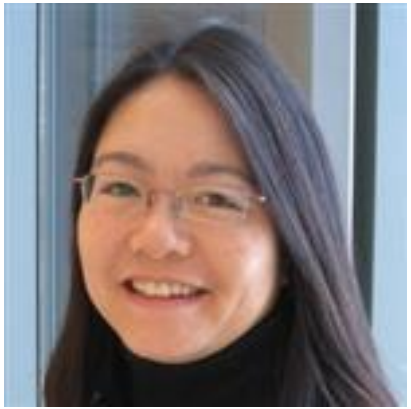
Brigham and Women's Hospital
Founding Member, Mass General Brigham

# Immunological Assessment Pre and Post Transplant

Melissa Y. Yeung, MD, FRCPC, FAST, AACHI
Medical Director, HLA Tissue Typing Laboratory
Associate Physician, Renal Division, Department of Medicine
Brigham and Women's Hospital
Assistant Professor of Medicine
Harvard Medical School

# Melissa Yeung, MD



University of Ottawa Medical School
Internal Medicine Residency, Maine Medical Center
Nephrology Fellowship, MGH/Brigham & Women's Hospital
Assistant Professor of Medicine, Harvard Medical School
- Clinical focus: kidney transplant, HLA/histocompatibility
- Research focus: regulatory B cells, alloantibody

# Disclosures

- Consultant: Alexion Pharmaceuticals, One Lambda Inc.
- Author and Reviewer, UpToDate Inc.
- Grant funding: Dialysis Clinic Inc., American Heart Association, National Institutes of Health

# Objectives

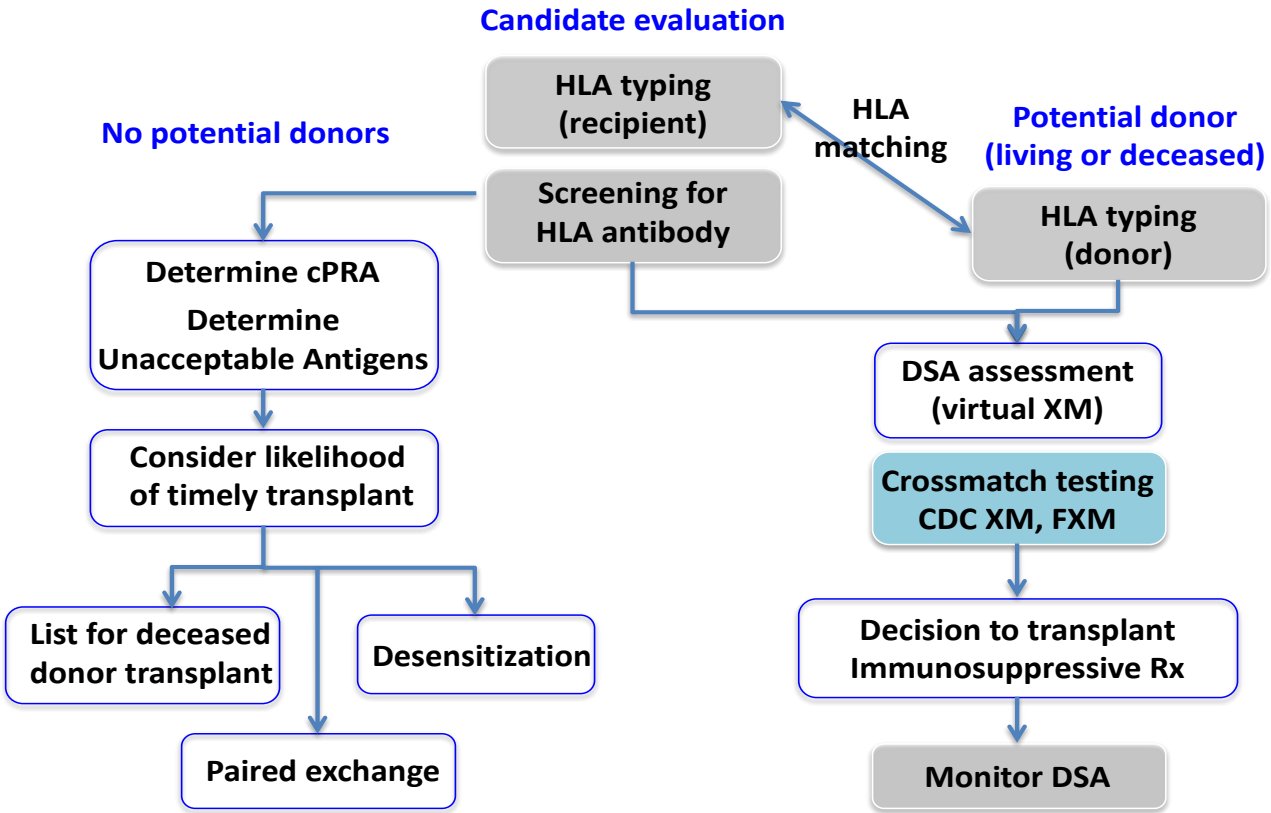
- Review the basics of HLA and histocompatibility
- Discuss the role of histocompatibility testing in determining immunological compatibility between a donor and recipient pair in kidney transplantation

