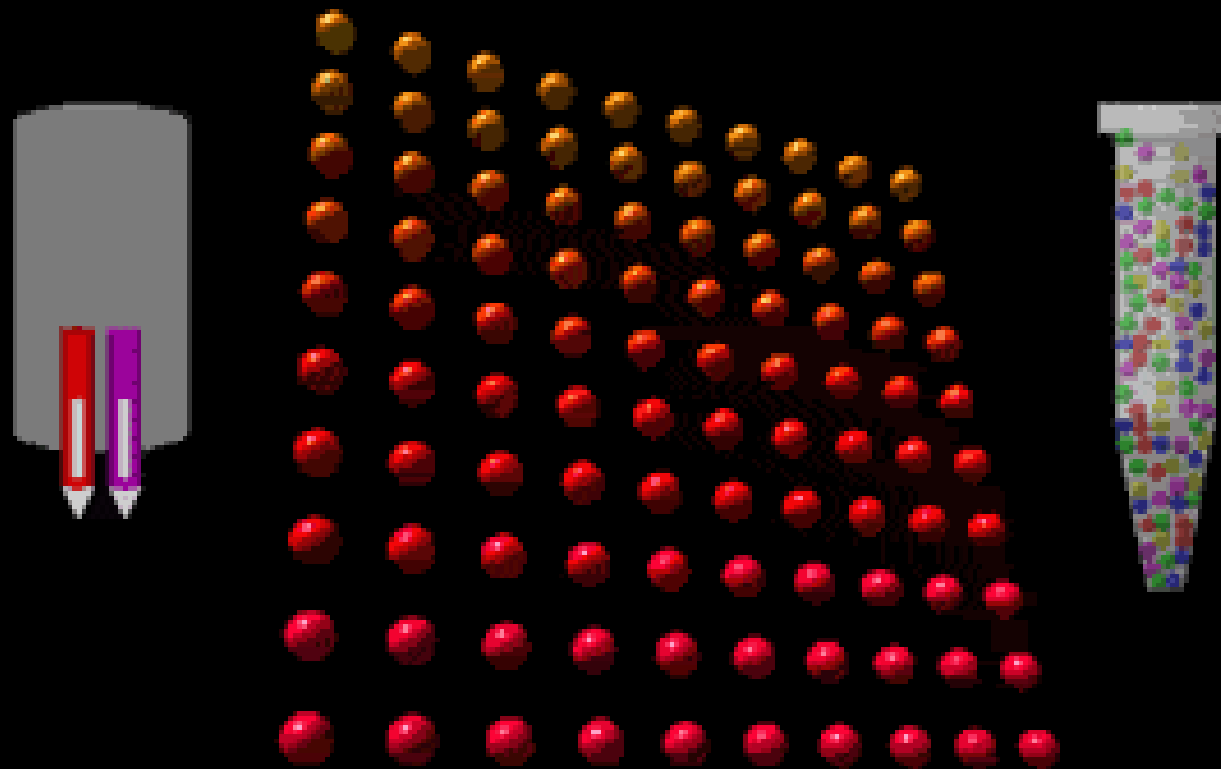


# Color-coded Microspheres



Unique microsphere sets are color-coded using a blend of different fluorescent intensities of two dyes.

