DONOR SPECIFIC ANTIBODIES [DSA]

DR VILESH VALSALAN

Consultant Nephrologist and Transplant Physician

ACADEMIC CORDINATOR

EXTRACORPOREAL NEPHROLOGY GROUP [ECNG]

INTRODUCTION

- Donor-specific antibodies [DSA] - biomarker predicting antibody-mediated rejection.[ABMR].
- Preformed DSA in sensitized patients can trigger hyperacute rejection, accelerated acute rejection and early acute ABMR.
- *De novo* DSA are associated with late acute ABMR, chronic ABMR and transplant glomerulopathy.
- "Benign" DSAs that may not be clinically relevant, because they are not associated with ABMR or graft failure.

SENSITIZATION

- Presence of DSA in recipient's serum to HLA antigens from the donor pool.
- Panel Reactive Antibody test [PRA] test is a marker of sensitization.
- PRA <20 % low risk and >80% is high risk for ABMR.
- Pregnancy , blood transfusion and previous transplant triggers sensitization.

PATHOGENESIS

- Donor antigen-presenting cells include macrophages, dendritic cells, and B cells.

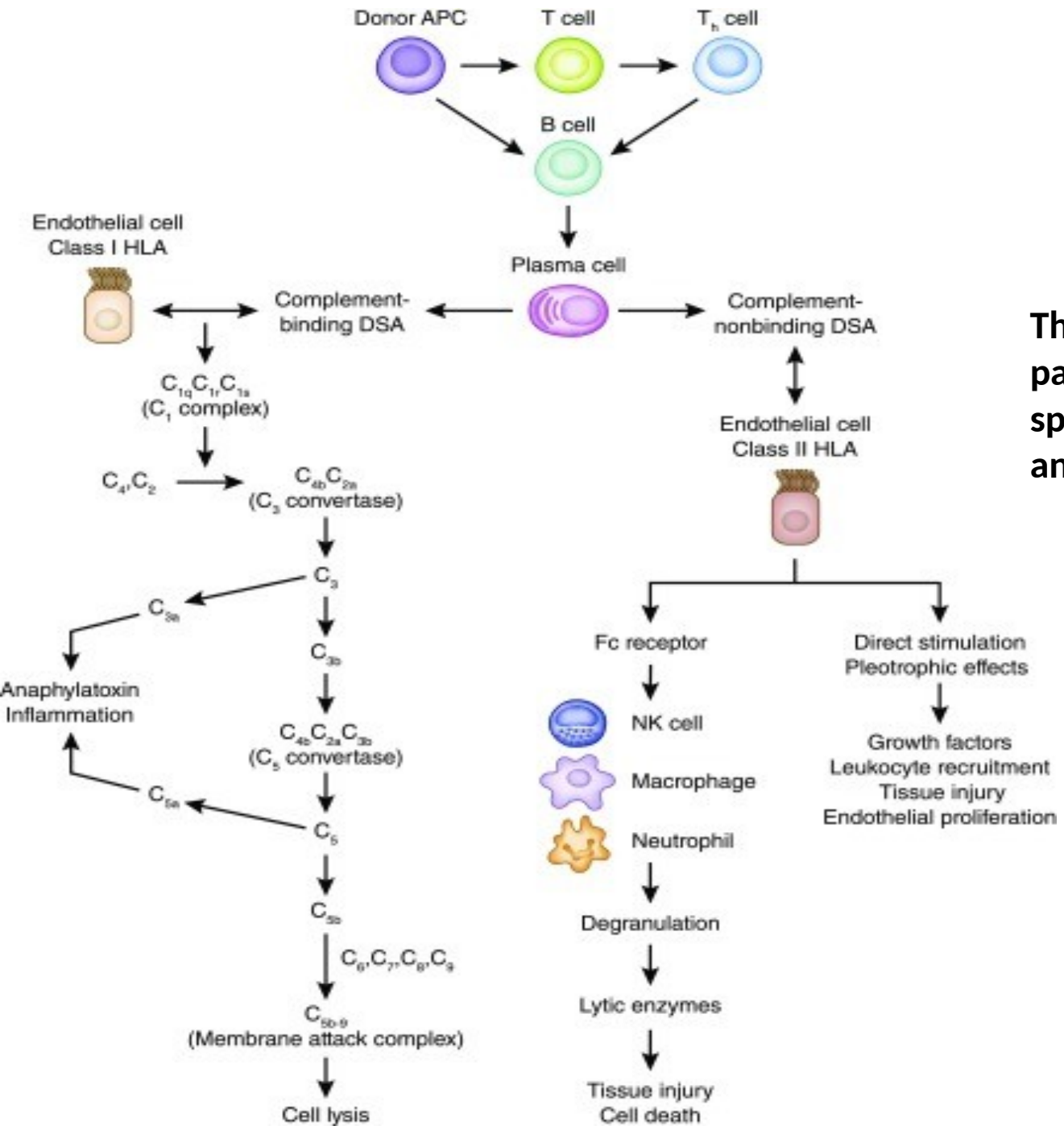
- Complement binding DSAs target the class 1 HLA on endothelial cells, activate the classic complement cascade, and deliver complement-dependent cytotoxicity in ABMR.

