Using All the Tools in the Toolbox to Define True HLA-DPB1 Antibody Reactivity

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Case description

Comprehensive pre-transplant HLA antibody testing is essential for immunological risk assessment, but discordant results across testing platforms cause challenges for clinical decision-making.

Here, we describe a kidney transplant candidate undergoing evaluation with a potential living donor who demonstrated conflicting HLA-DPB1 antibody results. Single antigen bead (SAB) testing from two vendors was positive for HLA-DPB1 with strong reactivity against the 96R epitope, including donor-specific DPB1*04:01, making the virtual crossmatch positive. However, cell-based flow-cytometric crossmatch using potential donor lymphocytes (heterozygous for DPB0401) and surrogate crossmatch with homozygous DPB0401 donor cells both resulted in a negative B cell crossmatch. FlowPRA testing was negative for HLA class II antibodies, supporting the negative cell-based crossmatch results.

Low antigen expression due to regulatory polymorphisms (rs9277534) A/G and/or reactivity to non-clinically relevant epitopes were considered possible explanations for the discordance. These findings suggested the absence of clinically relevant donor-specific antibodies (DSA). Yet, due to high reactivity to DPB0401 in SAB assays, remote sensitization history, and surgical complications, the transplant program decided not to proceed with this living donor and go ahead with another compatible living donor.

Methods

- HLA typing was performed using LABType™ rSSO from One Lambda Thermo Fisher Scientific.
- HLA antibody screening was performed using LIFECODES ® LifeScreen Deluxe (LMX) from Werfen-Immucor GTI Diagnostics, Inc.
- Serum samples were pre-treated with EDTA and tested for antibody specificity by LABScreen® Single Antigen (LSA) from One Lambda Thermo Fisher Scientific, and LIFECODES ® Single Antigen (LC-LSA) from Werfen-Immucor GTI Diagnostics, Inc.
- SAB results were acquired using Luminex Flexmap 3D instrument with no divider.
- Eplet analysis was performed using MatchMaker tool within the HLA Fusion™ software of One Lambda Thermo Fisher Scientific.
- Serum sample was sent to the HLA laboratory in Winnipeg for antibody assessment using FlowPRA.
- Flow Cytometer crossmatch was performed using patient serum and lymphocytes isolated from the potential donor or surrogate donor using the EasySep™ Direct Human Total Lymphocyte Isolation kit from STEMCELL Technologies.

HLA typing

HLA-Antigens	A	B	Bw	C	DRB1	DRB345	DQA1	DQB1	DPA1	DPB1
Patient	1	8	6	7	17	52	05	2	01	03:01P
	23	18	4	7	11	52	05	7	02	14:01P
Potential donor	2	7	6	7	7	53	02	2	01	04:01P
	3	7	6	7	15	51	01	6	02	05:01P

Serological equivalents are shown for HLA-A, B, Cw, DRB1, DR345 and DQB1, and molecular typing are shown for HLA-DQA1, DPA1 and DPB1.

Conclusion

This case highlights the challenges of determining the clinical significance of HLA-DPB1 antibody reactivity when solid-phase and cell-based assays produce discordant results. Low antigen expression and potential reactivity to cryptic or non-clinically relevant epitopes may contribute to the discrepancies. This case underscores the need for improved testing strategies to support clinical decision-making in transplantation.

Results

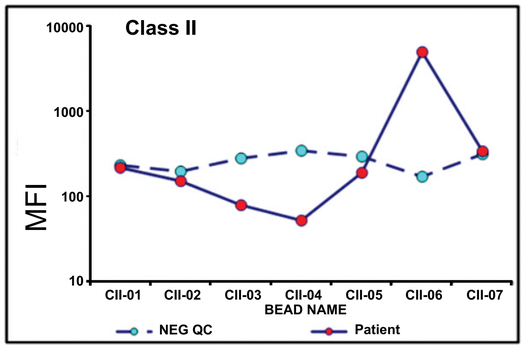


Figure 1. HLA Class II antibody reactivity screening was positive for the HLA-DPB1 bead.

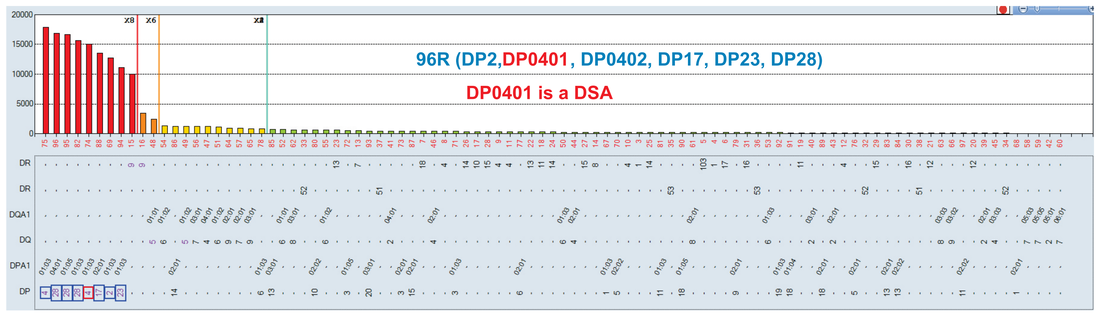


Figure 2. SAB testing from our primary vendor identified strong HLA class II antibody reactivity against the 96R epitope, including donor-specific DPB1*04:01.