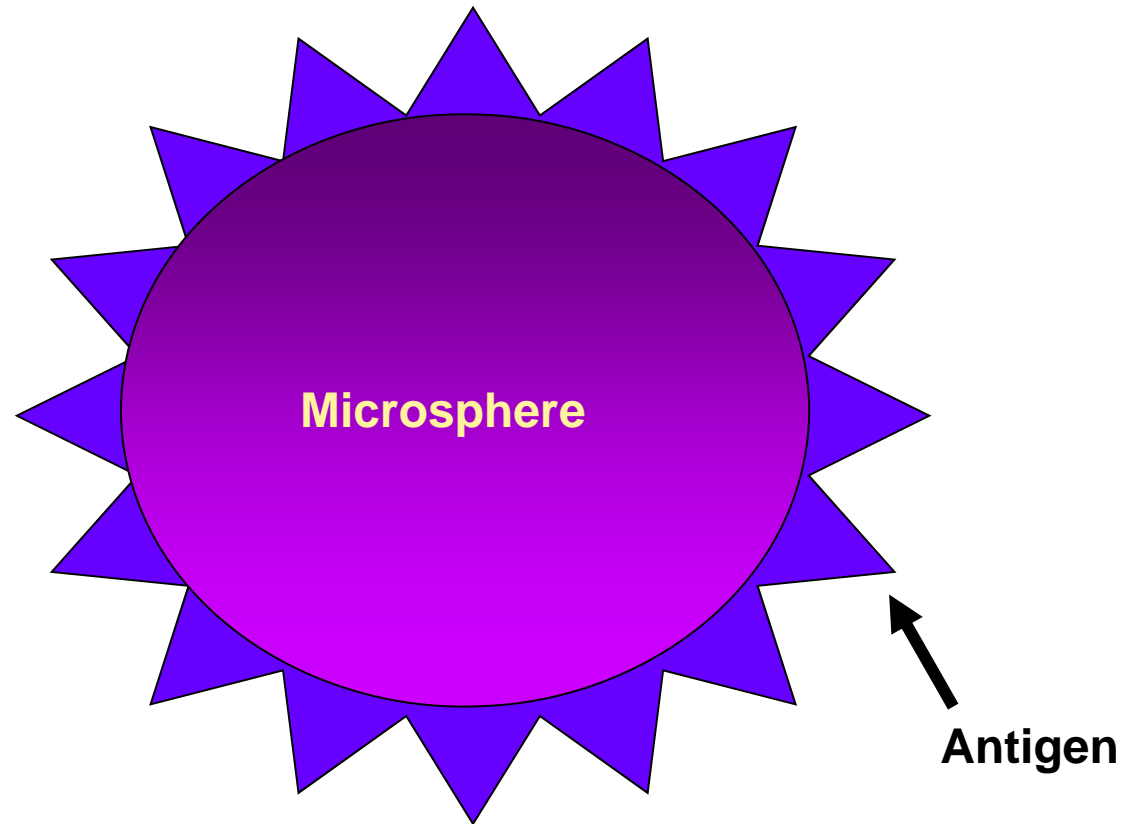
# 100 Color-codes = 100 Simultaneous Tests



Using this method, over 100 distinct  
microsphere sets can be created.