- Complement nonbinding DSAs recruit innate immune cells (NK cells, macrophages, and neutrophils) through Fc receptors and lead to antibody-dependent cellular toxicity.

PATHOGENESIS

- In addition, complement nonbinding DSAs have direct stimulation and pleotropic effects that cause tissue injury, cellular recruitment, and endothelial proliferation.

- The latter two mechanisms play an important role in ABMR with negative C4d deposit in peritubular capillaries as well as chronic ABMR, transplant glomerulopathy, and vasculopathy.



The three proposed pathogeneses of donor-specific antibodies (DSAs) in antibody-mediated rejection.

Comparison of the dominant characteristics of classes 1 and 2 DSA s

	Class 1 Donor-Specific Antibodies	Class 2 Donor-Specific Antibodies
HLA		
Antigens	A, B, and C	DR, DQ, and DP
Epitopes location	α-chain	α- and *β*-chains
Expression	All nucleated cells	Antigen-presenting cells
Preformed donor-specific antibodies		
Important	Very	Less
Positive crossmatch	T cells	B cells
Transplant decision	No transplant	Permissible
***De novo* donor-specific antibodies**		
Detection	Sooner	Later
IgG subclasses	IgG1, IgG3	IgG2, IgG4
Complement binding	Strong	Weak/no
Frequency	Fewer	Common, especially DQ
Antibody-mediated rejection		
Phenotypes	Acute	Chronic, subclinical
Presentation	Early	Later
Graft dysfunction	Rapidly	Slowly
C4d deposit	Positive	Negative
Treatment	More responsive	Less responsive
Graft loss	Early	Later

DSA CLASS and SPECIFICITY

- Preformed DSAs in sensitized patients can be class 1, class 2, or both.
- Positive T cell crossmatch secondary to cytotoxic IgG antibody, which is usually complement binding IgG1 or IgG3 subclass – not to proceed with transplant.
- The majority of *de novo* DSAs are class 2 antibodies, especially DQ.
- Class 1 *de novo* DSAs are usually detected sooner after transplant and more likely IgG1 and IgG3 subclasses. They are associated with acute ABMR and early graft loss.
- Class 2 *de novo* DSAs appear later – non complement binding IgG2 or IgG4 subclass , associated with chronic ABMR and transplant glomerulopathy.
- Eliminate class 2 DSA, especially the DQ, may not be successful and it can put patients at great risk of excessive immunosuppression without much benefit.
- C1q binding DSAs are associated with significantly higher risk of antibody-mediated rejection, severe tissue injury and graft loss.

C4d

- C4d is a degradation product of the classic complement pathway.
- C4d binds covalently to the endothelial basement membrane, thereby avoiding removal during tissue processing.

- Positive C4d deposit in peritubular capillaries serves as an immunologic footprint of ABMR.
- It is in a linear pattern and best shown by immunofluorescence in frozen tissue section.