# Why do we reject a transplanted organ?

**Recognize it as being "foreign"**

- Any protein that looks different from our own can elicit an immune response
- HLA molecules –very highly polymorphic ( >17,000 distinct HLA proteins! )
- ABO blood group antigens
- Other proteins are much more highly conserved

# Role of Histocompatibility Testing



# Case Examples: Histocompatibility Considerations

**Patient A**
35 year-old with ESRD on HD x5 years awaiting DD kidney transplant. cPRA 32%

**Patient B**
40 year-old with ESRD on HD x6 years awaiting DD kidney transplant. cPRA 98.2%

**Current clinical practice:**
list Unacceptable Antigens (UA) class 1 MFI >3000, class 2 MFI >3000
Local offers: T-CDC XM, FXM
T-CDC: if positive, absolute C/I
FXM: if positive, relative C/I (depends on strength, presence of DSA on SAB screen, donor characteristics, likelihood of another timely offer)

**What immunological risks are acceptable for each patient?**

# HLA Expression

**Class I HLA antigen (A, B, C)**

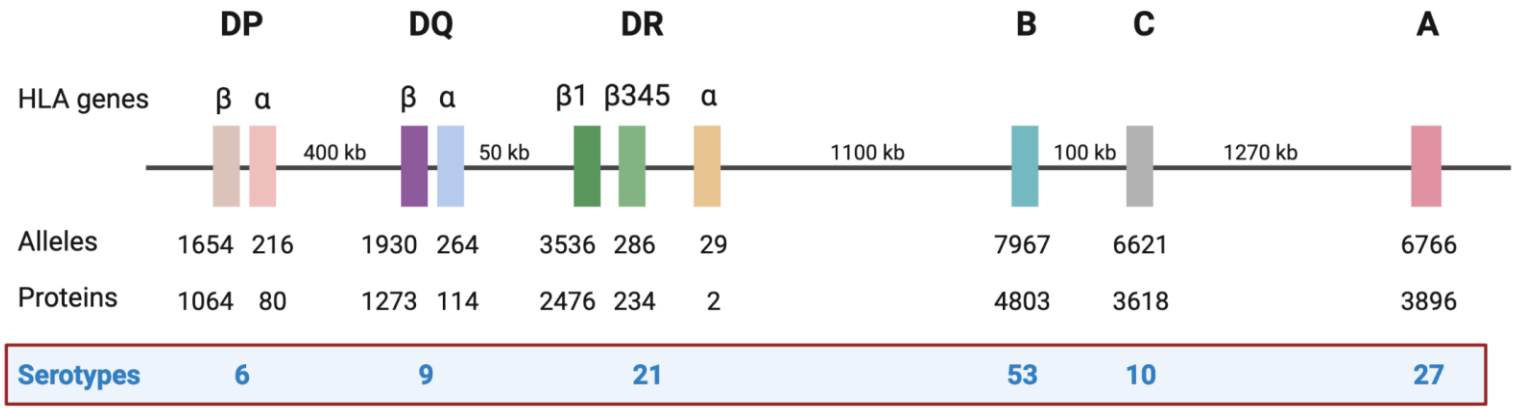
- expressed on all nucleated cells

**Class II HLA antigen (DP, DQ, DR)**

- expressed on antigen presenting cells (DCs, B cells), upregulated on endothelial & epithelial cells in inflammatory conditions

- Co-dominant expression
- Expression levels can modulate immune responses
  - HLA-C and HLA-DP cell surface expression levels are less than other loci