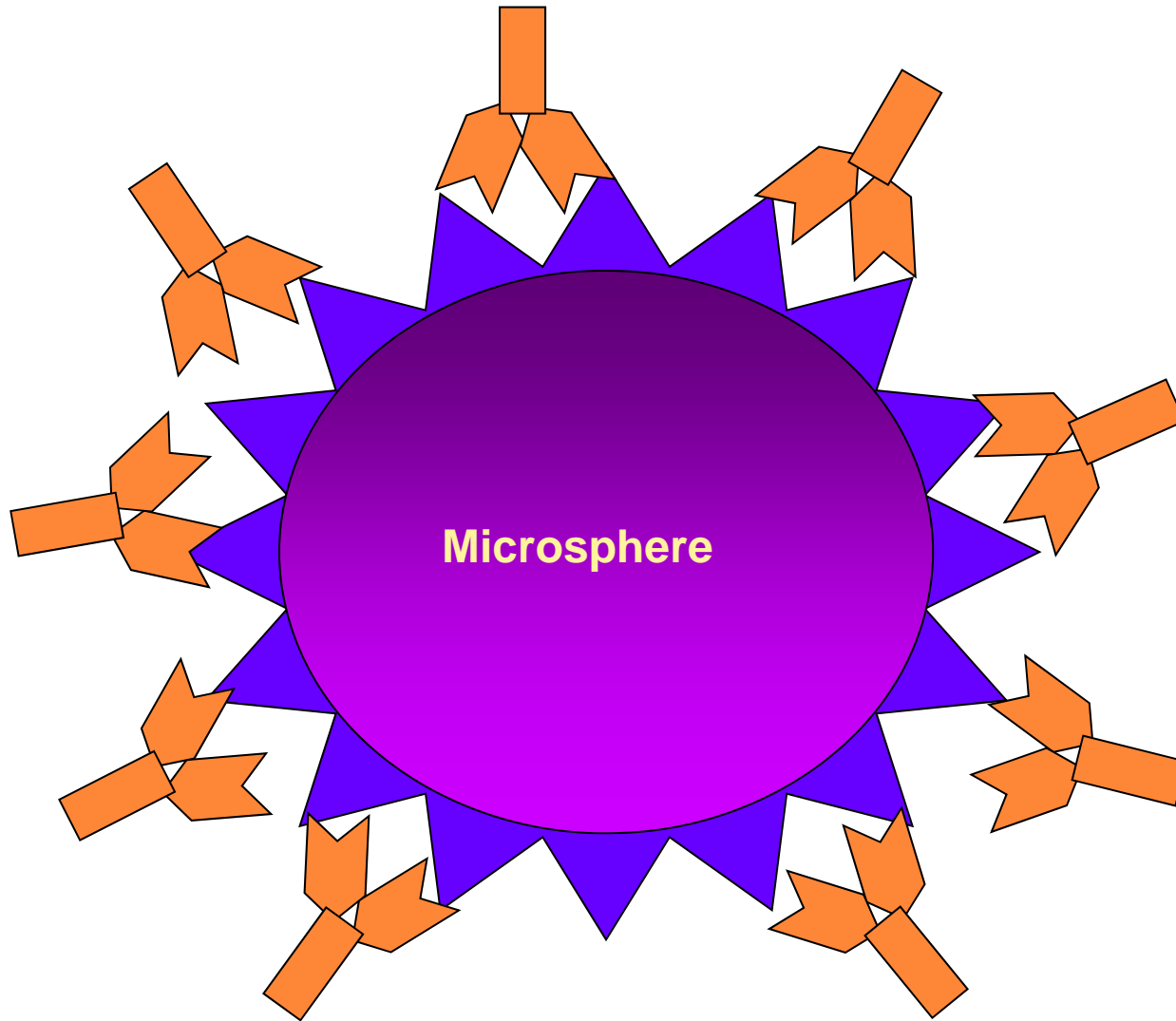


# HLA antigen is coated onto the microspheres

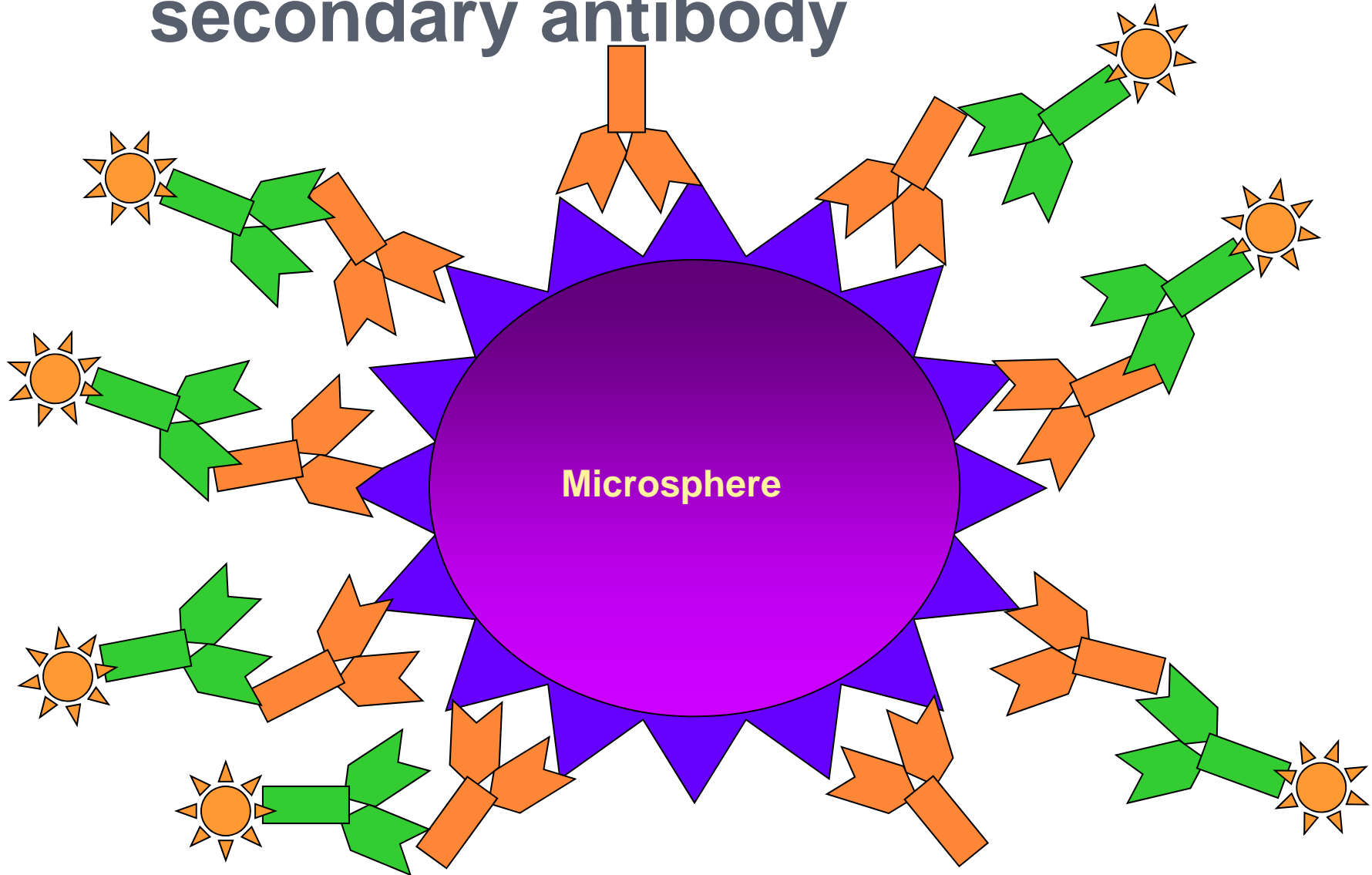


Each microsphere in the array is coated with a different single recombinant antigen or group of antigens from a cell line