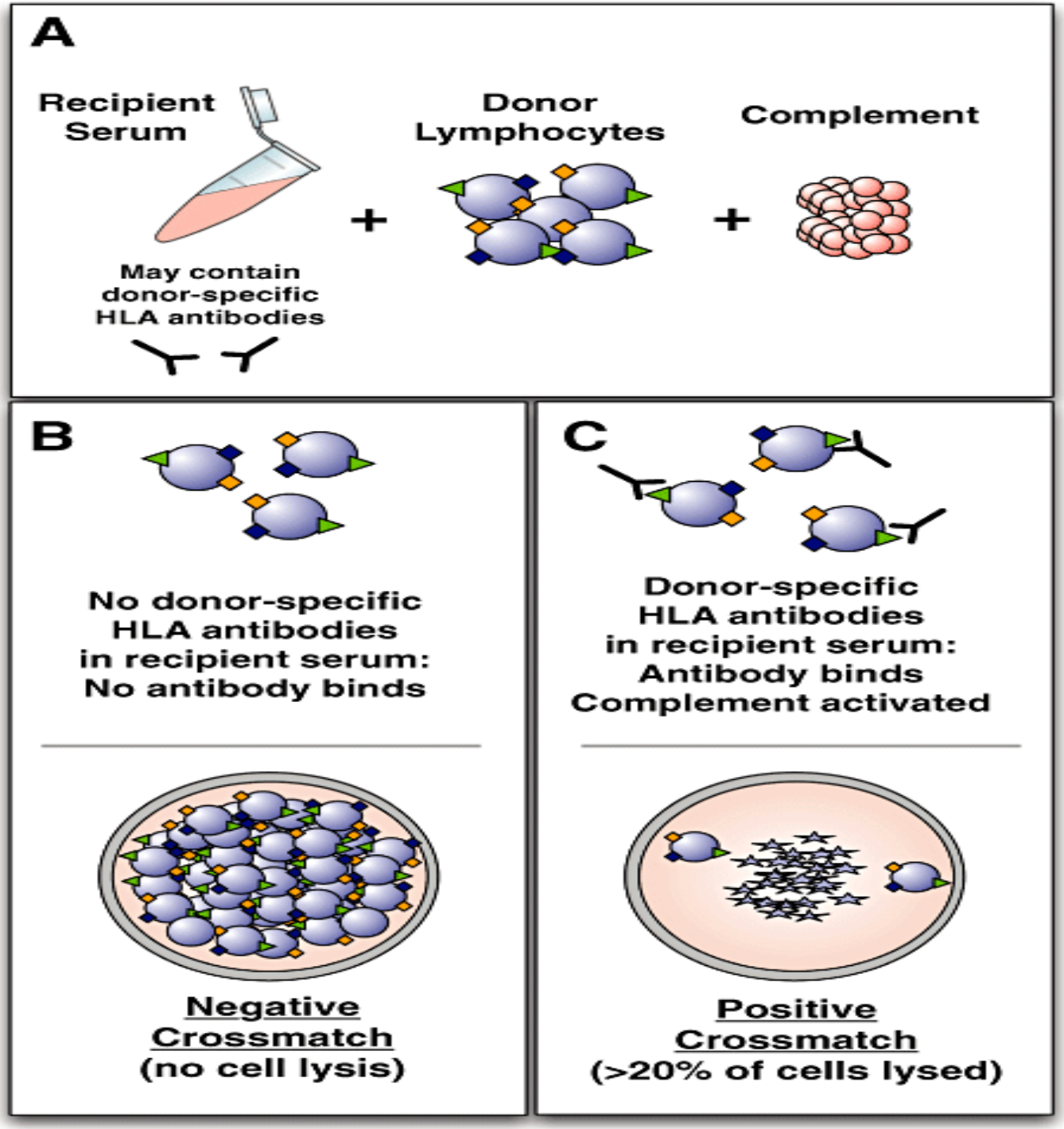
- Positive DSA but negative C4d staining :- 1. technique error (false negative) 2. non complement-activating DSA .
- Positive C4d deposit without DSA against HLA :- ABMR caused by non-HLA antibodies.

De Novo DSA

- Risk factors that develop *de novo* DSA :-

1. female sex of the recipient,
2. young age of the recipient,
3. viral infection (especially cytomegalovirus and Epstein-Barr virus),
4. class II HLA mismatching,
5. prior cellular rejection,
6. sensitizing events (blood transfusion, retransplantation, pregnancy, *etc*.) and
7. non-adherence to immunosuppressant medication.
8. Nephrectomy is considered as a factor that facilitates production of DSA.

- *De novo* DSA post-transplant has been reported to be associated with AMR, increased risk of graft loss and poor transplant outcomes.