# HLA Polymorphism: Over 17,000 distinct HLA proteins!

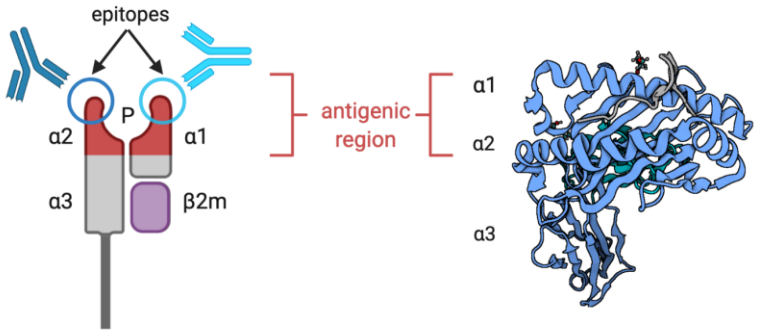


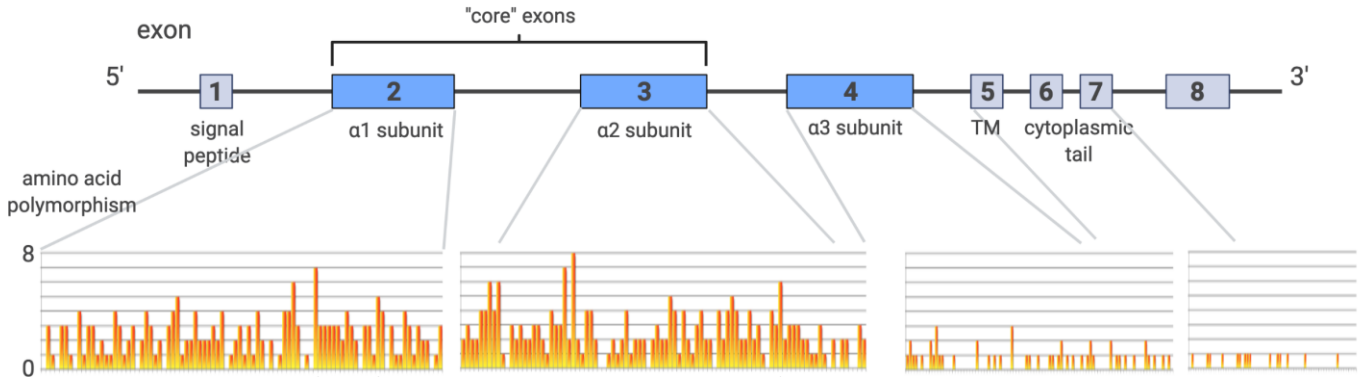

**>30,000 distinct HLA alleles**
**>18,000 distinct HLA proteins**

IMGT database 7/2021 (http://www.ebi.ac.uk/ipd/imgt/hla/stats.html)

