# Unexpected Positive Flow Crossmatches with Bimodal Distribution of the Donor Cells

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## INTRODUCTION

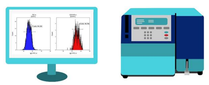
***Flow cytometry crossmatch*** (FCXM) is a critical tool for pretransplant immunologic risk assessment in solid organ transplantation, offering high sensitivity for detecting donor-specific antibodies that may compromise graft survival. Occasionally, ***unexpected positive*** FCXM results arise in recipients without HLA-DSA antibodies, prompting further immunologic investigation as they carry important clinical implications. One phenomenon that could lead to an unexpected positive FCXM is the appearance of a ***bimodal fluorescence distribution***, characterized by two distinct peaks in flow cytometry histograms.

Here, we aimed to investigate the frequency of bimodal distributions in donor cells, explore the benefits of conducting autologous FCXM in these instances, and examine the factors that may contribute to this bimodal distribution.

## PATIENTS AND METHODS

Flow cytometry crossmatches (FCXMs) performed for solid organ transplant patients.

Retrospective review of all flow crossmatch results performed in our laboratory between March 024 and March 2025.



Flow cytometer

FCXMs were performed on T and B cells isolated from peripheral blood using *EasySep* and treated with pronase.

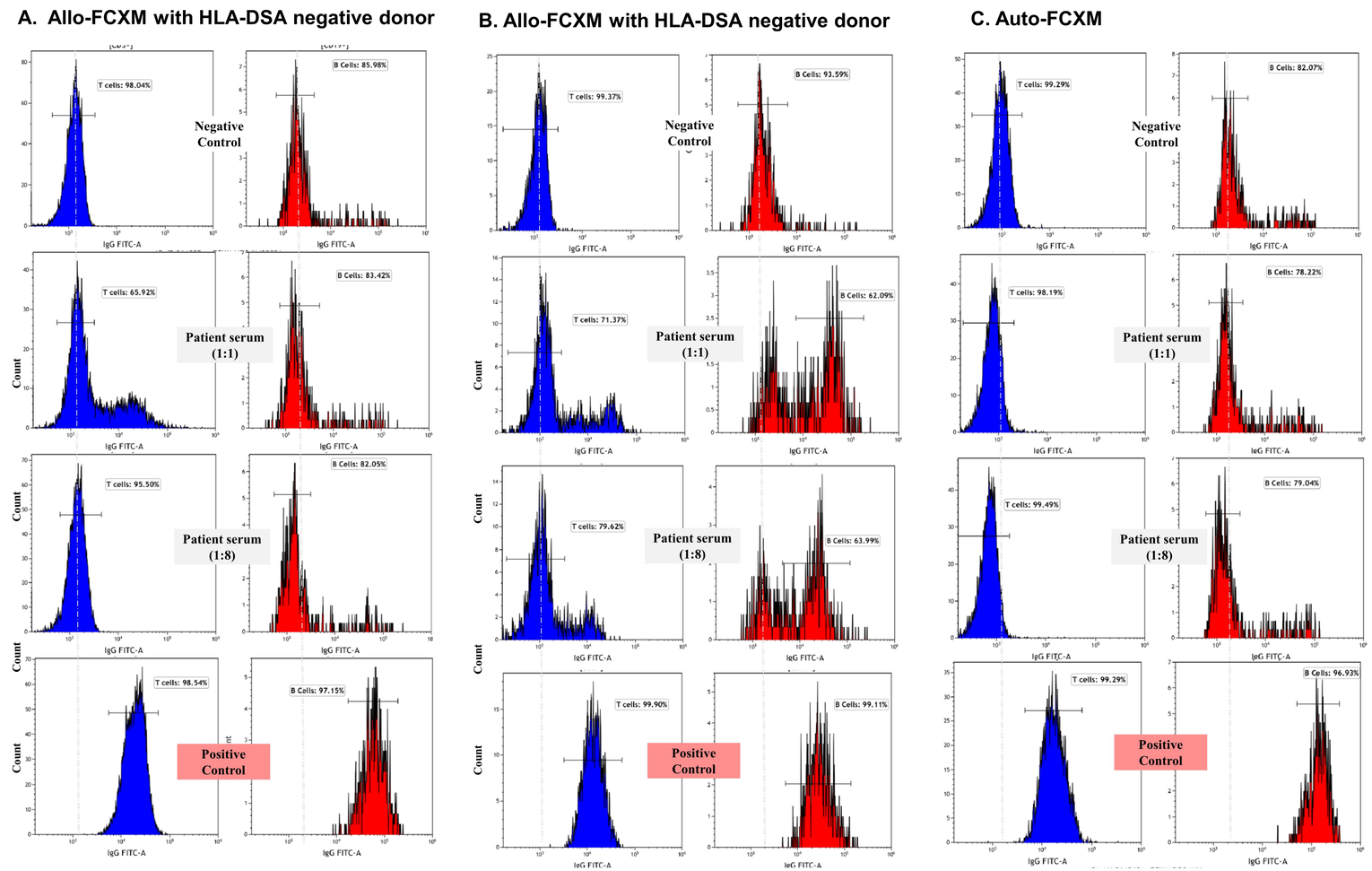
## RESULTS

A total of 458 FCXM results were reviewed, of which 57 cases (12%) showed a bimodal fluorescence distribution, defined by a distinct second peak comprising more than 20% of the total T and/or B cell population.

In 51 out of 57 cases, a second fluorescence peak was observed in the B cell population across wells containing patient serum, negative calibrator, and negative control, suggesting the presence of *memory B cell subpopulations*. This hypothesis was supported in 10 FCXMs by additional well staining in which the donor cells were stained solely with anti-human IgG FITC antibody, confirming the presence of IgG-positive B cells.

In 3 FCXMs we observed a ***patient serum-specific*** bimodal T cell distribution not seen in the negative calibrator and control wells *(Figure 1A)*. The remaining three cases exhibited bimodal distributions in both T and B cell populations, which were similarly attributed to factors present in the patient serum *(Figure 1B)*. Notably, in one of these cases, the bimodal pattern persisted even in the absence of pronase treatment, and autologous FCXMs performed for two of the patients yielded negative results *(Figure 1C)*.

**Figure 1. Flow crossmatch histograms in patients with bimodal fluorescence distribution**




A. Allo-FCXM vs. HLA-DSA negative donor exhibiting bi-modal T fluorescence distribution.

B. Allo-FCXM vs. HLA-DSA negative donor exhibiting bi-modal T and B fluorescence distribution.

C. Auto-FCXM of the patients with Bi-modal T and B fluorescence distribution in allo-FCXMs.

The bimodal architecture could not be explained by organ category, ABOi, autoimmunity, or therapeutic antibodies. Two of these patients are kidney patients and are still waiting for FCXM-compatible donors. The third, a lung transplant recipient, was transplanted across weak class I HLA-DSA and exhibited unexpectedly strong T and B cell positive FCXM with bimodal architecture. However, post-transplant monitoring showed clearance of the weak pre-transplant HLA-DSA; the patient's clinical course was complicated by severe early allograft rejection, suggesting potential significance of bimodal FCXM patterns in predicting adverse post-transplant outcomes.

## CONCLUSION

The bimodal fluorescence distribution observed in B cells is primarily attributed to the presence of memory cells. However, in a limited number of cases, a bimodal T and B cell fluorescence distribution can be observed due to patient-specific factors present in the serum, resulting in unexpected positive flow cytometry crossmatches with unclear clinical significance.

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