Effect of Ethylenediaminetetraacetic Acid (EDTA) Treatment of Recipient Sera on Flow Cytometry Crossmatch

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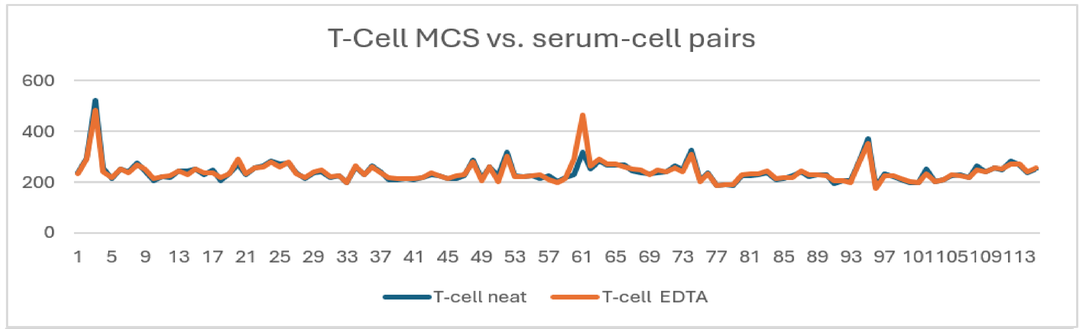
INTRODUCTION

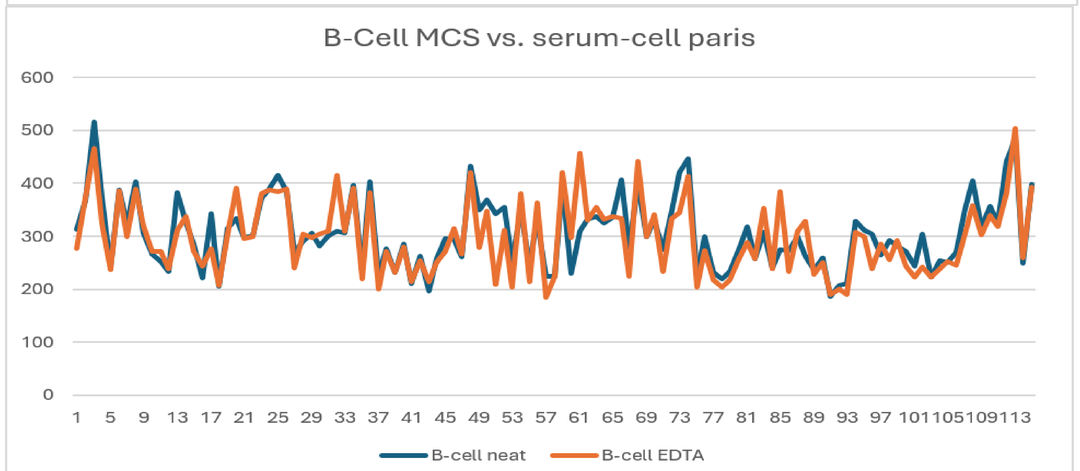
An increase in positive B-cell flow cytometry crossmatches (FCXM) without clinically significant donor-specific antibodies (DSA) prompted an evaluation of EDTA-treated sera to potentially reduce false B-cell reactivity.

MATERIALS & METHODS

Flow cytometry crossmatch: FCXMs were performed in 96-well plates (*Halifaster* technique) using pronase (Protease from *Streptomyces griseus*, Sigma Aldrich P5147) treated (15 min at 37°C) donor cells (150,000) incubated with recipient serum and controls (30 µL) for 20 min at room temperature. Cells were washed three times with 2% FCS/DPBS solution. Stain reagents added included fluorescein-labeled (FITC) goat anti-human IgG (10 µL), phycoerythrin (PE) anti-CD19 (5 µL, B cells), and peridinin chlorophyll protein (PerCP) anti-CD3 (5 µL, T cells). After 5 min incubation and two washes with 2% FCS/DPBS, samples were resuspended (150 µL of 2% FCS/DPBS) and analyzed on a Beckman Coulter CytoFLEX flow cytometer.

