Obinutuzumab Associated Interference Causing Unexpected Flow Cytometric Crossmatch Reactivity

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Case Study

This case highlights the potential for monoclonal antibody therapies to cause significant interference in histocompatibility testing, leading to false positive B cell crossmatches and clinical delays.

A 54-year-old male with membranous glomerulonephritis who was scheduled for a preemptive living unrelated kidney transplant received a transfusion. His calculated Panel Reactive Antibody (cPRA) was 0% prior to the transfusion, and the initial virtual crossmatch was negative. Following the transfusion, updated antibody testing identified low level, non-specific reactivity on the Single Antigen Bead (SAB) Class II assay. Surrogate crossmatches were performed to confirm reactivity. The Flow Cytometric Crossmatch (FCXM) yielded negative results for T cells but strongly positive results for B cells which did not correlate with the low-level reactivity observed in the antibody testing. Additionally, testing with the Lifecodes Single Antigen (LSA) assay was negative for all samples, confirming a discrepancy between the B cell FCXM and the antibody profile. Consequently, the transplant surgery was postponed to investigate the cause of this discrepancy.

Patient CD19/CD20 B cell %

		CD19+CD20+
Ref. Range & Units		%
07/13/26	07:48	11.2
06/25/26	08:20	20.4
04/07/26	15:23	0.4
03/05/26	16:32	0.0
02/03/26	16:21	0.1
01/06/26	16:29	0.0
10/25/25	02:51	0.6

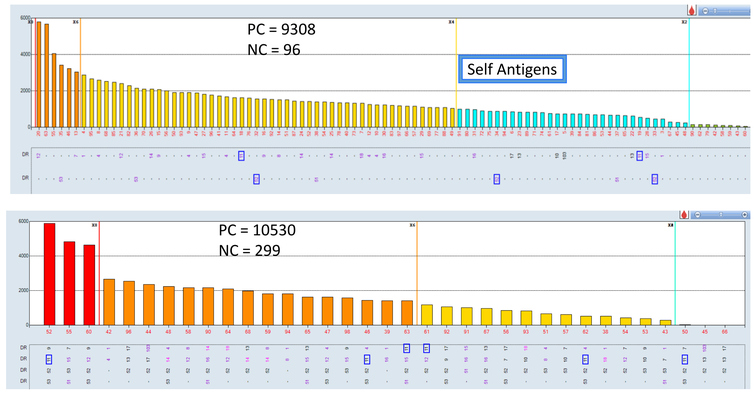
Results

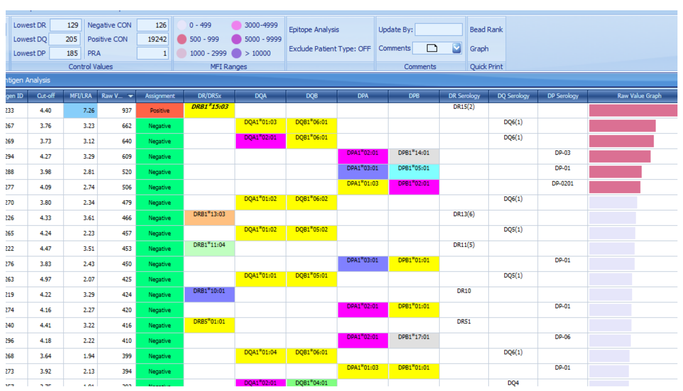
Antibody testing repeated at 1:4 dilution showed no additional reactivity. A new serum sample was collected and confirmed the same reactivity pattern. Auto FCXM performed was negative for T cells; however, B cell results were invalid because the sample contained only 0.01% B cells and 85% T cells. Five different surrogate donor cells were tested; all consistently yielded strongly positive B cell crossmatch results.

A review of the patient's history revealed interstitial lung disease previously treated with mycophenolate mofetil (MMF), prednisone, rituximab (last dose in 2024), cyclophosphamide, and most recently, obinutuzumab. Obinutuzumab is a monoclonal antibody designed to target CD20 and often used for patients who are refractory or intolerant to rituximab. It can directly induce cell death through a mechanism known as antibody dependent cellular cytotoxicity. The patient's last dose was administered in July 2025. According to the package insert, while the half-life is <32 days, sustained B cell depletion can persist for up to 12 months post infusion.

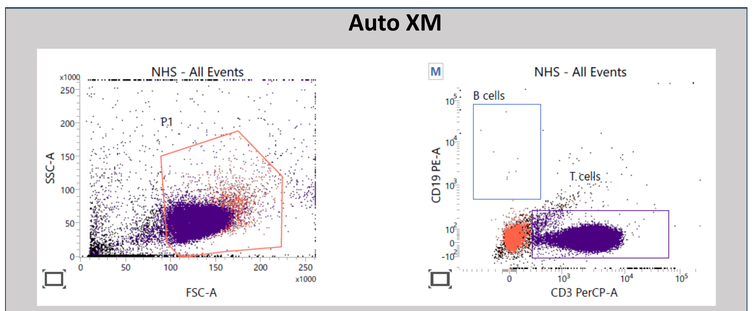
Antibody Data

Sample ID: 140691





FCXM



Statistics

Patient Auto XM: 11/25/25

Name	IgG FITC-A Median	Events	% Total	% Parent	% Grandparent
Tube_002:All Events	183	102,756	100.00	***	***
Tube_002:T cells	179	89,712	87.31	87.31	***
Tube_002:B cells	52,165	8	0.01	0.01	***

	LR Donor T Cell XM		LR Donor B Cell XM	
Patient Sample Date	MCS	Interpretation	MCS	Interpretation
9/3/2025	0	negative	11490	Positive
12/10/2025	0	negative	7356	Positive
1/6/2026	0	negative	2448	Positive
3/5/2026	0	negative	0	negative
MCS = Median Channel Shift			B cell Positive cutoff 349	

Discussion

Due to the low B cell count (<1% in October 2025), the transplant was delayed, and the patient started dialysis in November 2025. A follow up antibody test was performed on a sample from April 2026 returned to 0% cPRA with no nonspecific background. Subsequent FCXM using multiple patient samples confirmed that the nonspecific B cell reactivity was no longer present. Transplant with a living donor was performed and patient is doing well.

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