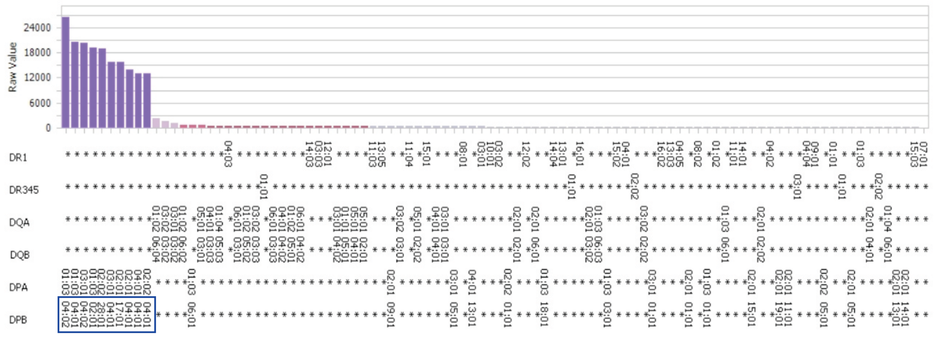


Figure3 . SAB testing from our secondary vendor showed a similar DPB1 antibody reactivity pattern.

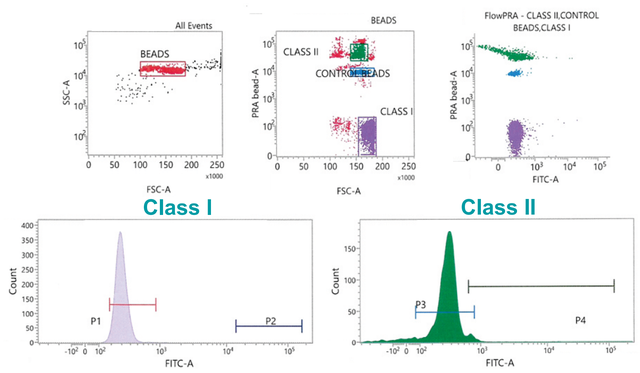


Figure 4. Antibody reactivity was not detected by FlowPRA

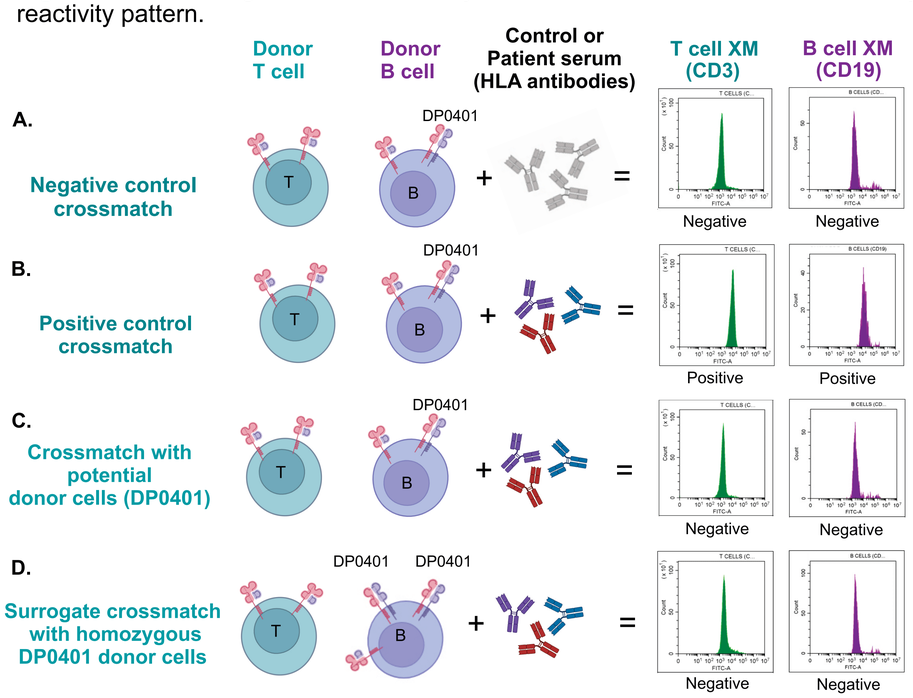


Figure 5. Cell-based flow-cytometric crossmatch including negative control (A), positive control (B), patient serum with potential donor lymphocytes (heterozygous for DPB0401) that resulted in a negative B cell crossmatch (C), and surrogate crossmatch using homozygous DP0401 cells also resulted in a negative B cell (D).

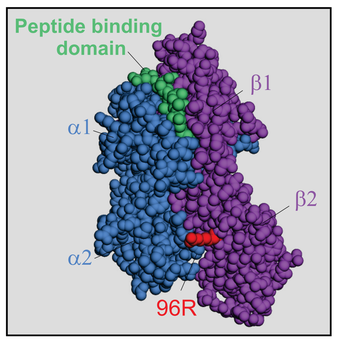


Figure 6. HLA-DP 3D shape showing the position of 96R epitope in the β2 chain. The image was obtained from pHLA 3D (https://www.phla3d.com.br/alleles/view/DPA1*01:03-DPB1*04:01/1).