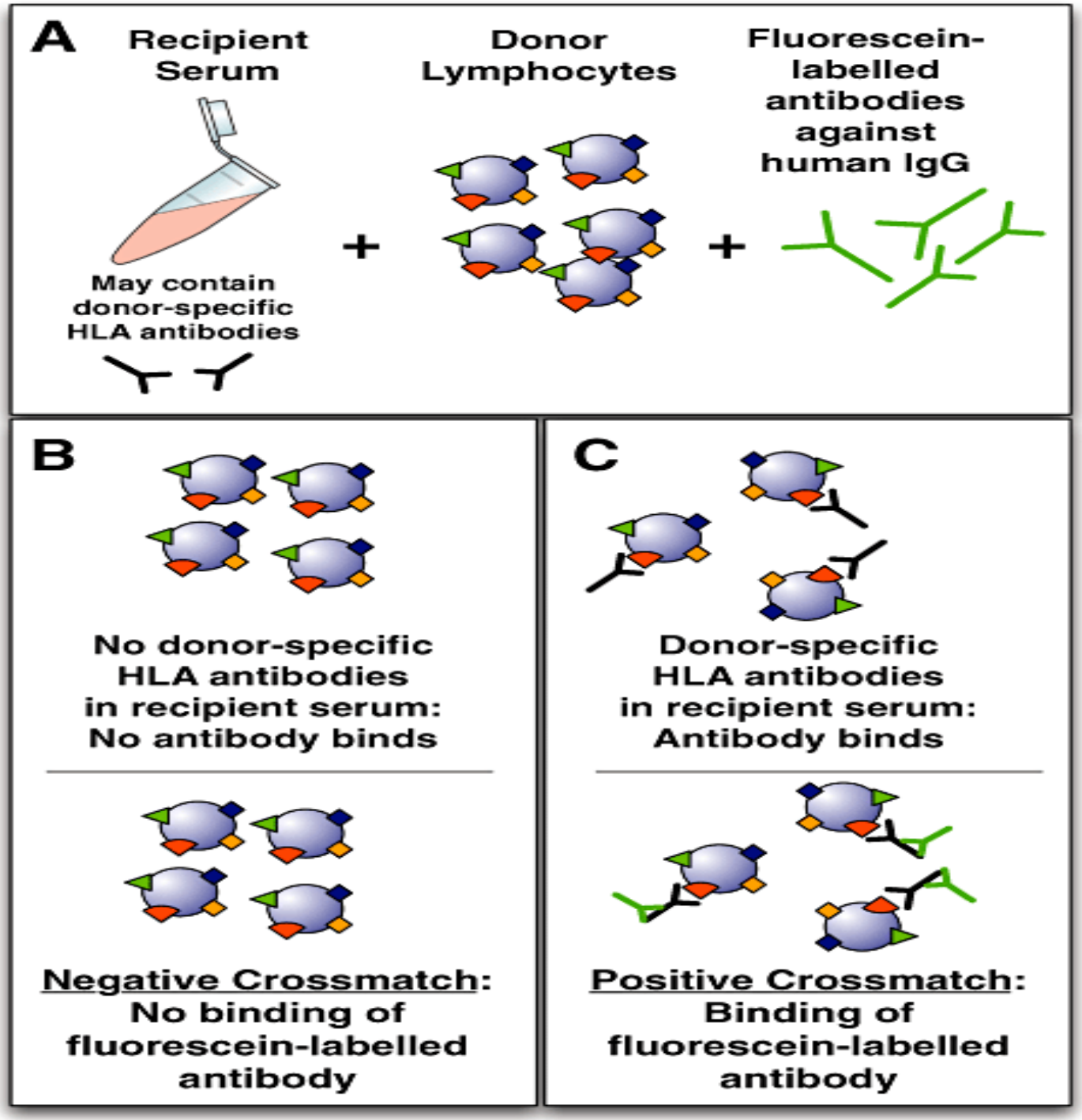


CDC CROSS MATCH

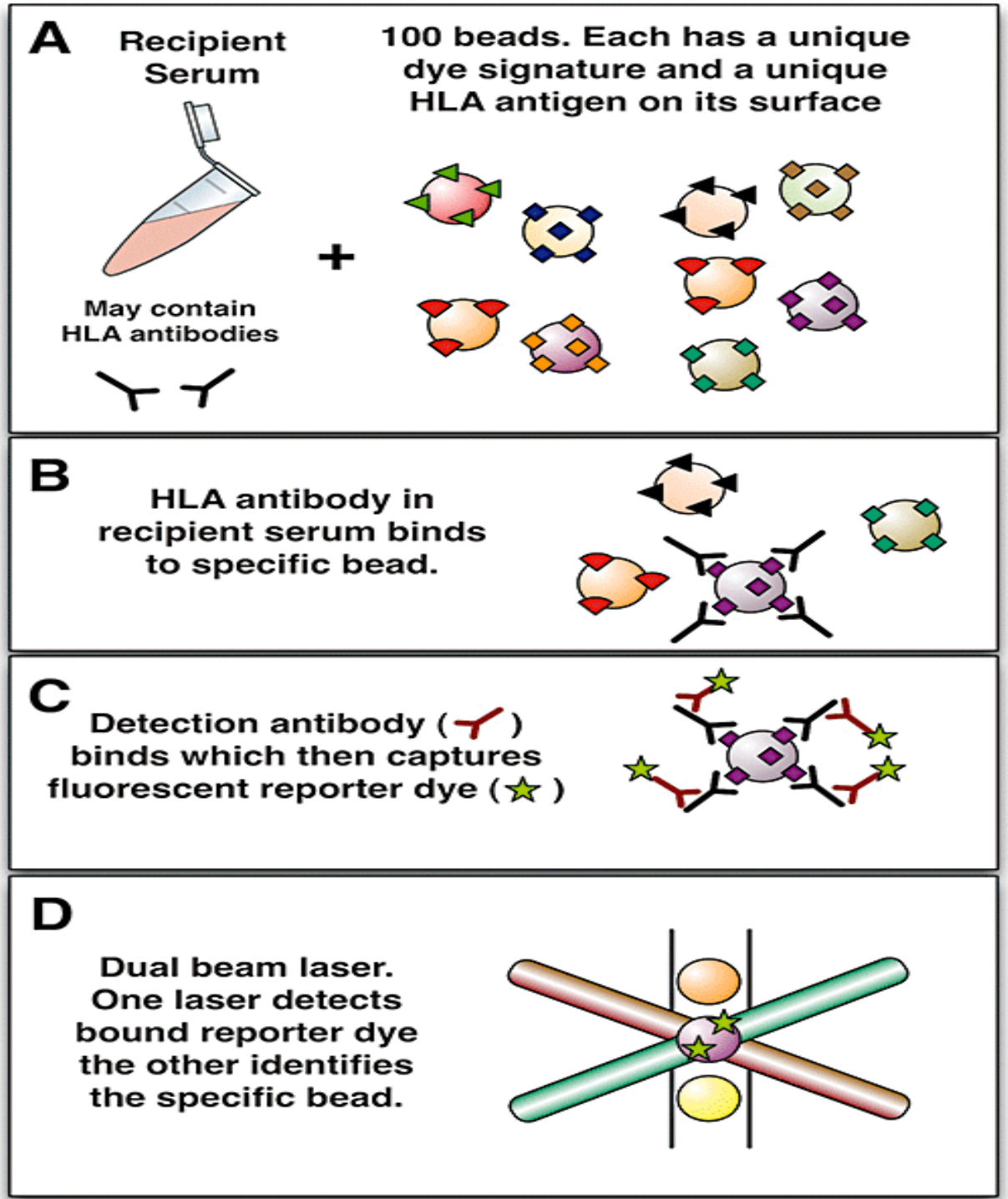
Interpretation of Crossmatch result

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

T-Cell XM	B-Cell XM	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.



FLOW CROSS MATCH



Virtual crossmatch.

MFI –MEAN FLUORESCENCE INTENSITY

1. MFI is a measure of the fluorescence emitted by a bead, indicating the amount of antibody bound to it.
2. Don't necessarily represent antibody strength or predict clinical outcomes.

MFI Range	Interpretation
< 1,000	Negative or very low; usually considered insignificant
1,000 – 3,000	Low-level antibodies; may warrant monitoring
3,000 – 5,000	Moderate antibodies; potential clinical impact
5,000 – 10,000	High-level antibodies; increased risk of rejection
> 10,000	Very strong antibodies; often contraindication for transplant or requires desensitization