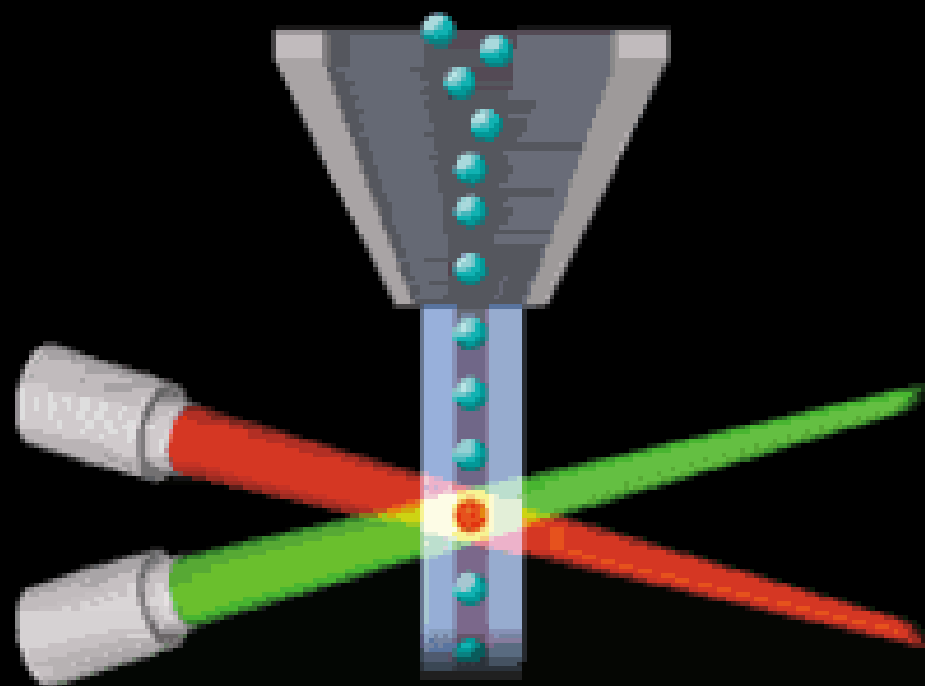
**Patient serum is incubated with  
the beads**



# Add PE conjugated anti-human Ig secondary antibody



## Microspheres in a Fluid Stream



Precision fluidics align the microspheres in single file, and pass them through the lasers one at a time.

