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# Virtual Crossmatch Compatible, With Unexpected Fluorescence Cytometric Crossmatch Positive, May Be Safe To Proceed with Transplantation Under Certain Circumstances

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## ABSTRACT

**AIM:** To assess Virtual Crossmatch (VXM) compatible, with unexpected Fluorecence Cytometric Crossmatch (FCXM) positive patient-donor results, for causes and whether it may be safe to proceed with transplantation

**METHODS:** VXM and FCXM results on renal transplantation patients from September 2022 through March 2025 were reviewed for discrepancies. Patient charts were reviewed for autoimmune conditions and infectious status, which may result in autoimmune or other antibodies (eg, heterophile antibodies), which could result in a "false" positive FCXM. Potential donor-specific antibodies (DSA) and FCXM median channel number shifts (CNS) were assessed. Epitope analyses were performed as needed to verify antibodies as potential DSA. Autologous FCXM (A-FCXM) is recommended, but not necessarily ordered by the transplantation service. T-test was used, with $p < 0.05$ considered significant.

**RESULTS:** VXM and FCXM results on renal transplantation patients from September 2022 through March 2025 were reviewed, with 4429 VXM and 1022 FCXM performed. Thirty-eight FCXM, of 28 patients, had an unexpected positive result (3.7%). Twenty-five patients had positive autologous FCXM, autoimmune disorders, or the presence of HIV to explain the unexpected positive allogeneic FCXM. Sixteen patients were transplanted without post-transplantation complications. Reasons for selection of these to transplant versus those not transplanted were not provided to the HLA staff.

**CONCLUSIONS:** FCXM remains valid for confirming compatibility, due to exposures resulting in DSA since the last anti-HLA antibody analysis. We have had a VXM-compatible patient with positive FCXM who had received fresh frozen plasma one week prior to the FCXM, unbeknownst to the transplantation service. Anti-HLA-B57 was detected, which went away after 5 months. There are many potential causes for unexpected positive FCXM. A-FCXM positivity can be reassuring for proceeding with transplantation. Otherwise, it may be safe to proceed with transplantation if non-HLA antibodies are suspected (such as due to HIV infection), and sensitizing events have not occurred since the last anti-HLA antibody assay.

## BACKGROUND

FCXM and VXM data from September 2022 through March 2025 were reviewed for discrepancies between VXM results and FCXM results, specifically, looking for compatible VXM resulting in a positive FCXM. VXM were also reviewed for differences between preliminary results (technologist performed) and final results (director reviewed) to rule out a wrong interpretation as the cause for an unexpected positive FCXM. Data were collected on deceased donor kidney patients. Thirty-eight FCXM of 28 different patients gave an unexpected positive result based on a compatible VXM. Patient information was reviewed to find possible explanations for positive FCXM. FCXM data were categorized based on 1) patient transplanted vs not transplanted, 2) T cell positive and B cell positive result, 3) T cell negative and B cell positive result, and 4) disease correlation. Median channel shift (MCS) for both T and B cells for the different categories were compared for statistically significant differences. Mean Fluorescence Intensity (MFI) values were reviewed between all serums used for physical crossmatch and compared between those that received transplantation and those that did not for a statistically significant difference.

## METHODS

- LABScreen™ single antigen determination using One Lambda® commercial kits
- LABType™ SSO typing using One Lambda® commercial kits
- Next Generation Sequencing (NGS) using CareDx® commercial kits
- Flow Cytometric Crossmatch (FCXM): 2SD T-cell positive, 3SD B-cell positive cutoffs
- Virtual Crossmatch (VXM) using Mtilda® VXMatch® software

## RESULTS

Between September 2022 and March 2025; 4429 VXM were performed, with 1022 FCXM. Only 38 FCXM (28 patients) had an unexpected positive result (3.7%). Twenty-five of the patients had reasons other than anti-HLA antibodies to explain a positive FCXM, (these included positive autologous FCXM, autoimmune disease, or HIV). Sixteen of the patients were transplanted with the donor from the unexpected positive FCXM; all patients are currently doing well post-transplantation. No significant differences were found in MCS between those transplanted and those that were not transplanted (Table 1). No statistically significant differences were found between serum MFI values in those transplanted and those not transplanted. MFI values for potential DSA for those transplanted were generally below 1000, with a few exceptions. In patients with higher MFI values an autologous FCXM was performed and found to be positive in most cases. The final results from a Director agreed with all the preliminary VXM, thus ruling out technical errors.

*Table 1. Mean Channel Shift of T and B cell flow cytometric crossmatch with unexpected positive results. (N =38)*

| Flow cytometric crossmatch results | T-Cell single dose | B-Cell Single dose | T-Cell double dose | B-Cell double dose |
|---|---|---|---|---|
| Overall Unexpected positive (38) | 82 | 159 | 109 | 170 |
| -T cell positive B cell positive (24)63% | 116 | 150 | 155 | 162 |
| -T cell negative B cell positive (14) 36% | 25 | 174 | 34 | 183 |
| Positive crossmatch resulting in a transplant (16) 42% | 105 | 169 | 118 | 179 |
| -Transplanted T cell positive B cell positive(9)23% | 149 | 170 | 165 | 184 |
| Transplanted -T cell negative B cell positive (5) 13% | 41 | 170 | 50 | 173 |
| Patient Hep C positive (3) 8% | 63 | 167 | 62 | 158 |
| Patient Hep C positive and transplanted (2) 5% | 59 | 176 | 60 | 166 |
| Patient Lupus (4) 11% | 100 | 180 | 115 | 198 |
| *Other autoimmune total (12) 32% | 81 | 170 | 93 | 180 |
| *Other autoimmune and transplanted (6) 16% | 92 | 165 | 103 | 175 |
| Not Transplanted total (22) 58% | 68 | 146 | 106 | 158 |
| Not Transplanted- T cell positive B cell positive (12) 32% | 101 | 141 | 164 | 151 |
| Not Transplanted -T cell negative B cell positive (10) 26% | 33 | 172 | 41 | 185 |

A T cell channel shift of ≥60 is positive, B cell channel shift of ≥100 is positive.

## CONCLUSIONS

A compatible VXM without elevated donor-specific HLA antibodies remains a good predictor of a negative FCXM (92% correlation in our long-term experience). While unexpected positive FCXM can occur, our data indicate that for most patients, the transplant could still occur successfully, when potential DSA have low MFI values (especially <300) and no sensitizing events have occurred, further, when the positive FCXM can be explained by underlying disease, infection, or autoimmunity. Additionally, a positive A-FCXM can be reassuring that the allogenic FCXM was likely a "false-positive". For the VXM to be successful, very recent anti-HLA antibody studies need to be performed. Clinical decision-making between the HLA service and the Transplantation service is also critical for proceeding with safe transplantation. Finally, it remains very important to fully review the individual history of each patient when making the decision to proceed with transplantation or not.