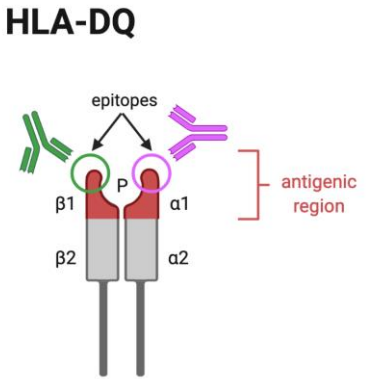
# Class I HLA polymorphism

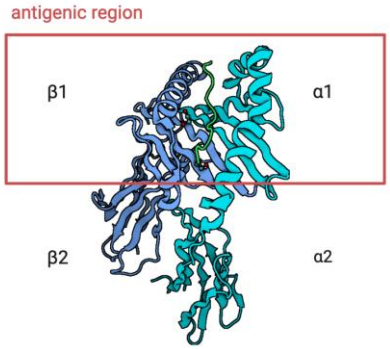
**HLA-A**

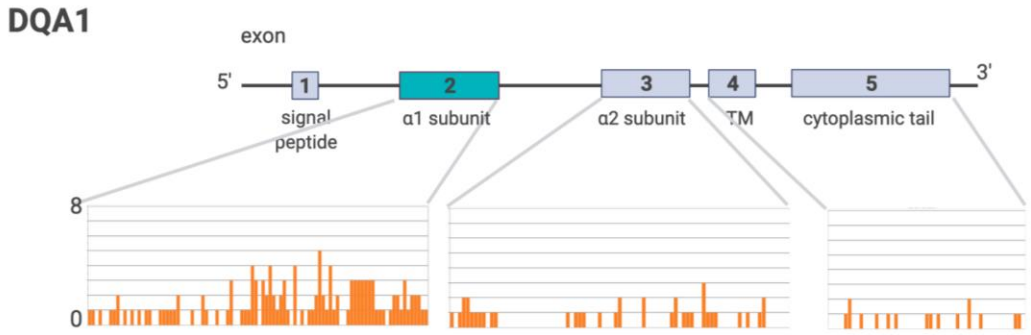


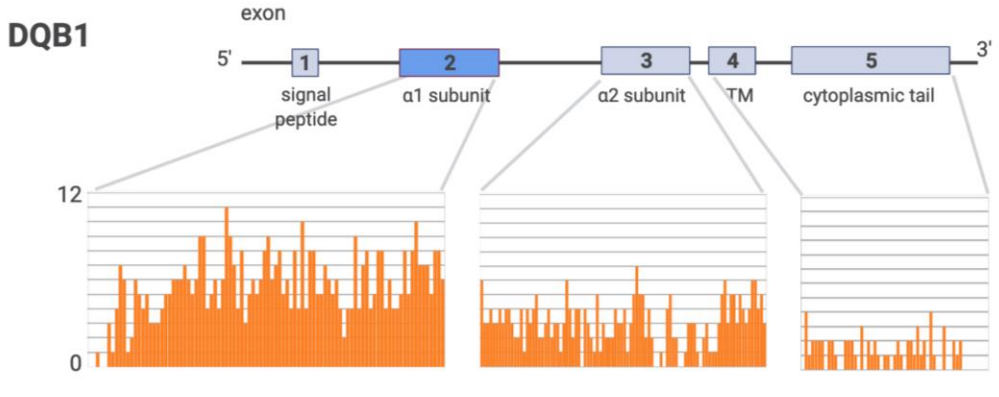



# Class II HLA polymorphism



# HLA typing methodology

## DNA-based molecular methods

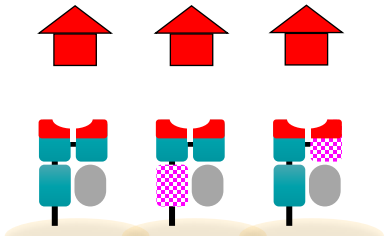
- Real-time PCR, SSP (sequence-specific primers), SSO (sequence-specific oligonucleotide probes)
  - Focuses on regions that are highly polymorphic (antigenic regions, “core exons”)
  - Resolve differences to the antigen/serologic level (low-resolution typing)

- Sequence-based typing (Sanger sequencing, next-generation sequencing)
  - Can sequence entire HLA gene
  - Resolves differences to the nucleotide level (high-resolution typing)

# Serological Antigen Equivalents

Epitope 1
Epitope 2
Antigenic region

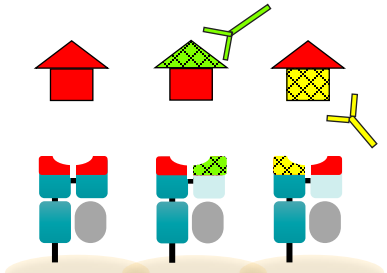
**What does the immune system “see” ?**



A*02:01 A*02:03 A*02:06

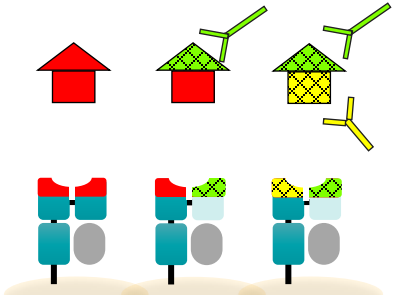
**A2 A2 A2**

- Serologically equivalent
- Named as same antigen
- Differences outside antigenic region



**A2 A11 A66**

- Serologically different
- Named different antigens



**B13 B7 B8**

**BW6**

- Serologically different but have a shared epitope
- Belong to a CREG

# HLA matching

- Each individual inherits one haplotype (set of HLA genes) from each parent

- Mismatch: an HLA antigen found on the cells of the donor (allograft), but not in the recipient
- 6 antigen match (zero mismatch): share same HLA-A, HLA-B and HLA-DR antigens, regardless of haplotype