## DATA ANALYSIS

- two parameters of information are collected per bead
  - inherent fluorescence of the bead
  - fluorescence of the reporter dye
- analysis is based upon those beads which carry the reporter dye
- software calculates values based on negative control bead and negative control serum



## MICROSPHERE ANTIBODY ANALYSIS

- antibody: yes or no?
  - mixed antigens on beads
  - class I and class II on separate beads
- PRA
  - antigens from single cell on each bead
  - class I and class II on separate beads
- antibody specificity
  - recombinant single antigen
  - each on a different bead



# BEAD CONSIDERATIONS

- High negative control bead:
  - sticky serum, anti-latex antibodies
  - adsorb with uncoated beads
- Low positive control bead:
  - interference from other factors
    - possibly IgM or immune complexes or C1
  - treat with DTT, EDTA, 56°C, hypotonic dialysis or spin column with filter
- Antibodies to cryptic epitopes:
  - acid wash beads
    - everything is denatured
  - iBeads
    - “clean beads” with minimal denatured antigen



# ARE THE ANTIBODIES CLINICALLY RELEVANT?

- most solid phase assays detect total IgG
  - not all IgG antibodies bind complement equally
- importance of crossmatching was demonstrated with a complement-dependent cytotoxicity assay

## The New England Journal of Medicine

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Volume 280

APRIL 3, 1969

Number 14

### SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION\*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

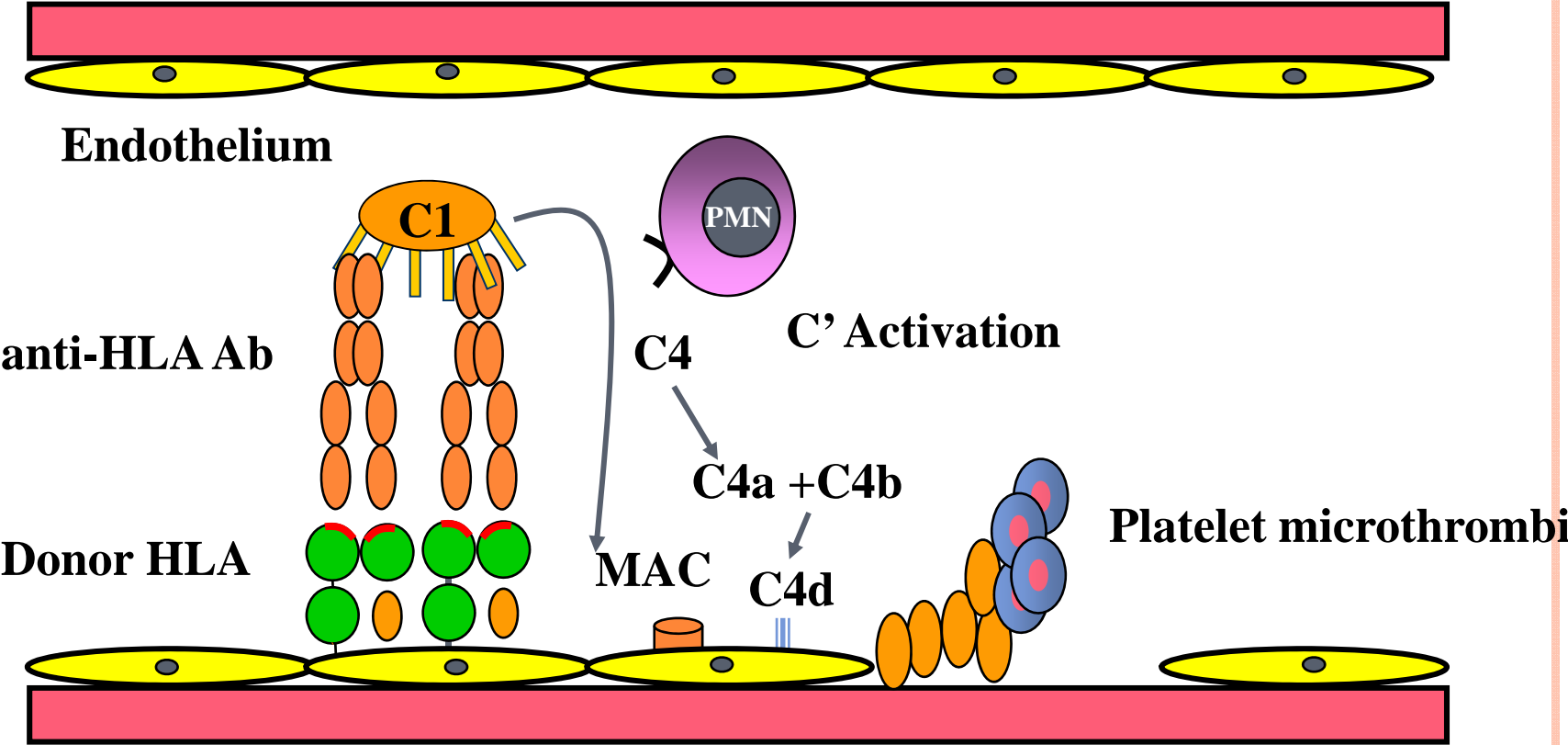
**Abstract** Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch ( $p$  less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females

and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.