Data collection: A total of 115 samples (67% autologous) were tested representative of ordinary samples (no selection criteria other than necessary quantity of sample applied). Median Channel Shift (MCS) values were compared between untreated serum and EDTA-treated serum (5 µL 100 mM EDTA + 95 µL serum). Standard positive/negative cutoffs for the negative controls were applied to determine “positive” or “negative” crossmatch result. Differences were calculated with mean and standard deviation. Cases where result “flips” from negative to positive or positive to negative occurred were reviewed for auto/allo status, presence of donor specific antibody, and proximity to pos-neg cutoff.





Case Studies:

B-cell flip to Positive:

Case 1 (238839 vs L009288): Allo crossmatch. Repeated three times. 0% CPRA. Only one of the three was positive. Virtual XM negative. Likely a spurious result.
Case 2 (235332 vs L009344): Allo crossmatch. 0% CPRA. Virtual XM negative. EDTA 29 MCS over cutoff. Neat serum 47 MCS under cutoff.
Case 3 (238111 vs L009436): Allo crossmatch. 0% CPRA. Virtual XM negative. Neat serum 18 under cutoff. EDTA treated serum 24 over the cutoff for a spread of 42 MCS change.

B-cell flip to Negative:

Case 1 (240413 vs. L009309). Allo crossmatch. Virtual crossmatch negative. 33% CPRA. Repeated three times. Untreated serum only positive in one run. EDTA treated all negative. Example of effective use of EDTA treated serum.
Case 2 (241353 vs L009354) Auto crossmatch. 9% CPRA. Auto-antibody not detected. Repeated negative. EDTA sample negative. Example of effective use of EDTA treated serum.
Case 3 (241721 vs L009383): Allo crossmatch. 0% CPRA. Repeated positive twice with untreated serum. EDTA treated serum negative. Virtual crossmatch negative. Historical Auto crossmatch negative. Very clear example of the utility of EDTA treatment.
Case 4 (241942 vs L009413) Auto crossmatch. 0% CPRA. EDTA treated sample negative, while untreated was positive (no repeat). Clear example of utility of EDTA treatment.
Case 5 (242072 vs L009435) Auto crossmatch. Auto-antibody not detected (40% PRA). Crossmatch with neat serum repeated negative. EDTA treated serum negative. Example of utility of EDTA treatment.
Case 6 (239108 vs L009444) Allo crossmatch. DSA @ 2475 MFI against donor (62% CPRA). Recipient had recent negative auto crossmatch. EDTA treated sample was only 1 mean channel shift below the positive-negative cutoff (consistent with the average lowering of EDTA treated serum MCS values). Neat serum was 34 MCS above the cutoff. Example of the need to run neat serum before EDTA treated serum to prevent possibility of false Negative result interpretation.

RESULTS

EDTA Treated Serum Vs. Untreated

Total crossmatches:115

	T-cell	B-cell
Increase MCS with EDTA:	64	41
Decrease MCS with EDTA:	51	74
Flips to positive with EDTA:	1	**3**
Flips to negative with EDTA:	1	**6**
Average change in MCS	+1.3	-7.9
SD of Average change MCS	17	37

CONCLUSIONS

EDTA treatment had minimal impact on T-cell FCXM (mean +1.3 MCS) and both examples where a flip occurred were very near cutoff. *Effect of EDTA treatment on T-cells appears insignificant.*

For B-cell FCXM, EDTA modestly reduced reactivity by ~8 MCS on pronase treated cells. Three cases showed negative-to-positive flips without DSA; repeat testing with untreated serum was negative. Six cases (3 allo, 3 auto) shifted from positive to negative with EDTA; five lacked DSA, supporting reduction of false positives hypothesis. One allo case with DSA (2475 MFI) fell just below cutoff after EDTA treatment but was clearly positive untreated (+34 MCS above cutoff).

Overall, *EDTA treatment helps resolve suspected false B-cell positivity even in pronase treated cells*, but it carries risk of false negatives if untreated serum is not used.

References:
Liwski, R. S. et al. *Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols.* Human Immunology. 2018 Jan; 79(1):28-38.
Putheti, Prabhakar et al. *EDTA Treatment of Serum Reduces T Cell and B Cell False Positivity in Flow Cytometry Crossmatching: A Multicenter Study.* HLA Immune Response Genetics. Volume 106, Issue 3. September 2025