- the greater the disparity between the donor and recipient, the more “foreign” the allograft appears = higher likelihood of developing an immune response
- Why not wait for a “perfect” 12/12 match?
  - Need to consider likelihood of finding such a match
  - impact on ethnic minorities with rarer antigens
  - Points and national sharing are given for zero antigen MM (A/B/DR)

# Detection of pre-formed HLA antibodies

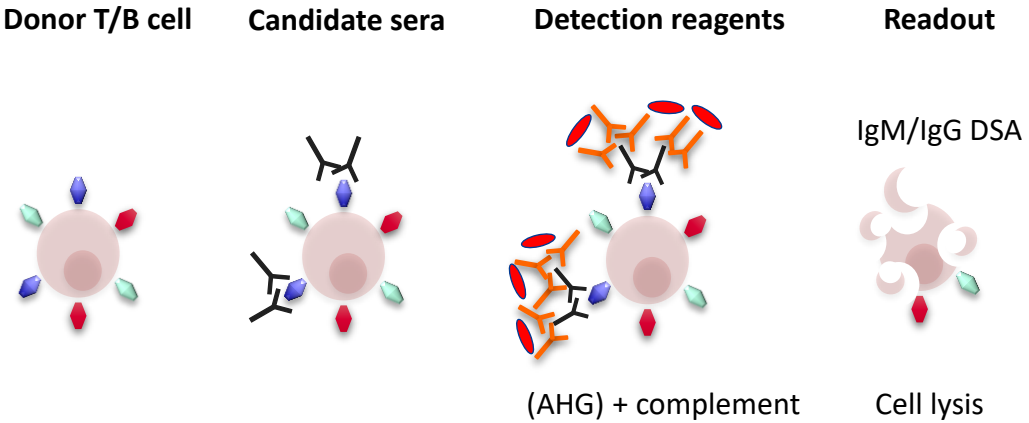
**Goal:**

- To determine the presence of any pre-formed donor-specific HLA antibody (DSA)
- Define its specificity to particular HLA antigen(s)
- Determine their relative amount/strength

**Current methodologies:**

- Complement-dependent cytotoxicity crossmatch (CDC XM)
- Flow crossmatch (FXM)
- Solid phase immunoassays (virtual XM)
  - Pooled/phenotype beads
  - Luminex single antigen bead (SAB) assays

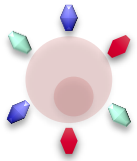
# Crossmatch Testing
## Complement-dependent cytotoxic crossmatch (CDC XM)

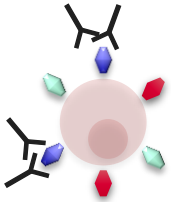


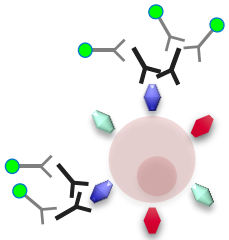

- Strong predictor of hyperacute rejection
- Detects both IgM and IgG anti-HLA antibodies
- T-CDC: class I HLA antibodies
- B-CDC: class I and II HLA Ab
- Also detects Ab directed against other non-HLA antigens present on T/B cells