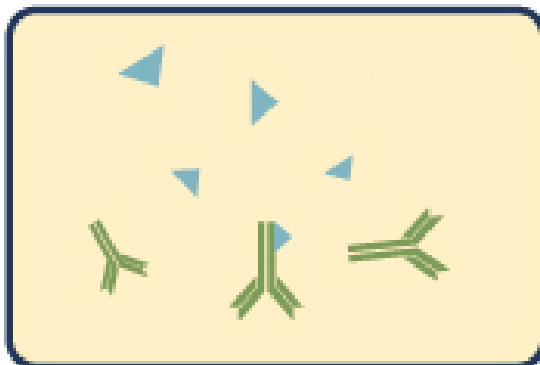


# ANTIBODY MEDIATED REJECTION

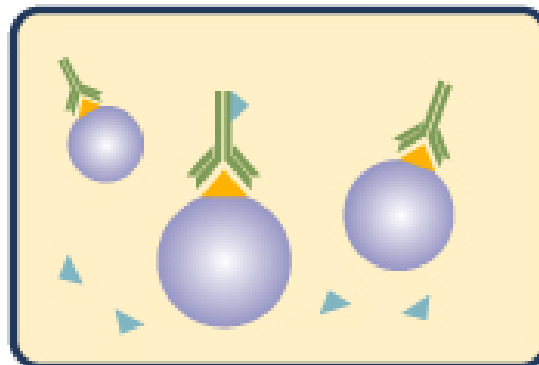


C1QSCREEN™

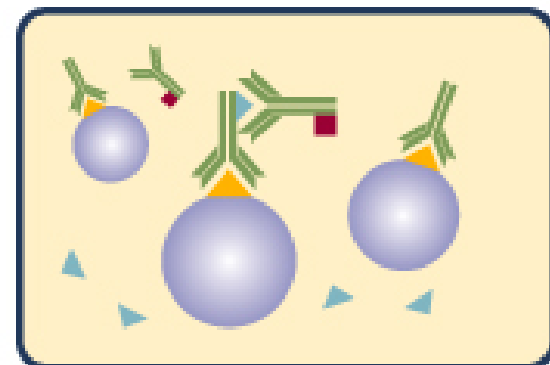
# Detection by PE Conjugated Anti-C1q



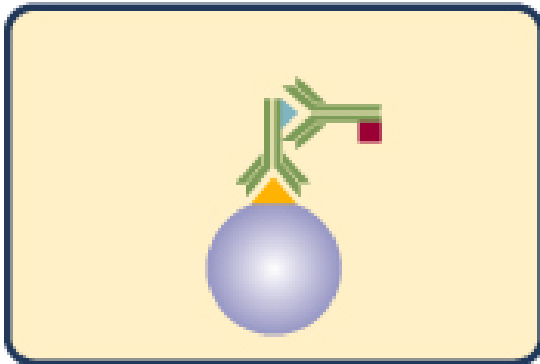
1. Add C1q to HI serum sample



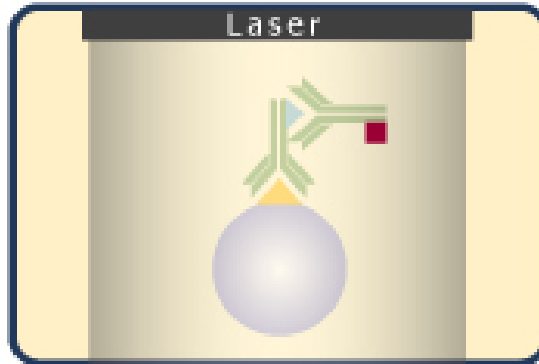
2. Add HLA antigen coated beads



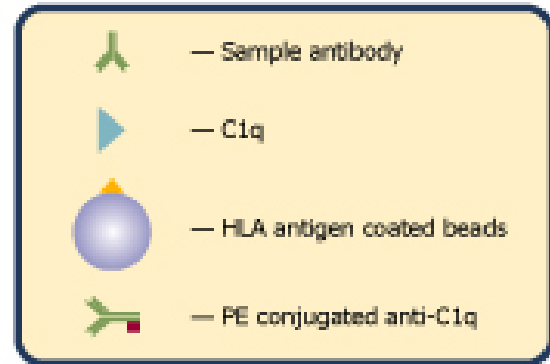
3. Add PE conjugated anti-C1q



4. Wash



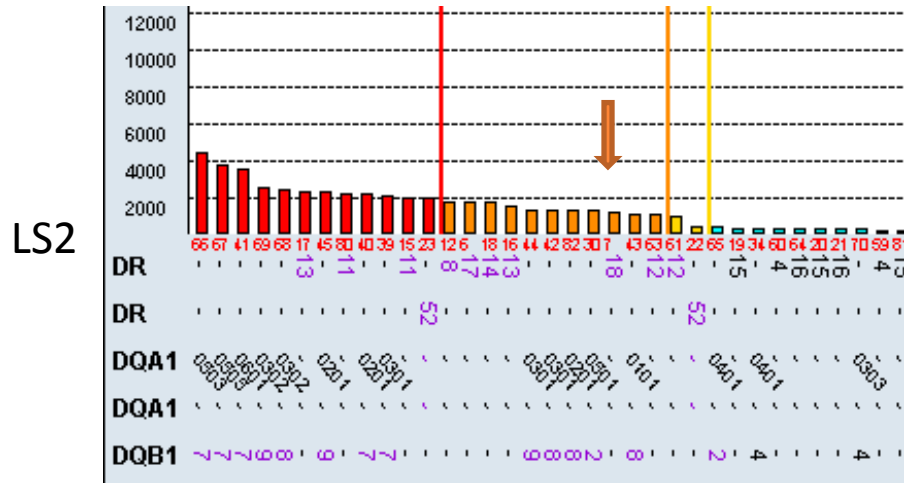
5. Read with LABScan™ 100



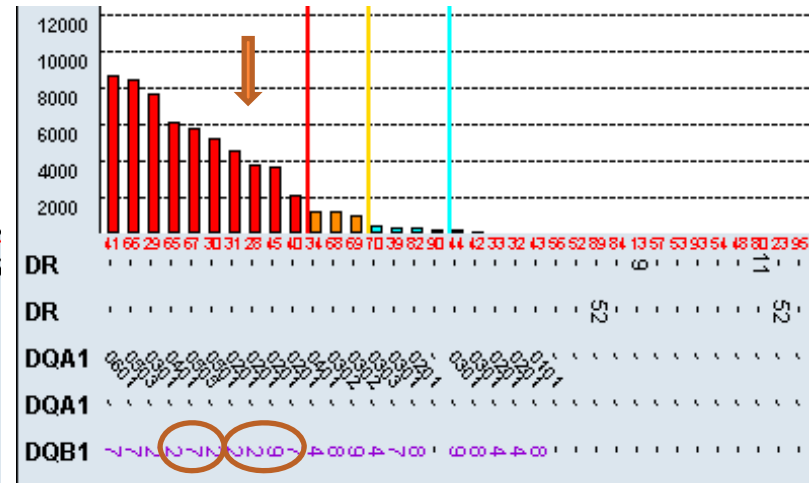
Legend