# Crossmatch Testing
## Flow cytometric crossmatch (Flow XM)

**Donor T/B cell** | **Candidate sera** | **Detection reagents** | **Readout**

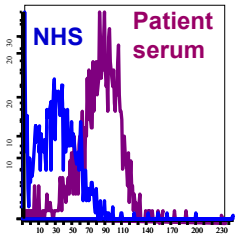






Fluorochrome conjugated Ab

IgG DSA



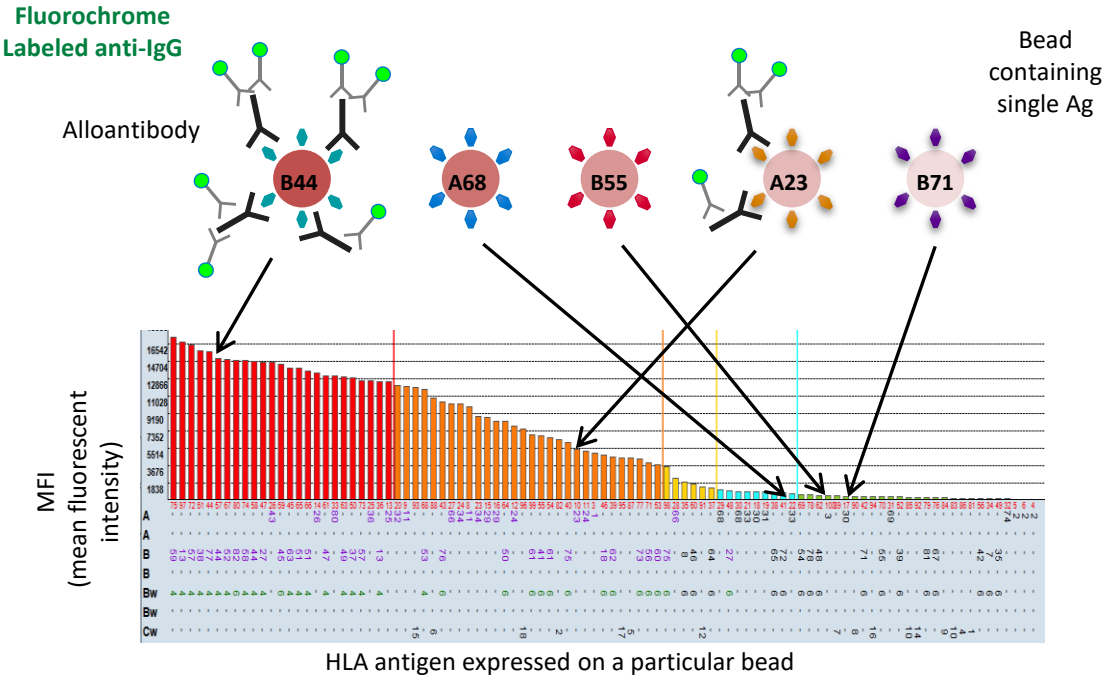

Fluorescent intensity

- Detects IgG anti-HLA antibodies
- Also detects Ab directed against other non-HLA antigens present on T/B cells

- Results expressed as a difference between fluorescent intensity shift of patient serum compared with normal human serum
  - Mean channel shift (MCS)
  - Direct fluorescent units (DFU)

- Threshold for positivity is set by lab
  - Lower threshold = more sensitive, higher false + rate
  - Higher threshold = less sensitive, higher false - rate

# Screening for anti-HLA antibodies
## Single antigen bead assay




1. Single antigen bead mix –each bead identified by different shade of fluorescent color
2. Add patient sera –if antibody is present, it will bind to the specific bead
3. Add a fluorochrome-labeled anti-IgG antibody
4. Detect by flow cytometric methods (“Luminex”) which beads are coated with antibody, and to what degree (semi-quantitative)

# cPRA (calculated Panel of Reactive Antibody)

- **Calculates likelihood of transplant by:**
  - comparing the specificity of the anti-HLA antibodies present in the potential recipient to…
  - the phenotypic prevalence of the particular HLA antigen in the donor population

- **a high cPRA means a lower chance for a transplant**
  - a cPRA of 80% means the patient will be ineligible (on the basis of having DSA) for 80% of deceased donor kidneys

- **Used in UNOS/OPTN kidney allocation system**
  - to create greater parity between waitlisted patients, regardless of their degree of sensitization by giving “priority points” based on increasing cPRA levels