# PATIENT TRANSPLANTED WITH DQ2 MISMATCH

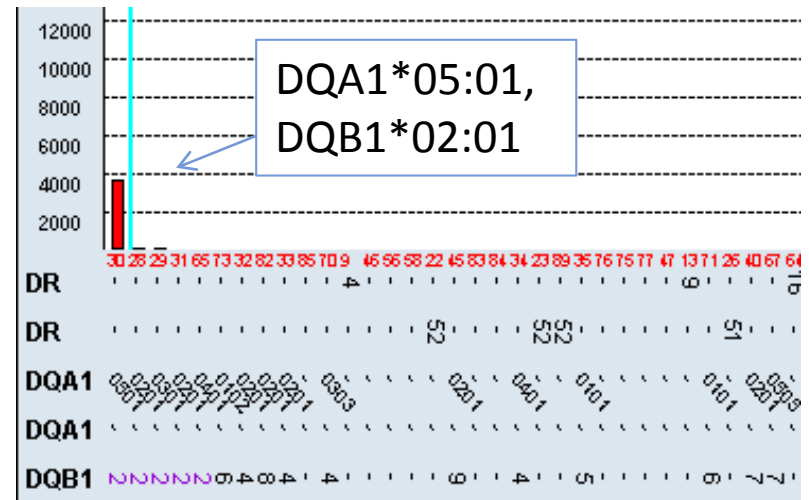
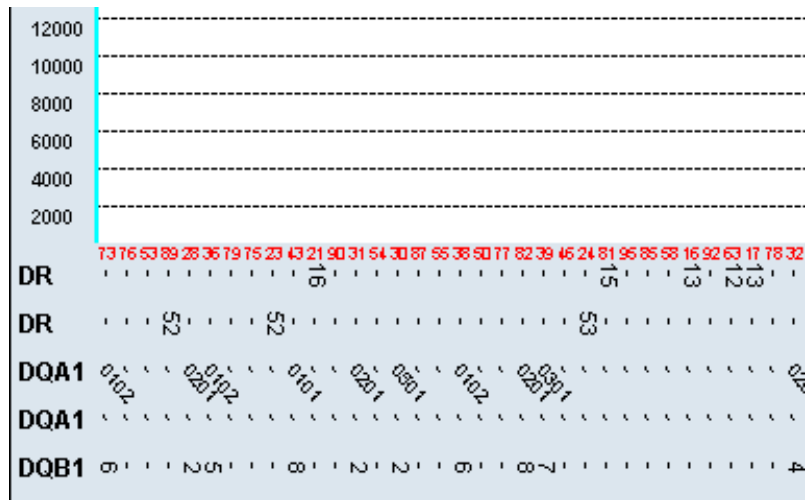
day of transplant



853 days post



LS2+  
C1q



LS1 was negative

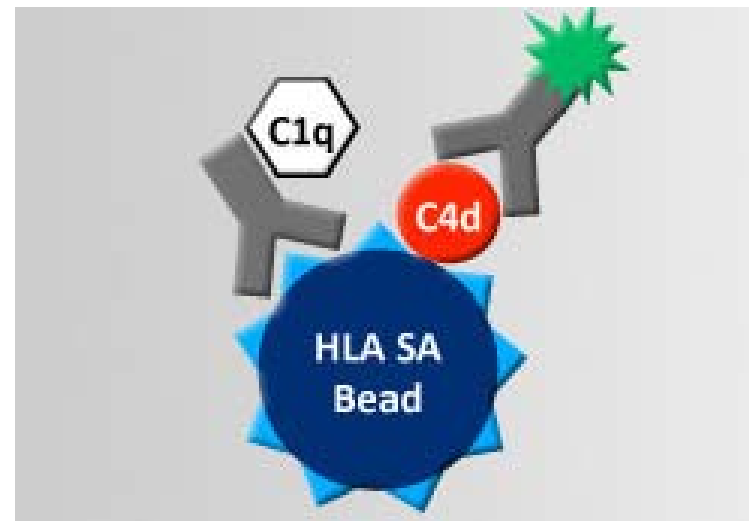
## POSSIBLE C1Q ISSUES

- antibody concentration effect
  - need Fc domains in close proximity to allow C1q binding
  - add AHG to provide additional Fc domains
    - Fuller et al, ASHI 2011 abstract 25-P
- interference by native C1
  - treat serum with DTT
    - wash well before adding C1q reagent
      - Spanjer et al, ASHI 2012 late breaking abstract
  - dilute or heat treat
    - Lunz et al, ASHI 2012 abstract 35-OR



## C4D LUMINEX ASSAY

- incubate SAB with patient serum
- add normal unsensitized male serum and incubate
  - source of human complement
- wash and incubate with Dylight-549-labeled anti-human C4d polyclonal antibody
- wash and analyze



Vienna Transplant and Complement Laboratory

## ACCURACY IN ANTIBODY DETECTION IS CRITICAL

- allograft survival
- virtual crossmatching
- desensitization
- diagnosis of rejection





obrigado

Dank U

Merci

mahalo

Köszi

спасибо

Grazie

Thank  
you

mauruuru

Takk

Gracias

Dziękuję

Děkuju

danke

Kiitos