# Identifying antibodies as donor-specific (DSA): “Virtual crossmatch”

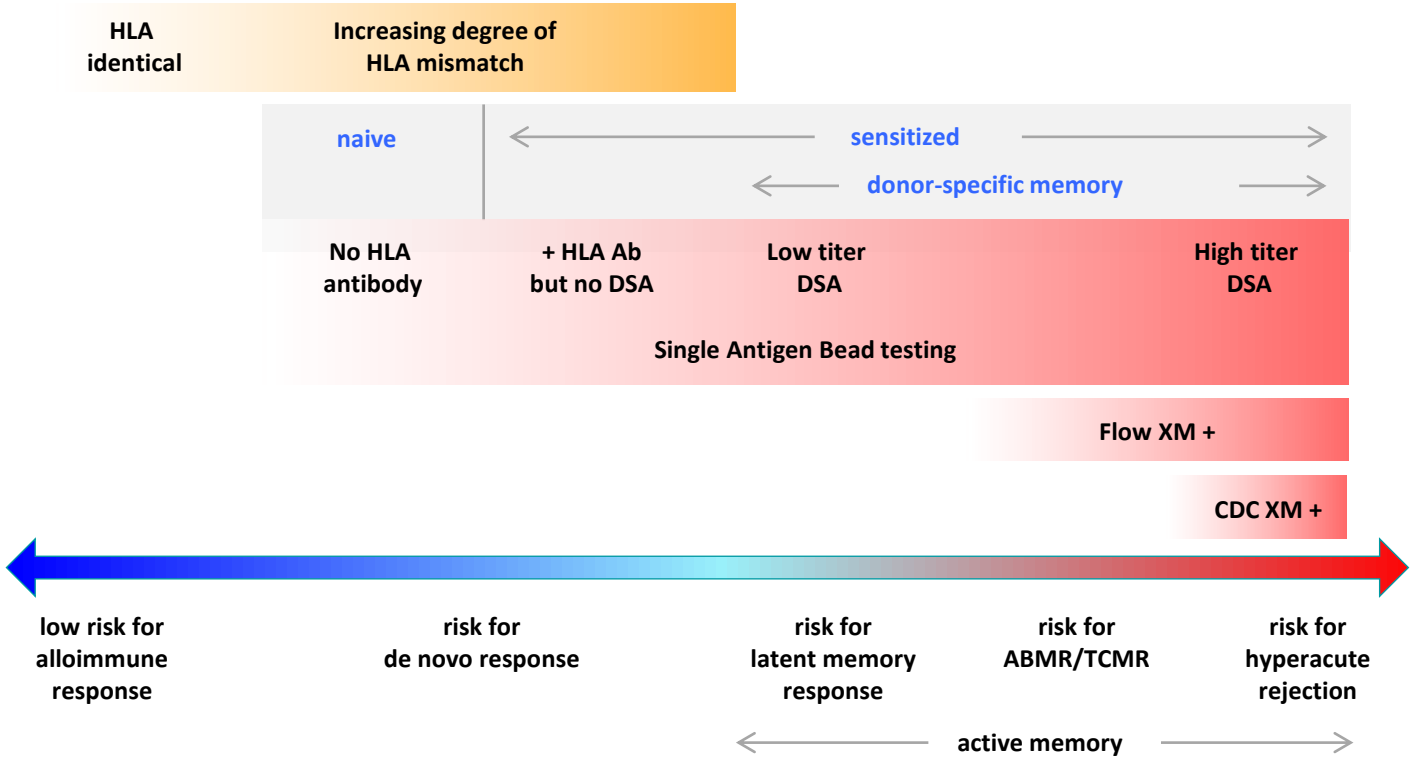
Compare the single antigen bead testing results to the HLA typing of the donor

Antibodies detected in candidate:
**A1, A11, B49, B44, DR4, DQ6**

Donor antigens:
**A11, A24, B51, B55, DR11, DR16, DQ7**

Donor Specific Antibody: **A11**

# Immunological Risk Assessment



# Comparison between tests

| Single Antigen Bead Testing | Flow XM | CDC XM |
|---|---|---|
| Pre-tx risk assessment<br>Post-tx monitoring | At time of transplant | At time of transplant |
| Beads | Donor cells | Donor cells |
| IgG | IgG | IgG and IgM |
| Anti-HLA<br>(denatured HLA Ag) | Anti-HLA<br>Non-HLA | Anti-HLA<br>Non-HLA |
| More consistent than Cell-based assays | Detects Ab that can bind to the donor cells | Detects Ab that can bind donor cells and fix complement |
| Highest sensitivity | High sensitivity | Lowest sensitivity |
| Clinical significance of low-level Ab unclear | False positive with poor quality cells | False positive with poor quality cells |

# Take Home Messages

- Current histocompatibility testing assesses for the presence/absence of pre-formed donor specific antibody (DSA)
- The various histocompatibility assays should be used in totality to provide a comprehensive assessment of immunological risk
- When relying on a virtual crossmatch alone, be cognizant that limitations in testing methodologies may make it difficult to conclude presence/absence of pre-formed donor specific antibody

# References


- Yeung MY. Overview of HLA sensitization and crossmatch testing in renal transplantation. In: UpToDate, Basow DS (Ed), UpToDate, Waltham, MA.